isabc 14
Toulouse•France

14th International Symposium on Applied Bioinorganic Chemistry

Conference Book

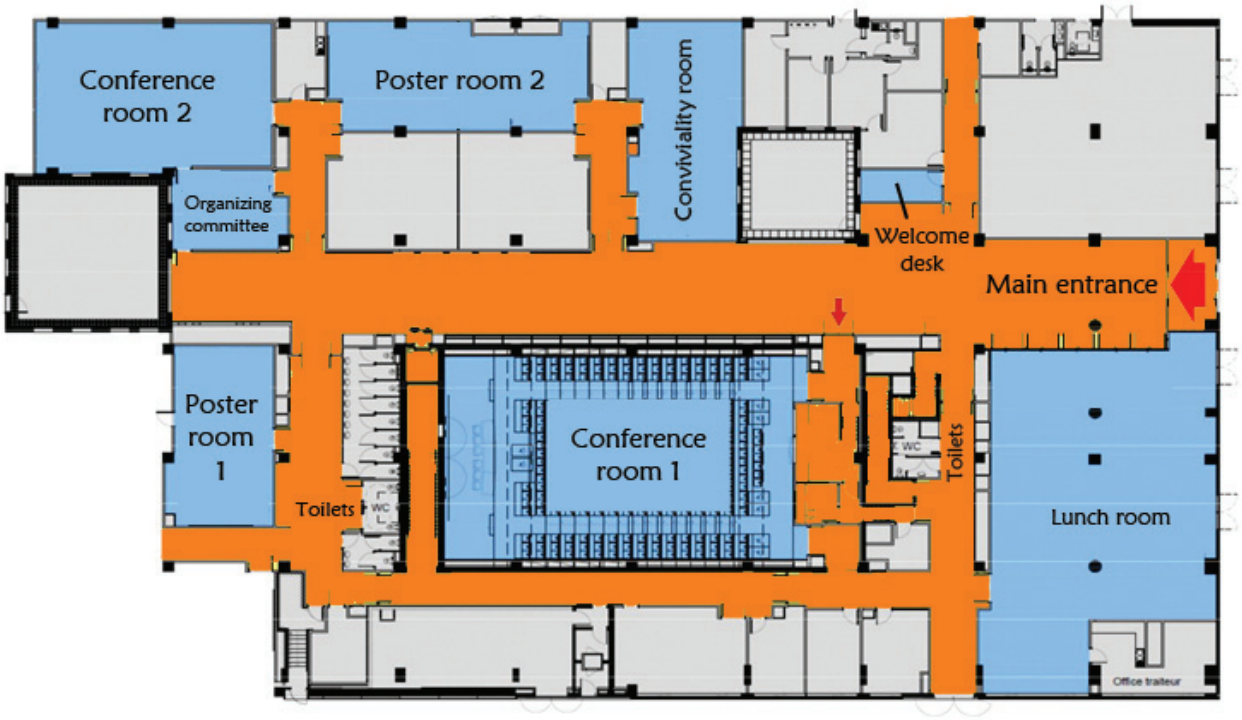
June 7th-10th, 2017

Content

Local organizing committee

Eric BENOIST, Synthèse et Physico-Chimie des Molécules d'Intérêt Biologique
Stefan CHASSAING, Institut des Technonologies Avancées en sciences du Vivant
Fabrice COLLIN, Laboratoire de Chimie de Coordination
Céline DERAEVE, Laboratoire de Chimie de Coordination
Fatima EL GARAH, Laboratoire de Génie Chimique
Peter FALLER, Institut de Chimie de Strasbourg
Emmanuel GRAS, Laboratoire de Chimie de Coordination
Christelle HUREAU, Laboratoire de Chimie de Coordination
Béatrice MESTRE-VOEGTLE, Laboratoire de Chimie de Coordination,
Pierre PAYOUX, CHU PURPAN, ToNIC - Toulouse Neuroimaging Centre
Rinaldo POLI, INP-Toulouse, Laboratoire de Chimie de Coordination
Sébastien RICHETER, Institut Charles Gerhardt, Montpellier
Jeroen SONKE, GET - Observatoire Midi-Pyrénées

Conference hall map



Welcome to ISABC 14 !

Dear Participants (Bio-Inorganic Chemists, Colleagues, Students…),

It is our privilege and a great pleasure to welcome you to the 14th International Symposium on Applied Bioinorganic Chemistry (ISABC14) in Toulouse, France from June 7th to 10th 2017.

This is already the 14th edition of this series, which started in 1990 with a symposium in Wuhan (Bejing, China). Since then, the meeting has been all over the world and now comes to Toulouse.

Toulouse, also called "the pink city (la ville rose)" is in the heart of South West of France and was the ancient capital of the Visigoths. In our days Toulouse is a very active and animated city, with more than 100 000 students. It is well known for its aeronautical industry and the scientific activities lead by the 22 000 researchers in different domains.

Toulouse has a long standing tradition in bioinorganic research and this is the second time an international Bioinorganic Chemistry congress is held in Toulouse. 17 years ago, at the turn of the millennium EuroBIC 5 (5th European Biological Inorganic Chemistry Conference) was held in July with about 500 international participants and chaired by Bernard Meunier. We hope to build on this successful conference in Toulouse.

In line with the ISABC tradition, an important aim for us was to keep the registrations fees (in particular for students) as low as possible. Thus, we made a special effort to find financial supports and economic arrangements. We are extremely thankful for all supports we obtained. This includes the professional sponsors and exhibitors as well the different scientific societies and public organizations. The sponsors are depicted on the back page of the booklet.

We would also like to thank all the invited lecturers who have accepted our invitations despite the limited support we could offer. All the persons that help(ed) us in different ways, starting from the local organization committee, the international advisory committee, the CNRS, in particular all the helpers from the administration of the LCC, the members of the poster Jury, the session chairs, etc. are warmly acknowledged.

ISABC14 will cover a wide range of topics in and around the field of Applied Bioinorganic Chemistry. Our aim is to stimulate scientific exchange and interactions between participants in an informal atmosphere. We hope to do so with an exciting program in a pleasant place fueled by local and high quality caloric and liquid brain nutrients.

Peter Faller
Christelle Hureau
chairs of ISABC 14

Conference programme

Wednesday June 7th

14h – 16h30	Registration
16h30-17h00	Introduction (*Conference room 1*)
17h00 – 17h45	Svetlana Lutsenko *(Conference room 1)*
17h45 – 18h30	Bill Martin *(Conference room 1)*
18h30 – 19h00	Opening Ceremony (*Conference room 1*)
19h30 – 21h00	Opening reception (*Lunch room*)

Thursday June 8th

9h-9h45	Franc Meyer *(Conference room 1)*	
	Session Metal-based drugs I *(Conference room 1)*	Session Metals in health *(Conference room 2)*
9h45- 10h05	G. Gasser (IL)	P. Carver (IL)
10h05-10h20	O. Iranzo (OP)	L. Bertini (OP)
10h20-10h35	A. Tinoco (OP)	M.A. Santos (OP)
10h35-11h00	A. Erxleben (KN)	R. Squitti (KN)
11h00-11h30	Coffee Break	
11h30-11h50	D. Crans (IL)	M.H. Lim (IL)
11h50-12h05	S. Bombard (OP)	F. Bellia (OP)
12h05-12h20	A. Terenzi (OP)	*12h05-12h30* L. Quintanar (KN)
12h20-12h35	B. Spingler (OP)	
12h30 – 14h	Lunch	
14h-14h45	Vince Pecoraro *(Conference room 1)*	
	Session Metals and biomolecules I *(Conference room 1)*	Session Imaging and sensors I *(Conference room 2)*
14h45-15h05	A. Duhme-Klair (IL)	C. Orvig (IL)
15h05-15h20	U. Schatzschneider (OP)	F. Zelder (OP)
15h20-15h35	J. Müller (OP)	C. Bonnet (OP)
15h35-16h00	E. Gumienna (KN)	K. Kikuchi (KN)
16h00-16h30	Coffee Break	
	Session: Metalloenzymes, inspiration, mimics, function and inhibition I *(Conference room 1)*	Session Metals and biomolecules II *(Conference room 2)*
16h30–16h50	J. Messinger (IL)	E. Freisinger (IL)
16h50-17h05	G. Berggren (OP)	N. Kulak (OP)
17h05-17h20	H. Kitagishi (OP)	H. G. Ly Thi (OP)
17h20-17h35	D. Pantazis (OP)	R. Sigel (OP)
17h35-17h55	S. Hirota (IL)	W. Bal (IL)
17h55-18h10		L. S. Mészaros (OP)
18h15-18h45	Flash Poster Presentations (*Conference room 1*)	
19h00-22h00	Poster Session A – “Local” stand-up dinner	

Friday June 9th

9h-9h45	Sandra Signorella *(Conference room 1)*	
	Session Metal-based drugs II *(Conference room 1)*	Session New Methods around metals in biology *(Conference room 2)*
9h45- 10h05	S. Bonnet (IL)	L. Hemmingsen (IL)
10h05-10h20	L. Cardo (OP)	T. Van Acker (OP)
10h20-10h35	A. Ahmedova (OP)	T. Kamachi (OP)
10h35-11h00	D. Gambino (IL)	*10h35-11h05* V. Balter (KN)
11h - 11h30	Coffee Break	
11h30-11h45	P. Gamez (OP)	S. Pallada (OP)
11h45-12h00	J. Walton (OP)	B. Busser (OP)
12h00-12h20	H. Gornitzka (OP)	E. Paredes (OP)
12h20-12h35	X. Wang (OP)	*12h20-12h40* A. Dey (IL)
12h35 – 14h	Lunch	
14h-14h45	Janet Morrow *(Conference room 1)*	
	Session Imaging and sensors II *(Conference room 1)*	Session Metals in environment, biogeochemistry, toxicology and origin of life I *(Conference room 2)*
14h45-15h05	Y. You (IL)	R. Austin (IL)
15h05-15h20	J. Molloy (OP)	A. Deniaud (OP)
15h20-15h40	C. Goze (IL))	P. Delangle (IL)
15h40-16h05	*15h40-15h55* A. Frei (OP)	R. Lobinsky (KN)
16h05-16h30	Coffee Break	
	Session Metalloenzymes, inspiration, mimics, function and inhibition II *(Conference room 1)*	Session metals and biomolecules III *(Conference room 2)*
16h30 – 16h50	C. Léger (IL)	D. Valensin (IL)
16h50-17h05	S. Menage (OP)	Y. Yamamoto (OP)
17h05-17h20	S. Herres-Pawlis (OP)	T. Moriuchi (OP)
17h20-17h35	J. Shearer (OP)	A. Krezel (OP)
17h35-17h55	U.P. Apfel (OP)	J. Rodriguez (OP)
18h00-18h30	Flash Poster Presentations (*Conference room 1*)	
18h30-20h00	Poster session B	
20h30	Gala Dinner	

Saturday June 10th

9h30-10h15	Gerard Jaouen *(Conference room 1)*	
	Session Metalloenzymes, inspiration, mimics, function and inhibition III *(Conference room 1)*	Session Metal-based drugs III *(Conference room 2)*
10h15-10h30	C. Cavazza (OP)	I. Ott (OP)
10h30-10h45	K. Oohora (OP)	B. Bertrand (OP)
10h45-11h00	M. Seemann (OP)	E. Bodio (OP)
11h-11h30	Coffee Break	
11h30-11h55	C. Policar (KN)	Session Metals in environment, biogeochemistry, toxicology and origin of life II *(Conference room 2)*
		C. Blindauer (KN)
11h55-12h10	V. Vu (OP)	A. Jancso (OP)
12h10-12h25	D. Raines (OP)	*12h10-12h30* D. Pignol (IL)
12h30–12h55	E. Reisner (KN)	*12h30–12h50* J. Duval (IL)
12h40–14h15	Lunch	
	Session Imaging and sensors III *(Conference room 1)*	Session Metal-based drugs IV *(Conference room 2)*
14h15-14h40	E. Borbas (KN)	M. Joao Romao (KN)
14h40-14h55	S. Petoud (OP)	L. Ronconi (OP)
14h55-15h10	M.C. Gimeno (OP)	N. Margiotta (OP)
15h10-15h30	F. Riobé (IL)	A. M. da Costa Ferreira (IL)
15h30 - 16h00	Coffee Break	
16h00-16h45	Takashi Hayashi (*Conference room 1*)	
16h45-17h15	Closing Ceremony	

Plenary conferences

Copper trafficking pathways in neuronal development and lipid balance

Yuta Hatori[1], Abigael Muchenditsi[1], Hannah Pierson[1], Martina Ralle[2], Katharina Schmidt[1], Lutsenko[1]

[1]*Department of Physiology, Johns Hopkins University, School of Medicine, Baltimore, MD, USA,* [2]Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland , OR, USA

Presenting author: Svetlana Lutsenko, lutsenko@jhmi.edu

Abstract

Copper homeostasis is essential for human growth and development. Two ATP-driven copper transporters ATP7A and ATP7B play the key role in balancing copper levels in tissues and activating essential copper-dependent enzymes. This presentation will give a brief overview of copper homeostasis in human cells; it will highlight distinct functions of ATP7A and ATP7B in tissues, discuss how cells regulate distribution of copper between different cellular compartments, and illustrate that dysregulation of copper balance markedly alters cholesterol and fat metabolism. Relevance of our findings to the pathogenesis of Wilson's disease, Menkes disease and other disorders of human copper misbalance will be summarized.

References

1) Hatori Y, Yan Y, Schmidt K, Furukawa E, Hasan NM, Yang N, Liu CN, Sockanathan S, Lutsenko S. "Neuronal differentiation is associated with a redox-regulated increase of copper flow to the secretory pathway" (2016) Neuronal differentiation is associated with a redox-regulated increase of copper flow to the secretory pathway. *Nat Commun.* 16;7:10640
2) LutsenkoS "Copper trafficking to the secretory pathway.2016 Metallomics.;8(9):840-52.
3) Hamilton JP, Koganti L, Muchenditsi A, Pendyala VS, Huso D, Hankin J, Murphy RC, Huster D, Merle U, Mangels C, Yang N, Potter JJ, Mezey E, Lutsenko S. Activation of liver X receptor/retinoid X receptor pathway ameliorates liver disease in Atp7B(-/-) (Wilson disease) mice. (2016) *Hepatology.* 63(6):1828-41
4) Muchenditsi A, Yang H, Hamilton JP, Koganti L, Housseau F, Aronov L, Fan H, Pierson H, Bhattacharjee A, Murphy RC, Sears CL, Potter JJ, Wooton-Kee CR, Lutsenko S. Targeted inactivation of copper-transporter Atp7b in hepatocytes causes liver steatosis and obesity in mice. (2017) Am J Physiol Gastrointest Liver Physiol. doi: 10.1152/ajpgi.00312.2016

The bioinorganic physiology of the the last universal common ancestor

William F. Martin

Institute for Molecular Evolution, University of Düsseldorf, 40225 Düsseldorf, Germany; email : bill@hhu.de

Abstract

The concept of a last universal common ancestor of all cells (LUCA, or the progenote) is central to the study of early evolution and life's origin, yet information about how and where LUCA lived is lacking. We investigated all clusters and phylogenetic trees for 6.1 million protein coding genes from sequenced prokaryotic genomes in order to reconstruct the microbial ecology of LUCA. Among 286,514 protein clusters, we identified 355 protein families (~0.1%) that trace to LUCA by phylogenetic criteria. Because these proteins are not universally distributed, they can shed light on LUCA's physiology. Their functions, properties, and prosthetic groups depict LUCA as anaerobic, CO_2-fixing, H_2-dependent with a Wood-Ljungdahl (WL) pathway, N_2-fixing, and thermophilic. LUCA's biochemistry was replete with FeS clusters and radical reaction mechanisms. Its cofactors reveal dependence upon transition metals, flavins, S-adenosyl methionine (SAM), coenzyme A, ferredoxin, molybdopterin, corrins, and selenium. Its genetic code required nucleoside modifications and SAM-dependent methylations. The 355 phylogenies identify clostridia and methanogens, whose modern lifestyles resemble LUCA's, as basal among their respective domains. LUCA inhabited a geochemically active environment rich in H_2, CO_2, and iron. The data support the theory of an autotrophic origin of life involving the Wood–Ljungdahl pathway in a hydrothermal setting. It appears that LUCA lived from exergonic reactions of gasses that were converted via transition metal catalysts to soluble organic compounds.

References

1) Weiss MC, Sousa FL, Mrnjavac N, Neukirchen S, Röttger M, Nelson-Sathi S, Martin WF: The physiology and habitat of the last universal common ancestor. Nature Microbiology (2016) 1:16116.

Bioinspired small molecule activation for energy-related catalysis

Franc Meyer[a]

[a] *Universität Göttingen, Institut für Anorganische Chemie, Tammanstrasse 4, D-37077 Göttingen, Germany; email : franc.meyer@chemie.uni-goettingen.de*

Abstract

Most scenarios for storing energy in, and retrieving energy from, chemical bonds involve the activation and transformation of small ubiquitous molecules (O_2, H_2O, H_2, N_2, CO_2, CH_4); water splitting, generation of dihydrogen, and hydrogenation of dinitrogen and carbon dioxide are some of the pivotal reactions relevant to the global energy challenge. For mediating these challenging reactions, nature has evolved sophisticated enzymes that usually contain one or more metal ions within their protein active sites. Understanding the functional principles of these bioinorganic systems offers great inspiration for the development of new types bioinspired metal-based catalysts, which in the end may or may not emulate structural features of the natural cofactors. Some of our recent work along these lines will be presented, focusing on (1) the stabilization and catalytic application of biorelevant iron-based intermediates in organometallic complexes with macrocyclic N-heterocyclic carbene scaffolds[1,2] and (2) the activation of small molecules via H_2 elimination from oligometallic dihydride complexes akin to the key N_2 binding step in nitrogenase's FeMoco.[3]

References

1) (a) S. Meyer, I. Klawitter, S. Demeshko, E. Bill, F. Meyer, *Angew. Chem. Int. Ed.* **2013**, *52*, 901-905; (b) S. Ye, C. Kupper, S. Meyer, E. Andris, R. Navrátil, O. Krahe, B. Mondal, M. Atanasov, E. Bill, J. Roithová, F. Meyer, F. Neese, *J. Am. Chem. Soc.* **2016**, 138, 14312-14325; (c) C. Kupper, B. Mondal, J. Serrano-Plana, I. Klawitter, F. Neese, M. Costas, S. Ye, F. Meyer, *manuscript in preparation.*
2) (a) C. Kupper, A. Schober, S. Demeshko, M. Bergner, F. Meyer, *Inorg. Chem.* **2015**, *54*, 3096-3098; (b) C. Kupper, J. A. Rees, S. Dechert, S. DeBeer, F. Meyer, *J. Am. Chem. Soc.* **2016**, 138, 7888-7898.
3) D. H. Manz, P.-C. Duan, S. Dechert, M. John, R. Mata, F. Meyer, *manuscript in preparation.*

Development of Metallacrowns into Lanthanide Based Luminescent Agents

Vincent L. Pecoraro,[a] Ivana Martinić[b], Tu N. Nguyen[a], Jacob Lutter,[a] Beatriz Bermudez-Lopez,[a] Svetlana V. Eliseeva[b], Stéphane Petoud[b,c],

[a] Department of Chemistry, University of Michigan, Ann Arbor, MI USA 48109-1055; [b]Centre de Biophysique Moléculaire, CNRS UPR 4301, 45071 Orléans Cedex 2, France [c)] Department of Inorganic, Analytical and Applied Chemistry, University of Geneva, CH-1211 Geneva 4, Switzerland; email : vlpec@umich.edu

Abstract

The field of optical biological imaging is growing explosively in recent years due to technological advances related to detection techniques and image treatment. Luminescent probes allow for the visualization and/or quantification of biological objects or events with a high detection sensitivity and resolution at the cellular level. Lanthanide(III)-based complexes are a class of luminescent compounds that possess fascinating and unique optical properties due to the electronic structure of the lanthanide(III) cations (Ln^{3+}) they incorporate. More specifically, Ln^{3+} exhibit f-f emission bands from the visible to the near-infrared (NIR) range and a number of complementary properties with respect to the fluorescent probes: sharp emission wavelengths that are highly insensitive to the microenvironment, large energy differences between the absorption and emission bands and high resistance toward photobleaching (allowing long term or repetitive quantitative experiments). Metallacrowns are the inorganic analogues of organic crown ethers and have been shown to be excellent vehicles for the encapsulation of lanthanides. We have reported previously the design, synthesis, characterization and luminescence properties of Ln^{3+} "encapsulated sandwich" metallacrown complexes (MC) based on Zn^{2+} ions and bivalent aromatic hydroximate ligands $(L^{2-})^{1,2}$ that sensitize NIR emitting Ln^{3+}= Nd, Er, Yb and $Ln^{3+}Ga^{3+}$ 12-MC-4 structures[3] that sensitive the full range of lanthanide emission across the visible and NIR regions. In both classes of complexes, we observed some species with the highest quantum yield values (in comparison to NIR emitting Ln^{3+}-based complexes containing C-H bonds) and longest luminescence lifetimes in the solid state and in methanol solutions due to an efficient Ln^{3+} sensitization and a strong protection against non-radiative deactivation pathways (resulting from overtones of high energy C-H, N-H, and O-H vibrations located in solvent molecules and ligands close to the Ln^{3+} ion). We will discuss how the pyrazinehydroxamic acid (H_2pyzHA) analogue that forms Ln^{3+}[12-$MC_{Zn(II),pyzHA}$-4]$_2$[24-$MC_{Zn(II),pyzHA}$-8] (Ln^{3+}[Zn(II)MC_{pyzHA}], Ln^{3+}= Yb, Nd) may be used as a highly photostabile probe that and is able to label preferentially necrotic cells[4] and may be used in one step dual fixation and staining of human cells. A second class of molecules containing Ga^{3+} with the stoichiometry Ga_8Ln_2 will also be discussed.

References

1) Jankolovits, J.; Andolina, C.M.; Kampf, J.W.; Raymond, K.N.; Pecoraro, V.L. "Assembly of NIR Luminescent Lanthanide Host(Host-Guest) Complexes Using a Lanthanide-Metallacrown Sandwich Motif" *Angew. Chem*., **2011**, *50*(41), 9660-9664.

2) Trivedi, E.R.; Eliseeva, S.V.; Jankolovits, J.; Olmstead, M.; Petoud, S.; Pecoraro, V.L. "Highly Emitting Near Infrared Lanthanide "Encapsulated Sandwich" Metallacrown Complexes with Excitation Shifted Toward Lower Energy" *J. Amer. Chem. Soc*. **2014**, 136, 1526−1534.

3) Chow, C. Y.; Eliseeva, S. V.; Trivedi, E. R.; Nguyen, T.; Kampf, J.W.; Petoud, S.; Pecoraro, V.L. "Ga^{3+}/Ln^{3+} Metallacrowns: A Promising Family of Highly Luminescent Lanthanide Complexes that Covers Visible and Near-Infrared Domains" *J. Amer. Chem. Soc*., **2016,** *138***,** 5100-5109.

4) Martinìc, I.; Eliseeva, S. V.; Nguyen, T.; Pecoraro, V.L.; Petoud, S. "Near-Infrared Optical Imaging of Necrotic Cells by Photostable Lanthanide-Based Metallacrowns," submitted to *J. Amer. Chem. Soc*.

Rationally Designed Mimics of Manganoenzymes: Small Molecules Activation and Applications in Catalysis

Sandra Signorella

IQUIR (Instituto de Química Rosario), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina ; email : signorella@iquir-conicet.gov.ar

Abstract

Manganese is a particularly useful catalytic center due to its unrivaled repertoire of redox chemistry. Inspired by Mn-biosites, a number of catalytic systems in which manganese can interact with small oxygenated molecules have been tested for biochemical, industrial and environmental purposes.[1-4] Manganoenzymes are involved in the protection of living organisms from reactive oxygen species (superoxide dismutase, catalase), in the activation and reduction of O_2 (ribonucleotide reductase, lipoxygenase), and in O_2 formation from water at the Mn_4Ca cluster of Photosystem II. Despite the critical role of Mn in these biological processes, the fundamental understanding is far from complete and biomimetic small-molecule chemistry continues to play a key role in furthering mechanistic insights.

The number and type of ligands, the local charge, the nuclearity of the active site, are among the factors that introduce a way of tuning the redox potential of the metal center to face redox reactions. Recent synthetic advances have led to the isolation and characterization of a number of Mn model complexes, and reactivity studies in conjunction with spectroscopy and theory have helped to understand some structural and electronic properties of the metal centers that modulate their reactivities. Scavenging of reactive oxygen species by manganese sites can occur through a direct oxidation/reduction reaction or via a high-valent reactive species;[1] but, also, peroxide can be activated to form a more powerful oxidant, which in turn can be applied for oxidation of a substrate.[2] Several Mn-complexes in the presence of H_2O_2 exhibit both catalase activity and a high ability for substrate oxidation, depending on the reaction conditions. Also, by changing reaction conditions, simple mononuclear Mn complexes can disproportionate superoxide, peroxide, or activate O_2 to generate more powerful oxidants,[3] and various diMn complexes can exhibit catalase activity or ability to oxidize water to O_2.[4]

In this talk, I will contrast antioxidant activity and small molecule activation ability of several Mn systems, and discuss key factors that control these processes.

References

1) C. Palopoli, G. Gomez, A. Foi, F. Doctorovich, S. Mallet-Ladeira, C. Hureau, S. Signorella, J. Inorg. Biochem., 2017, 167, 49-59.
2) M. Oszajca, M. Brindell, Ł. Orzeł, J. M. Dąbrowski, K. Śpiewak, P. Łabuz, M. Pacia, A. Stochel-Gaudyn, W. Macyk, R. van Eldik, G. Stochel, Coord. Chem. Rev. 327-328, 2016, 143-165.
3) S. Sahu, D. P. Goldberg, J. Am. Chem. Soc. 2016, 138, 11410−11428.
4) J. D. Blakemore, R. H. Crabtree, G. W. Brudvig, Chem. Rev., 2015, 115, 12974−13005.

Applications for the selective interaction of metal complexes with nucleic acids

Janet R. Morrow,[1] Naomi Bryner,[1] Akanksha Patel,[1] Rajendiran Venugopal[1]

[1] *Department of Chemistry, University at Buffalo, State University of New York; email: jmorrow@buffalo.edu*

Abstract

Optical sensing of metal ions within cells has many challenges. Foremost among these is the requirement of the metal ion to bind selectively to the sensor and to transduce a change in fluorescence emission. While there are many successful approaches for small molecule sensors of Zn(II) or Cu(I), studies with Fe(II) have received little attention. Our group is studying new approaches to this area of research by using nucleic acids to improve binding selectivity of the metal ion and also to promote a turn on fluorescence response. Towards this goal, new ligands that more selectively bind Fe(II) over Zn(II) have been developed. Past work published by our group has shown that ligands appended with fluorophores produce a metal ion selective fluorescence response in the presence of certain DNA or RNA structures.[1-4] For example, selective binding of Zn(II) complexes to thymine groups enables the creation of nucleic acid sensors that have thymine within loop structures including those in G-quadruplexes. In some cases, a three component mixture of ligand, metal ion and nucleic acid self-assembles to light up in response to Zn(II), but not Fe(II) or Cu(II).[1] In other experiments, a conformation change is induced to register the metal ion binding response.[3,4] New optical probes that demonstrate a large fluorescence enhancement upon binding to DNA structures in the presence of these metal ions will be presented as a method for improved binding selectivity and fluorescence turn on response in optical sensing applications.

References

1) Siters, K. E.; Fountain, M. A. Morrow, J. R., "Selective binding of Zn^{2+} complexes to human telomeric G-Qudruplex DNA" Inorg. Chem. **2014**, 53, 11540-1151.
2) Siters, K. E.; Sander, S. A.; Devlin, J.; Morrow, J. R. "Bifunctional Zn(II) Complexes for Recognition of Non-Canonical Thymines in DNA Bulges and G-Quadruplexes" *Dalton Trans.* **2014**, 3708-3716.
3) S. A. Sanders, A. K. VVan Hall J. R. Morrow, "Zn^{2+} Selective Switch of Duplex to Hairpin DNA" Inorg. Chem. **2015**, 54, 3084-3086.
4) Sanders, S. A; Morrow, J. R. "Zn(II) Complexes that trigger a DNA conformational Switch" *Inorg. Chim. Acta.* **2016**, 452, 90-97.

Inorganic anticancer drugs : example of the ferrocifen family.

Gérard JAOUEN, [a]

[a]*Chimie Paris Tech PSL, UPMC Univ Paris 06, IPCM, F-75005 Paris, France ; email : gerard.jaouen@upmc.fr*

Abstract

Owing to the poor outcomes seen in several types of cancers (*e.g*. epithelial ovarian cancer, EOC) the search for new active principles outside the established avenues is a burning current concern. In our search for innovative organometallic species able to overcome drug resistance to proapoptotic stimuli we have discovered the remarkable ferrocifen family bearing a redox motif [ferrocenyl-ene-phenol] selectively activated on cancer cells, and therefore revealing their redox environment. Biologically these species can operate via mechanisms related to both apoptosis and senescence depending on several parameters. This multitargeting property can inhibit resistance. Among the usable organometallic complexes, iron derivatives occupy a privileged position associated with the particular nature of ferrocene, which is a non-toxic, compact and stable aromatic metallocene with redox properties and a bioisostere of benzene.

It was important to elucidate the chemical behavior of the key metabolites generated oxidatively from the metallocifens in relation to their antitiproliferative effects. This study produced multiple surprises and will be discussed in depth.Interestingly metallocifen mechanisms depend on several parameters such as the nature of the metal, the shape of the molecular carbon skeleton and the kind of substituents.Examples of drugdelivery of thesespecies by usinglipidnanocapsules (LNCs) for*in vivo*studieswillalsobeprovided.

References

1) Jaouen, G.; Vessieres, A.; Top, S. *Chem. Soc. Rev.* **2015,***44* (24), 8802-8817.
2) Wang, Y.; Pigeon, P.; Top, S.; McGlinchey, M. J.; Jaouen, G. *Angew. Chem. Ed. Int.* **2015,***54* , 10230
3) Scalcon, V.; Top, S.; Lee, H. Z. S.; Citta, A *et al*., *J. Inorg. Biochem.* **2016,** 165,146-151.
4) Wang, Y.; Top, S.; Pigeon, P.; Vessieres, A.; Mansuy, D.; Jaouen, G., *Angew. Chem. Ed. Int.* **2016,***55* (35), 10431-10434.

Modification of Metalloproteins to Generate New Biocatalysts

Takashi Hayashi

Department of Applied Chemistry, Osaka University, Suita 565-0871, Japan

email : thayashi@chem.eng.osaka-u.ac.jp

Abstract

Hemoproteins which are versatile and ubiquitous metalloproteins contain an iron porphyrin cofactor, heme, located within the interior of the proteins. The cofactor interacts with the protein matrix and functions as a catalyst, sensor, or gas binding molecule and so on. One of the heme cofactors, protoheme IX (heme *b*), is non-covalently bound within the heme pocket. Thus, the heme *b*-containing hemoproteins can generally be converted into the colorless apo-form under acidic conditions, and the addition of heme *b* or a related metal complex into a solution of the apoprotein triggers refolding to generate the corresponding reconstituted proteins. Our group has focused on this process and devoted our efforts to obtain a series of modified hemoproteins with metal porphyrinoids as an artificially created cofactor, because it is found that the heme pocket after the removal of native heme is useful for an attractive scaffold of a metallocomplex-binding site to produce a new biocatalyst.[1] In this presentation, several examples of functionalized hemoproteins containing artificial cofactors will be reported:

!) *Improvement of original function:* Myoglobin, an oxygen storage protein, has a heme *b* cofactor in the globin protein. Myoglobin reconstituted with iron porphycene, a constitutional isomer of iron porphyrin, shows extremely large affinity for the O_2 binding. In addition, the O_2/CO discrimination is remarkably opposite to that of native myoglobin.

Horseradish peroxidase (HRP) is one of the famous heme-containing peroxidases. The insertion of iron porphycene into apoHRP enhances the enzymatic activity and the compound I-like intermediate formed by the addition of H_2O_2 is detectable.

2) *Conversion of original function:* Cytochrome *c* is a famous electron transfer protein with heme *c*. After the removal of heme c, the addition of $Fe_2(CO)_9$ into the solution of the apoprotein provides a diiron carbonyl cluster supported by two cysteine residues in the heme pocket. This hybrid composite catalytically generates H_2 gas in the presence of a Ru complex as a photosensitizer.

Myoglobin has no hydroxylase activity which is seen in a series of cytochrome P450s. However, manganese porphycene in myoglobin promotes inert alkane hydroxylation. Furthermore, the modification of the myoglobin heme pocket by mutagenesis is found to control the enantioselectivity of the hydroxylation reaction.

3) *Construction* of *bioorganometallic catalyst:* Nitrobindin, an NO binding heme protein, has a robust β-barrel structure, which could be a suitable scaffold for an organometallic complex. For example, our group linked a RuCp complex into the β-barrel to obtain a non-natural bioorganometallic composite. This biohybrid catalyst is found to promote the polymerization of acetylene and provides the trans-rich polymer, indicating that the protein matrix regulates the structure of the product.

References

1) T. Hayashi, in *Coordination Chemistry in Protein Cages*: T. Ueno and Y. Watanabe, eds; Wiley, Hoboken, 2013; pp 87–110.

Oral communications

Metals in health and diseases

Relationships between plasma metal ion concentrations of iron, copper, and zinc and the development of infectious diseases in ambulatory and hospitalized patients.

Carver, Peggy L.,[a,b]

[a]*The University of Michigan College of Pharmacy, Department of Clinical Pharmacy;* [b] *University of Michigan Medicine; email: peg@umich.edu*

Divalent metals are important components of nutrition, are required for cellular growth and metabolism, and may play a role patients' susceptibility to infection. [1,2] Subtle alterations of divalent metal homeostasis occur in the course of infectious diseases which aim, from the host perspective, either to reduce the availability of respective metals to microbes, or to use toxic metal accumulation to eliminate pathogens. Although patients with increased bone marrow stores of iron have an increased risk of bacterial and fungal infections, the effect of increased or decreased plasma levels of other metal ions is poorly understood. There are no known correlations between with Cu levels and the development of infectious diseases. In Zn-deficient patients, supplementation has shown substantial health benefits in in select high risk populations. However, an increased plasma Cu/Zn ratio has been demonstrated in select immune compromised patient populations, including those with tuberculosis, chronic hepatitis B, and in patients undergoing dialysis. [2,3]

Methods: We assessed correlations between metal ion concentrations and bacterial and fungal infections in diverse patient populations: (1) Fe levels in cardiac patients administered large intravenous doses of iron; (2) in acutely ill hospitalized patients, and (3) in stable, otherwise healthy patients receiving long-term parenteral nutrition (TPN). Data collection included all fungal and bacterial infections, doses and plasma levels of metal ions, and documented infections. Multivariate analyses, recurrent events survival analysis, and a Glimmix procedure to analyze correlation between plasma levels of the metal ions and infections.

Results: In stable, healthy TPN patients, there is a significant dose-response relationship between weekly metal ion doses and serum concentrations for Zn, Cr, and Mn, but not for Se, Cu, or Fe. An increased risk of infections was associated with increased plasma Cu/Zn ratios ($P<0.002$). In acutely ill hospitalized patients, an increased risk of infection correlated with red blood cell transfusions, iron saturation, and renal failure and the Cu/Zn ratio was significantly different in infected vs uninfected patients. In patients with acute decompensated heart failure, administration of high dose, intravenous iron administration to patients whose iron saturation (TSAT) was >20% was associated with higher rates of infections than patients in whom iron saturation was ≤ 20%.

Conclusions: Understanding correlations between iron administration, iron saturation, and plasma Cu/Zn ratios and the development of infectious diseases may help minimize the risk of life-threatening infections in both healthy and acutely ill hospitalized patients. Future studies need to interrelationships between metal ions. They should be prospective, and incorporate assessments of multiple metal ions, over time, in patients at risk of infection.

References

1. Btaiche, I.F.; Carver; P.L.; Welch, K.B. JPEN J Parenter Enteral Nutr. 2011, 35:736.
2. Carver PL. Metal ions in life sciences. 2013;13:1-28.
3. Weiss G, Carver PL. Clinical microbiology and infection 2017. DOI 10.1016/j.cmi.2017.01.018

Redox reactivity of Copper-Amyloid peptide complexes toward ROS production. A computational insight on the redox competent metal coordination in O_2 and H_2O_2 reduction.

Luca Bertini,[a]Tommaso Prosdocimi,[b]Luca Mollica,[c] Federica Arrigoni,[a]Jacopo Vertemara,[a] Giuseppe Zampella,[a]Luca De Gioia,[a]

[a]*University of Milano-Bicocca , Department of Biotechnology and Biosciences*
Piazza dellaScienza 2 – Milano, Italy

[b]*Italian Institute of Technology (IIT) -Center for Nano Science and Technology (CNST)*
Via Pascoli, 70/3 Milano, Italy

[c]*CompuNet, Istituto Italiano di Tecnologia, via Morego, 30, I-16163 Genova, Italy*

email : luca.bertini@unimib.it

Abstract

Oxidative stress has been implicated to play a crucial role in the pathogenesis of a number of diseases, including neurodegenerative disorders such as Alzheimer disease (AD). The main pathological feature of AD are the amyloid β (Aβ) peptide plaques deposits that cause neuronal cell death.According to the oxidative stress hypothesis, the interactions of redox active copper ions with Aβ peptide are linked to production of reactive oxygen species (ROS).An essential prerequisite in understanding this aspect is the study as detailed as possible of the ROS formation mechanism from O_2up to the hydroxyl OH radical. Considerable efforts have been made to investigate this issue.The first step was the electrochemistry investigation of the Cu(I)-Aβ/Cu(II)-Aβ redox process. This study revealed that the reduction did not occur directly between Cu(I)/Cu(II) resting states but through an higher energy in-between state (1). The features of this redox competent state remained matter of debate but recent studies have proposed coordination s that involve the Asp1 N-terminal and of the histidine residues (2). In the present study, using molecular dynamic and quantum chemistry techniques, we investigate the differences among several Cu(I)-Aβ models in terms of reactivity towards O_2 and H_2O_2 reductions, showing that only a single coordination mode but rather a group of coordination modesare redox active, including those already proposed at the experimental level.

References

1) Balland V, Hureau C, Savéant JM , Electrochemical and homogeneous electron transfers to the Alzheimer amyloid-β copper complex follow a preorganization mechanism, (2010) Proc. Natl. Acad. Sci. 107, 17113
2) Cassagnes L.-E.; Hervé V.; Nepveu F.; Hureau C.; Faller P.; Collin F. The Catalytically Active Copper-Amyloid-Beta State: CoordinationSite Responsible for Reactive Oxygen Species Production, Angew. Chem. Int. Ed. (2013), 52, 11110; Prosdocimi T.; De Gioia L.; Zampella G.; Bertini L.; On the Generation of OH radical species from H_2O_2 by Cu(I) Amyloid Beta peptide model complexes. A DFT investigation. J. Biol.Inorg.Chem. (2016) 197

Exploiting hydroxyphenylbenzimidazol-based hybrids for metal-modulation in potential anti-Alzheimer´s drugs

Asha Hiremathad,[a,b] Luca Piemontese,[a,c] Daniel Tomás,[a] Rangappa S. Keri,[b] Sílvia Chaves,[a] M. Amélia Santos[a]

[a] *Centro de Química Estrutural, Instituto Superior Técnico, Universidade Técnica de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal;*[b] *Centre for Nano and Material Sciences, Jain University, Jain Global Campus, Jakkasandra post, Kanakapura Road, Ramanagara District, Bangalore 562112, India;* [c] *Dipartimento di Farmacia–Scienze del Farmaco, Università degli Studi di Bari "Aldo Moro", Via E. Orabona 4, 70125 Bari, Italy; masantos@ist.utl.pt.*

Alzheimer´s Disease (AD) is the most serious age dependent degenerative disorder characterized by progressive loss of memory and cognitive deficits, and with no cure so far [1]. The main brain hallmarks of the AD patients are the senile plaques, the neurofibrilary tangles and the deficit of acetylcholine in the brain. However, other pathological features play important roles in the disease process, namely in upstream events, including enhanced brain oxidative stress and disruption of metal homeostasis [2]. Many targets have been explored aimed to control AD process or effects, such as the extracellular β-amyloid (Aβ), the intracellular τ-protein and acetylcholinesterase (AChE). AChE inhibitors have been the most explored targets as, among the five FDA approved drugs four are AChE inhibitors. However, the available drugs only improve symptoms but do not have really disease modifying effects [3].

The evidenced multi-factorial nature of AD has been considered one of the main reasons for the absence of cure so far. Thereby, we have assisted to the development of multi-target candidate drugs, aimed at interfering with multiple disease pathways. Many multipotent compounds have been designed and studied based on repositioning well known AChEi classical anti-AD drugs, such as tacrine and donepezil [4].

On the continuation of our interest on the development of multifunctional compounds as potential anti-AD drugs with capacity for the metal modulation [5], we present herein the results of the study of a series of compounds which include a hydroxyphenylbenzimidazol (HOBIM) moiety attached to a molecular moiety able to inhibit AChE, namely a tacrine or a donepezil mimic. The enclosed HOBIM moiety, besides improving the AChE inhibitory activity of the "old " drugs, because of the bimodal interaction within the active site of the enzyme, provides the new hybrid compounds with metal (Cu, Zn) chelation ability, anti-oxidant activity and also capacity for anti-Aβ aggregation. Thereby these new hybrids appear as potential anti-AD drugs.

References

[1] A. Wimo, M. Guerchet, G.-C. Ali, Y.-T. Wu, A.M. Prina, B. Winblad, L. Jönsson, Z. Liu, M. Prince, *Alzheimer's & Dementia* **2017**, *13*, 1-7.

[2] C.L. Masters, R. Cappai, K.J. Barnham, V.L. Villemagne, *J. Neurochem.* **2006,** *97*, 1700.

[3] M.L. Crismon, *Ann. Pharmacother*, **1994**, *28*, 744–751.

[4] M.A. Santos, K. Chand, S. Chaves, *Fut. Med. Chem*. **2016**, *8*, 2113-2142.

[5] M.A. Santos, K. Chand S. Chaves, *Coord. Chem. Rev.* **2016**, *327-328*, 287-303.

ABBERANT COPPER HOMEOSTASIS IN ALZHEIMER'S DISEASE

[a]Rosanna Squitti, PhD

[a] *Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni di Dio- Fatebenefratelli, Brescia, Italy, rsquitti@fatebenefratelli.eu*

Abstract

Essential metals are vital elements for human biology. Iron, copper, and zinc are all essential for life. Two-thirds of the proteins used by our body use these metals. Thus, the biology of these metals has an important impact on our health and the breakdown of their homeostasis often leads to disease. They are very useful when kept under control, as catalyst of a huge number of biological processes, but become extremely dangerous when their levels become deregulated producing oxidative stress in Haber-Weiss and Fenton like chemistry or in deficiency contests.

An involvement of iron, zinc and copper in AD is extensively documented by several meta-analyses that have been published in the last few years.

Considering as whole the meta-analyses of copper in AD demonstrated the existence of a copper failure, consisting in copper decreases in the brain [1], copper increases in the blood [2], and copper increases in the serum fraction of copper not bound to ceruloplasmin [3]. It has be acquired the knowledge that this labile non-Cp-Cu fraction characterizes a percentage of AD patients [4], in serum and in the brain [5], where it may accelerate the Aβ disease cascade

ATP7B is a copper chaperone that loads the metal into the serum copper-protein ceruloplasmin during its synthesis, escorts superfluous copper into bile, and represents a key protein in copper failure. Impaired function of this ATPase is associated with a well-known inborn error of copper metabolism, Wilson's disease (WD).

Four specific SNPs of the ATP7B gene and a WD rare mutation have a statistical association with AD. These SNPs are informative on the ATP7B gene structure. They contribute to characterize a copper subtype of AD, which is getting credit in the latest years [4].

References

[1] Schrag M, Mueller C, Oyoyo U, Smith MA, Kirsch WM (2011) Iron, zinc and copper in the Alzheimer's disease brain: a quantitative meta-analysis. Some insight on the influence of citation bias on scientific opinion. *Prog Neurobiol* **94**, 296-306.

[2] Wang ZX, Tan L, Wang HF, Ma J, Liu J, Tan MS, Sun JH, Zhu XC, Jiang T, Yu JT (2015) Serum Iron, Zinc, and Copper Levels in Patients with Alzheimer's Disease: A Replication Study and Meta-Analyses. *J Alzheimers Dis* **47**, 565-581.

[3] Squitti R, Simonelli I, Ventriglia M, Siotto M, Pasqualetti P, Rembach A, Doecke J, Bush AI (2014) Meta-analysis of serum non-ceruloplasmin copper in Alzheimer's disease. *J Alzheimers Dis* **38**, 809-822.

[4] Squitti R, Ventriglia M, Gennarelli M, Colabufo NA, El Idrissi IG, Bucossi S, Mariani S, Rongioletti M, Zanetti O, Congiu C, Rossini PM, Bonvicini C (2016) Non-Ceruloplasmin Copper Distincts Subtypes in Alzheimer's Disease: a Genetic Study of ATP7B Frequency. *Mol Neurobiol*.

[5] James SA, Volitakis I, Adlard PA, Duce JA, Masters CL, Cherny RA, Bush AI (2012) Elevated labile Cu is associated with oxidative pathology in Alzheimer disease. *Free Radic Biol Med* **52**, 298-302.

Multiple Interconnected Pathological Factors in Alzheimer's Disease

Mi Hee Lim

Department of Chemistry, Ulsan National Institute of Science and Technology; email : mhlim@unist.ac.kr

Abstract

Alzheimer's disease (AD), associated with degeneration of neurons and synapses in the brain, leads to motor impairment and eventual fatality. Neurodegeneration could be related to various interconnected features, including (i) plaque formation from amyloid-β (Aβ) peptide fragments, (ii) metal ion dyshomeostasis and miscompartmentalization, as well as (iii) inflammation and increased oxidative stress due to overproduction of reactive oxygen species (ROS). The inter-relations between some of these pathological factors have been investigated. Metals are found entangled in the Aβ plaque and likely contribute to Aβ neurotoxicity and oxidative stress. ROS have been shown to increase the rate of Aβ plaque formation. Our understanding of the correlation between these elements and AD neuropathogenesis has been very limited, however. There is currently no cure for AD; therapies are focused on symptomatic relief targeting the decrease in the levels of acetylcholine, only one of the multiple factors causing the disease.[1] To find a cure for AD, we require a better understanding of the relationship between the various causative factors of this devastating disease. Towards this goal, we need suitable chemical tools capable of targeting and regulating its multiple underlying factors simultaneously.[1,2] Herein, our rational design and preparation of our chemical tools will be discussed with our investigations of their reactivities with targets *in vitro* as well as their efficacy *in vivo*.[2]

References

1) *Acc. Chem. Res.* **2014**, *47*, 2475-2482; *Chem. Soc. Rev.* **2017**, doi: 10.1039/C6CS00731G.

2) *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 21990-21995; *Chem. Sci.* **2015**, *6*, 1879-1886; *J. Am. Chem. Soc.* **2014**, *136*, 299-310; *J. Am. Chem. Soc.* **2015**, *137*, 14785-14797; *Nat. Commun.* **2016**, *7*, 13115; *J. Am. Chem. Soc.* **2017**, DOI: 10.1021/jacs.6b09681.

Getting insight into the IDE-based proteostasis of amyloid β: new mutational studies

Francesco Bellia, [a,b] Giuseppe Grasso,[b] Valeria Lanza,[a] Danilo Milardi,[a] Enrico Rizzarelli, [a,b]

[a] Institute of Biostructure and Bioimaging – CNR, 18 P. Gaifami, 95126 Catania (Italy); [b] University of Catania, 6 A. Doria, 95126 Catania (Italy); email : francesco.bellia@cnr.it

Abstract

The dyshomeostasis of amyloid-beta peptide (Aβ), the consequent peptide aggregation and accumulation in cerebral senile plaques cause the onset of Alzheimer's disease (AD), a disorder that affects several million people worldwide [1].

The enzymatic degradation of Aβ plays a key role on the peptide homeostasis. A partial or total failure of the Aβ-degrading enzyme could reasonably have a negative impact on the physiological fate of the amyloid peptide.

The interaction between insulin-degrading enzyme (IDE) [2] and amyloid substrates are modulated by transition metal ions [3], i.e. copper(II) and zinc(II); they also change the proteolytic cleavage sites of other natural substrates of IDE, such as insulin and amylin [4,5]. However, the molecular mechanism of IDE function, including the structural details of the interaction several metal ions, remains elusive.

In this context, we performed conformational studies of IDE mutants in order to elucidate how transition metal ions (copper and zinc) can affect the protein folding. Moreover, by means of a mass spectrometry-based proteomic approach, we investigated the effect of IDE mutations on the extent of Aβ hydrolysis, as well as on the hydrolytic pattern, also in the presence of the metal ions of interest.

References

1) Bush A.I. Trends Neurosci., 2003, 26, 207.
2) Farris et al., PNAS, 2003, 100, 4162.
3) Grasso G. et al., Chem. Eur. J., 2011, 17, 2752.
4) Bellia et al., J. Mass Spectrom., 2013, 48, 135.
5) Bellia et al., J. Mass Spectrom., 2014, 49, 274.

Copper and protein aggregation: From amyloids in diabetes to non-amyloids in cataracts disease

Liliana Quintanar

Department of Chemistry, Cinvestav (Centro de Investigación y de Estudios Avanzados), Mexico City, México; lilianaq@cinvestav.mx

Abstract

Several degenerative diseases are associated to the deposition of protein aggregates. Examples include the amyloid aggregates of β-amyloid peptide, α-synuclein, prion protein, and islet amyloid polypeptide (IAPP), which are associated to Alzheimer´s, Parkinson´s, prion diseases, and type 2 diabetes, respectively.[1-3] Redox-active metals, such as copper, have been implicated in protein aggregation, and may provide a link between aggregation, oxidative stress and cell death. Our research group studies the interaction of copper ions with proteins that have been implicated in degenerative diseases, using spectroscopic tools to understand the impact of the metal ion in protein folding, stability and aggregation properties. In this presentation, two stories on the effects of copper ions in protein aggregation will be contrasted.

On one hand, the coordination chemistry features of Cu(II) binding to IAPP will be discussed, as they provide a structural basis for the inhibitory effect of copper in its amyloid aggregation.[4] IAPP is an intrinsically disordered peptide, and the amino acid residues involved in metal ion binding are also key residues for the formation of β-sheet structures and amyloid fibrils. Thus, copper-induced IAPP conformers would display a higher energetic barrier to form amyloid fibrils, hence explaining the inhibitory effects of copper.[5]

In contrast, Cu(II) ions can induce the non-amyloid aggregation of human lens γ-crystallins, a process associated to cataracts disease.[6] γ-crystallins are among the most stable proteins in the human body; however, copper ions coordinate to these β-sheet-rich proteins and impact their folding and stability, inducing the formation of partially folded intermediates that are prone to non-amyloid aggregation.[7] These two stories illustrate the relative strength of copper coordination features over the stability of β-sheet structures, while providing important structural insights into the bioinorganic chemistry of these degenerative diseases.

This research has been funded by: the National Council for Science and Technology in Mexico (CONACYT grants 128255 and 221134), MIT-Mexico Seed Funds Program, and Marcos Moshinsky fellowship 2016.

References

1) DeToma, A. S. et al. *Chem Soc Rev* **2012,** *41*, 608-21.
2) Binolfi, A. et al. *Coord Chem Rev* **2012,** *256*, 2188-2201.
3) Faller, P. et al. *Acc Chem Res* **2014,** *47*, 2252-9.
4) Rivillas-Acevedo, L. et al. *Inorg Chem* **2015,** *54*, 3788-96.
5) Sanchez-Lopez, C. et al. *Inorg Chem* **2016**, *55*, 10727-10740.
6) Moreau, K. L.; King, J. A. *Trends in Mol Med* **2012,** *18*, 273-282.
7) Quintanar, L. et al. *ACS Chem Biol* **2016,** *11*, 263-272.

Metal-based drugs

Novel Ru(II) Polypyridine Complexes as Photosensitizers in Photodynamic Therapy

Gilles Gasser, [a]

[a] *Chimie ParisTech, PSL Research University, 75005 Paris, France; email : gilles.gasser@chimie-paristech.fr*

Abstract

The need for novel photosensitizers (PSs) in photodynamic therapy (PDT) is evident taking into account the drawbacks that most of the currently approved PSs have (e.g. lack of water solubility, photobleaching, low clearance from the body, etc.). These shortcomings can be traced back to the fact that all approved PSs are phtalocyanine- or porphyrin-based compounds. In order to tackle these drawbacks, new types of compounds are currently being examined as novel PSs in PDT. Among them, substitutionally inert Ru(II) polypyridyl complexes have been found to be extremely promising alternatives with one of such compounds to enter into clinical trials in the very near future.[1]
During this talk, we will present our recent results on the preparation, characterization and biological evaluation of such metal complexes as PSs in PDT including in two-photon excitation PDT.[2-4]

References

1) C. Mari *et al*. (2015) *Chem. Sci., 6,* 2660-2686
2) C. Mari *et al*. (2014) *Chem. Eur. J., 44,* 14421–14436.
3) A. Frei *et al*. (2015), *J. Med. Chem., 57*, 7280-7292.
4) H. Huang *et al*. (2015), *Angew. Chem. Int*. *Ed*. *54*, 14049-14052.

Exploring the DNA Cleavage and Cytotoxic Activity of Copper(II) Complexes of Phenanthroline and Histidine Containing Ligands

Sílvia M. G. Leite,[a] Luís M. P. Lima,[a] Sofia Gama,[b] Filipa Mendes,[b] Maylis Orio,[c] Isabel Bento,[a] António Paulo,[b] Rita Delgado,[a] Olga Iranzo[a,c]

[a] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal; [b] Centro de Ciências e Tecnologias Nucleares, IST, Universidade de Lisboa, Estrada Nacional 10, 2695-066 Bobadela LRS, Portugal; [c] Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, Marseille, France; email: olga.iranzo@univ-amu.fr

Abstract

Copper compounds have become an interesting alternative to complexes of platinum and platinum group metals as anticancer drugs. Their development has been prompted by the aims of overcoming the therapeutic drawbacks encountered for the family of platinum drugs and of developing biometal-based drugs with improved pharmacological properties.[1-3] However, copper(II) complexes are much less structurally predictable than other first-row transition metal complexes due to their labile character and preference for distorted coordination geometries. Therefore, the design of the ligand is crucial in order to control the formation of different species as a function of pH and to craft the copper(II) centers in the right environment for the desired biological activity.

Under these premises, two new ligands containing a phenanthroline unit covalently attached to the amino acid His, having an amide C-terminal (HL1) or a carboxylic C-terminal (H_2L2), were prepared, characterized and their copper(II) coordination properties studied using potentiometry, spectroscopy techniques (UV-Vis and EPR), mass spectrometry (ESI-MS) and DFT calculations.[4] The data showed the formation of single copper complexes, $[CuL1]^+$ and [CuL2], with high stability within a large pH range (from 3.0 to 9.0 for $[CuL1]^+$ and from 4.5 to 10.0 for [CuL2]). In both complexes the Cu^{2+} ion is bound to the phenanthroline unit, the imidazole ring and the deprotonated amide group, and displays a distorted square pyramidal geometry as confirmed by single crystal X-ray crystallography. Interestingly, despite having similar structures, these copper complexes show different redox potentials, DNA cleavage properties and cytotoxic activity against different cancer cell lines (human ovarian (A2780), its cisplatin-resistant variant (A2780cisR) and human breast (MCF7) cancer cell lines). These results highlight the effect of different pendant functional groups (carboxylate vs amide), placed out of the coordination sphere, in the properties of these copper complexes.

References

1) Duncan C.; White A. R. *Metallomics* 2012, 4, 127–138.
2) Santini C.; Pellei M.; Gandin V.; Porchia M.; Tisato F.; Marzano C. *Chem. Rev.* 2014, 114, 815–862.
3) Brissos R. F.; Caubet A.; Gamez P. *Eur. J. Inorg. Chem.* 2015, 16, 2633–2645.
4) Leite S.; Lima L.; Gama S.; Mendes F.; Orio M.; Bento I.; Paulo A.; Delgado R.; Iranzo O. *Inorg. Chem.* 2016, 55, 11801–11814.

Expanding the therapeutic potential of the iron chelator deferasirox in the development of Ti(IV) anticancer complexes with a dual mechanism of attack

Arthur D. Tinoco,[a] Sergio A. Loza-Rosas, Alexandra M. Vázquez, Sofía C. Pérez, Yamixa Delgado, Kavita Gaur, Kennett I. Rivero, Lenny J. Negrón, Timothy B. Parks, Charlene Munet-Colón

[a] *University of Puerto Rico-Río Piedras San Juan, PR 00931; email : atinoco9278@gmail.com*

Abstract

With cancer cells having a higher requirement for iron than normal cells, the use of iron chelators to decrease iron bioavailability is a promising anticancer approach. Our finding of a difference in how the blood protein serum transferrin (sTf) binds Fe(III) and Ti(IV) elucidates a new anticancer drug design opportunity to couple Fe(III) chelation with the highly potent cytotoxic properties of Ti(IV).[1] This opportunity builds on a template using Ti(IV) complexes of chemical transferrin mimetic (cTfm) ligands. Deferasirox, a FDA-approved drug to treat iron overload disease, serves as a cTfm ligand that models STf coordination to Fe(III) and favors Fe(III) binding versus Ti(IV). This metal affinity preference enables deferasirox to facilitate the release of Ti(IV) intracellularly in exchange for Fe(III). The Ti(IV)-deferasirox complex at pH 7.4, [Ti(Deferasirox)2]2-, exhibits the highest aqueous stability observed for a potent cytotoxic Ti(IV) species. UV-Vis and 1H NMR studies demonstrate that this stability is maintained in the presence of biomolecular Ti(IV) binders such as citrate, STf, and albumin, which have been shown to induce partial or total dissociation, affecting the activity of other cytotoxic Ti(IV) complexes. Kinetic transmetalation studies show that Ti(IV) remains coordinated to deferasirox in the presence of Fe(III)-bound STf but its dissociation is induced by a labile Fe(III) source. This work reveals a mechanism to transport Ti(IV) into cancer cells and intracellularly induce Ti(IV) release by capturing Fe(III) from labile sources and attenuating Fe bioavailability. Cellular studies support the Fe(III) depletion mechanism of Ti(IV)-cTfm compounds and reveal potential intracellular Fe(III) target sites and the transformation of the iron into a toxic species.

References

1) Tinoco, A. D., Saxena, M., Sharma, S., Noinaj, N., Delgado, Y., Quiñones González, E. P., Conklin, S.E., Zambrana, N., Loza-Rosas, S. A., and Parks, T. B. Unusual Synergism of Transferrin and Citrate in the Regulation of Ti(IV) Speciation, Transport, and Toxicity, J. Am. Chem. Soc. 2016, 138, 5659-5665.

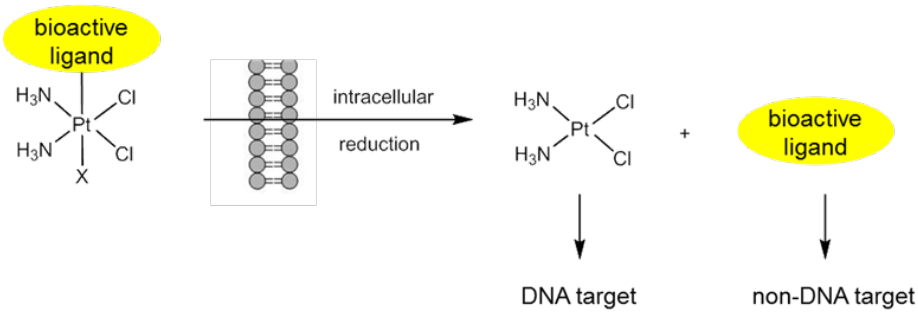
Multiple-threat Pt(IV) Anticancer Drugs

Andrea Erxleben

[a] *School of Chemistry, National University of Ireland, Galway, University Road, Galway, Ireland; andrea.erxleben@nuigalway.ie*

Abstract

Cisplatin and its second and third generation derivatives carboplatin and oxaliplatin are without doubt the most important anticancer drugs developed to date [1]. Despite the tremendous clinical success of these three platinum(II) drugs, toxicity, severe side effects and inherent and acquired resistance of certain tumors lead to significant limitations of platinum chemotherapy. The design of dual-threat platinum(IV) anticancer agents is a promising approach to enhance efficacy and to reduce the risk of tumors developing resistance in the course of treatment. In contrast to Pt(II), Pt(IV) forms octahedral complexes and the "axial" positions can be used to attach a biologically active ligand to a cisplatin-, carboplatin- or oxaliplatin-scaffold. On intracellular reduction the Pt(IV) pro-drug releases the active Pt(II) species that targets DNA and the axial ligand that then interacts with its non-DNA target, thus acting synergistically with the Pt(II) species and limiting cross-resistance.



This Keynote Lecture gives an overview on our recent work aimed at the development of Pt(IV) complexes with bioactive redox modulators, histone deacetylase inhibitors and cyclooxygenase inhibitors as axial ligands. In particular, the combination of DNA platination with the induction of oxidative stress through a redox active ligand will be highlighted as a promising strategy for achieving high selectivity and cytotoxic activity in cisplatin-resistant cancer cell lines [2].

While one of the axial positions is occupied by the bioactive ligand, the second axial site is often used to attach a biologically innocent ligand such as benzoate or acetate to modulate and optimize the lipophilicity, redox potential and cellular uptake. We have recently developed triple-threat anticancer agents by coordinating a second, different bioactive ligand to the vacant axial site.

References

1) T. C. Johnstone, K. Suntharalingam, S. J. Lippard, Chem. Rev. (**2016**) 116, 3436 and refs. therein.
2) D. Tolan, V. Gandin, L. Morrison, A. El-Nahas, C. Marzano, D. Montagner, A. Erxleben, Sci. Rep. (**2016**), doi:10.1038/srep29367.

Data mining of metalloenzymes: using coordination chemistry of vanadium in protein phosphatases for inhibitor design for diabetes

Debbie C. Crans [a,b] *and* Craig C. McLauchlan [c]

[a] *Dept. Chemistry, Colorado State University 1 ;* [b] *Cell and Molecular Program, Colorado State University 2* [c)]

Department of chemistry, Illinois State University3

email : Debbie.Crans@ColoState.edu; Craig.McLauchlan@Illinois.State.edu

Abstract

For over a century it has been known that vanadium compounds have antidiabetic properties. In addition to this they are inhibitors of phosphorylases, phosphatases and protein phosphatases.[1] The inhibitory properties of vanadium are attributed to the favorable five-coordinate geometry of phosphate ester hydrolysis transition states.[2] Five coordinate geometries for transition metal ions such as vanadium, however, are often found to be in an intermediate form between square planar geometry and trigonal bipyramidal geometry. The latter is optimal for a associative phosphate ester hydrolysis mechanism as it shares complementarity with the five-coordinate exploded transition-state geometry of an dissociative hydrolysis reaction mechanism.[3,4] We will conduct an analysis of the structural parameters obtained from X-ray crystallography of various five-coordinate vanadium complexes with a VO_4X core geometry (X = C, N, O, Cl, F, S)[3] and compare them to the vanadium-based inhibitors crystalized inside various phosphorylases.[4a,b] To evaluate the importance of small structure perturbation within inhibitor complexes and explore the potential uses of peptides for stability/interaction, each structure will be compared to the geometry of small molecules. Other aspects of drug delivery such as crossing membranes raise additional challenges. We have addressed this through a) studies with model membranes[5] to investigate the structural preferences of inhibitors and b) analyzed delivery uptake in both animal and human studies.[6] We will present a review detailing the advances within this field and discuss possible solutions for working with these metal-containing systems. Combined, these studies suggest that if effective delivery of potentially active antidiabetic compounds, such as the organic vanadate peptidic substrates, could be achieved, they could alleviate the current toxicity problems reported for the salts and some of the complexes as well as yield dramatic enhancements of antidiabetic vanadium compounds. This information can now be used in studies of vanadium compounds in oncolytic immunotherapy.

References

1) (a) K.H. Thompson, J. Lichter, C, LeBel, M.C. Scaife, J.H. McNeill, C. Orvig, *J. Inorg. Biochem.* **2009**, *103*, 554-558, (b) G.R. Willsky, L.-H. Chi, M.E. Godzalla, III, P.J. Kostyniak, J.J. Smee, A.M. Trujillo, J.A. Alfano, W. Ding, Z. Hu and D.C. Crans, *Coor. Chem. Rev.,* **2011,** *19-20,* 2258-2269.

2) (a) B.I. Posner, R.J. Faure, J.W. Burgess, A.P. Bevan, A. P., D. Lachance, G. Zhang-Sun, I.G. Fantus, J.B. Ng, J. B., D. Hall, B.S. Lum, *J. Biol. Chem.* **1994**, *269***,** 4596-4604; (b) C.C. McLauchlan, J.D. Hooker, M.A. Jones, Z. Dymon, E.A. Backhus, B.A. Greiner, N.A. Dorner, M.A. Youkhana, L.M. Manus, *J. Inorg. Biochem.* **2010**, *104,* 274-281; (c) L. Lu, X. Gao, M. Zhu, S. Wang, Q. Wu, S. Xing, X. Fu, Z. Liu, M. Guo, *BioMetals* **2012**, *25*, 599-610.

3) 3. D.C. Crans, M.L. Tarlton, C.C. McLauchlan, *Eur. J. Inorg. Chem.* **2014**, *27*, 4450-4468.

4) 4. (a) C.C. McLauchlan; B.J. Peters; G.R. Willsky; D.C. Crans, *Coord. Chem. Rev.*, **2015**, *301-302*, 163-199; (b) D. C. Crans, *J. Org. Chem.* **2015**, *80*, 11899-11915;(c) I. Sanchez-Lombardo, S. Alvarez, C.C. McLauchlan and D.C. Crans, *J. Inorg. Biochem.* **2015**, *147*, 153-164.

5) 5. (a) P.W. Winter, A. Al-Qatati, A. Wolf-Ringwall, S. Schoeberl, A.K. Van Orden, B.G. Barisas, D. A. Roess and D.C. Crans, *Dalton Transactions,* **2012**, *41(21),* 6419-6430; (b) A.G. Sostarecz, E. Gaidamauskas, S. Distin, S.J. Bonetti, N.E. Levinger and D.C. Crans, *Chem. Eur. J.,* **2014**, *20*, 5149-5159; (c) D.C. Crans, A.M. Trujillo, P.S. Pharazyn and M.D. Cohen, *Coor. Chem. Rev.,* **2011**, *19-20*, 2178-2192.

6) 6. **a)** G.R. Willsky, K. Halvorson, M.E. Godzala III, L.-H. Chi, M. Most, P. Kaszynski, D.C. Crans, A.B. Goldfine and P.J. Kostnyniak, *Metallomics,* **2013**, *5,* (11) 1491-1502; b) K. A. Doucette, K. N. Hassell and D. C Crans, *J. Inorg. Biol. Chem.* **2016**, 165, 56-70.

Hybrid platinum complexes ligands of G-quadruplex as probes for telomere targeting

Ali S.[a], Betzer, JF.[b], Kellermann, G[a], Poupon J.[c], Marinetti A[b], Teulade-Fichou M.P.[d] and Bombard S.[a, d].

[a] INSERM UMR-S 1007, Université Paris Descartes; Paris, France ; [b] Institut de Chimie des Substances Naturelles, CNRS UPR2301, Université Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette ; [c] Laboratoire de Toxicologie-Biologie, Hôpital Lariboisière, Paris, France ; [c] Institut Curie, Centre de Recherche, CNRS UMR9187-U1196, Université Paris-Sud, Université Paris-Saclay France ; e-mail : sophie.bombard@curie.fr

Abstract

Telomeres are DNA-protein structures that cap chromosome ends protecting them from recognition by the DNA repair machinery. In mammalians, among the six telomeric proteins belonging to the shelterin, TRF1 and TRF2 are both proteins able to bind directly to the double stranded telomeric DNA and are involved in telomere length regulation and chromosome end protection. Since telomeric DNA consists of highly repetitive short sequences of adjacent guanine residues $(TTAGGG)_n$, and able to fold in G-quadruplex structures, it is a potential target for platinum complexes. Series of hybrid platinum complexes such as Pt-MPQ, Pt-ttpy (ttpy= tolyl-terpyridine) [1] and NHC-Pt- PDC[2] able to recognise, stabilize and cross-link preferentially G-quadruplex structures, *in vitro*, have been designed. We then focused our studies on their cellular activities, in particular on their capacity to target telomeres. Our results show that telomere targeting by platinum complexes (telomere uncapping and damage) can be improved when they are linked to a G-quadruplex ligand and provide new advances in telomere biology[3].

References

1) Bertrand, H., Bombard, S., Monchaud, D., Talbot, E., Guédin, A., Mergny, J.L., Grünert, R., Bednarski, P.J., and Teulade-Fichou M.P.(2009). Exclusive platination of loop adenines in the human telomeric G-quadruplex. Org. Biomol. Chem., 7, 2864–2871

2) Betzer, JF, Chtchigrovsky, M., Hamon, F., Kellermann, G., Ali, S., Calméjane, MA, Roque, S., Poupon, J, Teulade-Fichou MP, Marinetti, A., Bombard, S (2016). Linking of antitumour trans NHC-Pt(II) complexes to G-quadruplex DNA ligand for telomeric targeting, Bioconjugate chemistry,27, 1456-.1470

Fluorescent mono- and bis-platinum(II) boxes binding G-quadruplex motifs

Olaya Domarco,[a] Marcos D. García,[a] Carlos Peinador,[a] Bernhard K. Keppler,[b,c] Walter Berger[c,d], Alessio Terenzi[b,c]

[a)] Departamento de Química Fundamental and Centro de Investigacións Científicas Avanzadas, Facultade de Ciencias, Universidade da Coruña, E-15071 A Coruña, Spain;[b)] Institute of Inorganic Chemistry, University of Vienna, Waehringerstr. 42, A-1090 Vienna, Austria;[c)] Research Platform "Translational Cancer Therapy Research", University of Vienna and Medical University of Vienna, Vienna, Austria; [d)] Department of Medicine I, Institute of Cancer Research and Comprehensive Cancer Center, Medical University Vienna, Borschkegasse 8a, A-1090 Vienna, Austria. email : alessio.terenzi@univie.ac.at

Abstract

Pt^{II} supramolecular architectures with square or rectangular shapes display remarkable applications in various fields, especially as molecular hosts or catalysts.[1] Interestingly, coordination-driven self-assembly allows for the efficient synthesis of nanometric aggregates, creating supramolecules that can potentially mimic the dimensions of protein DNA recognition motifs.[2]

We have recently reported on the DNA binding properties of three 4,4'-bipyridine-based Pt(II) dinuclear square boxes of different size. We showed that these Pt compounds possess biological activity against cancer cells and heavily influence the expression of genes known to form G-quadruplexes (G4s) in their promoter regions according to their size.[3]

We exploited our self-assembly strategy for the fast and modular generation of libraries of Pt(II) metallacycles with improved binding affinity and selectivity towards G4 motifs. By means of competitive FRET melting assays, UV-vis and circular dichroism titrations, we studied the DNA binding abilities of ten Pt-boxes of different shape, charge and size. One mono- and two bis-platinum(II) boxes resulted selective for G4 structures over B-DNA. Additionally, these three compounds presented a nice fluorescence profile in water solution (emission at ≅ 440 nm) indicating the possibility to track them inside the cell. Biological assays to test the effect of these compounds on cancer cells are currently ongoing.

AT has received funding from the Mahlke-Obermann Stiftung and the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 609431.

References

1) R. Chakrabarty, P. S. Mukherjee and P. J. Stang, Chem. Rev., 2011, 111, 6810
2) A. Terenzi; C. Ducani; V. Blanco; L. Zerzankova; A.F. Westendorf; C. Peinador; J.M. Quintela; P.J. Bednarski; G.Barone and M.J. Hannon, Chem. Eur. J. ,2012, 18, 10983
3) O. Domarco, D. Lötsch, J. Schreiber, C. Dinhof, S. van Schoonhoven, M. D. García, C. Peinador, B. K. Keppler, W. Berger and A. Terenzi, Dalton Trans., 2017, 46, 329

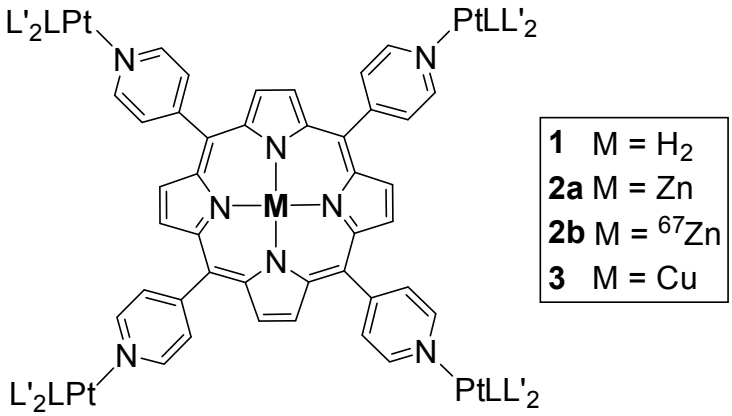
The Influence of the Central Metal Atom in Platinum-Porphyrin Conjugates on their Phototoxicity against Human Cancer Cells

Michele Larocca[a], Riccardo Rubbiani[a], Anu Naik[a], Gilles Gasser[a,b], Bernhard Spingler[a]

[a] *University of Zurich, Department of Chemistry, 8057 Zurich, Switzerland;* [b] *Chimie ParisTech, PSL Research University, Laboratory for Inorganic Chemical Biology, 75005 Paris, France; email : spingler@chem.uzh.ch*

Abstract

Our group has reported recently about the very promising *in vitro* light-induced anticancer properties of novel tetraplatinated porphyrins **1**.[1] The dark and light toxicity against human cancerous and non-cancerous cell lines (MRC-5, HeLa, A2780 and CP70) was determined by the resazurin assay. IC_{50} values were obtained after 4 h incubation, followed by 15 min irradiation at either 420 nm or 575 nm respectively. This family of platinum-porphyrin conjugates **1** had only minor dark toxicity, however upon visible light irradiation, IC_{50} values down to 19 ± 4 nM could be observed. These values correspond to an excellent phototoxic index (PI = IC_{50} dark / IC_{50} light) of greater than 5000.



We have now started to study similar systems that contain a metal in the central position of the porphyrin (**2a** and **3**). We will report and discuss the influence of the metal on the singlet oxygen yield, cellular dark and (photo)toxicity as well as cellular localisation. For the latter we employed the isotopically labelled ^{67}Zn complex **2b** in order to determine, by ICP-MS, the cellular distribution of ^{67}Zn and platinum, which in turn allowed us to study the stability of the platinum - pyridine nitrogen bond within the cells. We included the copper(II) **3**, since we previously discovered the first phototoxic copper(II) complex of a porphyrin.[2]

Acknowledgements

We thank for financial support by the University of Zurich and the Swiss National Science Foundation.

References

1) Naik, A.; Rubbiani, R.; Gasser, G.; Spingler, B. *Angew. Chem. Int. Ed.* **2014**, *53*, 6938.
2) Antoni, P. M.; Naik, A.; Albert, I.; Rubbiani, R.; Gupta, S.; Ruiz-Sanchez, P.; Munikorn, P.; Mateos, J. M.; Luginbuehl, V.; Thamyongkit, P.; Ziegler, U.; Gasser, G.; Jeschke, G.; Spingler, B. *Chem. Eur. J.* **2015**, *21*, 1179.

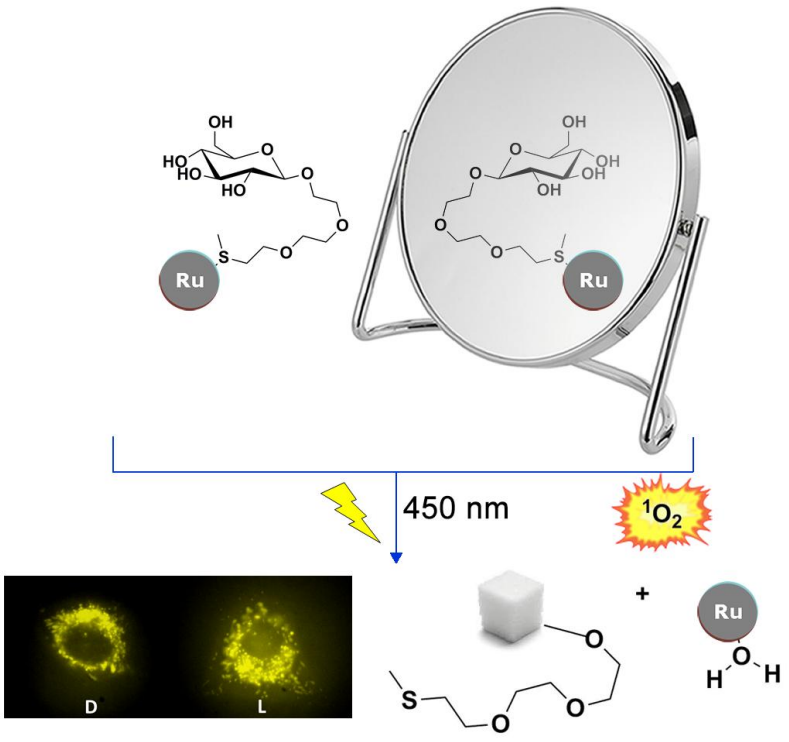
Sugar-Metallodrug Conjugates in a Mirror: Mitochondrial Targeting of a Light-Activated Ruthenium Anticancer Prodrug using D- and L-Glucose Conjugation

Lucien N. Lameijer, Samantha L. Hopkins, Tobias G. Brevé, Sven H.C. Askes, Sylvestre Bonnet

Leiden University, Leiden Institute of Chemistry, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands; email : bonnet@chem.leidenuniv.nl

Abstract

Light-activated ruthenium polypyridyl anticancer prodrugs often suffer from poor water solubility, poor selectivity, and/or ill-defined intracellular targets. Coordination of a D- or L-glucose thioether ligand (SRR') to the highly lipophilic ruthenium complex $[Ru(tpy)(dppn)(OH_2)]^{2+}$ ($[\mathbf{1}]^{2+}$, tpy = 2,2':6',2''-terpyridine, dppn = benzo[*i*]dipyrido-[3,2-*a*:2',3'-c]phenazine) solved all problems at once. The two enantiomers $[Ru(tpy)(dppn)(SRR')](PF_6)_2$ ($[\text{D-}\mathbf{2}](PF_6)_2$ and $[\text{L-}\mathbf{2}](PF_6)_2$) were soluble in water, which allowed for probing the influence of the chirality of the glucose moiety on uptake, toxicity, and intracellular localization of the prodrug without changing any other physico-chemical properties. Both compounds showed mild but different cytotoxicity in A549 (human lung carcinoma) and MCF-7 (human breast adenocarcinoma) cancer cells in the dark, whereas similarly high cytotoxicities were observed following low doses of visible light irradiation (3.1 $J.cm^{-2}$ at 455 nm). Irrespective of chirality the slightly emissive Ru complexes were found in the mitochondria, where two modes of action may contribute to light-induced cell death. On the one hand, the glucose-thioether ligand is photosubstituted by water, thus releasing $[\mathbf{1}]^{2+}$ that interacts with DNA at a exceptionally high 400:1 bp:Ru ratio. On the other hand, both $[\mathbf{2}]^{2+}$ and $[\mathbf{1}]^{2+}$ produce massive amounts of singlet oxygen, leading to very efficient photodynamic DNA cleavage.



Reference

1) Lameijer et al, Chemistry a European Journal, 2016, 22,18484 –18491.

Metallo supramolecular cylinders prevent the formation of HIV-1 TAR-TAT complex and inhibit the viral activity *in cellulo*.

Lucia Cardo,[a] Isabel Naworth,[b] Peter J. Cail,[a] Jane A. McKeating[b] and Michael J. Hannon.[a]

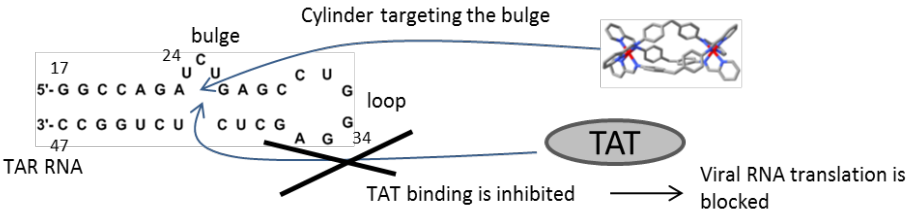
[a] School of Chemistry and [b] Institute of Immunology and Immunotherapy, Centre for Human Virology, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom; email : l.cardo@bham.ac.uk

Abstract

The Trans Activation Response region (TAR) is a highly conserved 59-nucleotide stem-loop RNA located at the 5' end of HIV-1 viral genome, which includes a six residues loop, a trinucleotide pyrimidine bulge and an extensive duplex structure (scheme below). The pyrimidine bulge interacts with the arginine rich motif of the virus-encoded Transactivator protein (TAT) forming a complex (together with other cofactors) that is essential for the correct transcription of viral mRNA. The structural characterisation of TAR-TAT complex has been studied extensively for decades and the design of synthetic agents able to prevent the formation of this complex and compromise viral transcription, is a promising strategy in anti-HIV therapy.[1] In particular, the inhibition of viral genome translation could affect the high level of genetic variation and prevent the development of virus resistance, which is very typical of HIV-1 and one main limit in current therapies.[2]

During the last years, our group has focused on developing a large family of metal-based supramolecular architectures, where coordination and supramolecular chemistry are important tools to target specific motifs of RNA and DNA by tuning shape and dimension of potential binders. In particular, di-nuclear metallo supramolecular helicates (cylinders) perfectly fit into nucleic acid junctions and bulges.[3-5] The biophysical data presented here, prove that octahedral metal-based cylinders selectively bind at the bulge of TAR RNA and inhibit the formation of TAT-TAR complex. Furthermore, studies in CD4+ T cells, in the presence of the virus, show that cylinders own remarkable anti-HIV activity (depending on the metal employed) and relatively low cytotoxicity, which is a very important feature when designing antiviral drugs.

Due to the high structural and functional variability of RNAs, the number of synthetic molecules known as targeting specific RNA motifs, is still relatively low compared to protein inhibitors or DNA binders.[6] Herein, metal based cylinders show interesting recognition properties of TAR RNA bulge, which makes them promising candidates in the field of anti-retrovirus therapy.



References

1) Fardokht A. Abulwerdi, Matthew D. Shortridge, et al., J. Med. Chem., 2016, 59, 11148−11160.
2)Jessica E. Wynn and Webster L. Santos, Org. Biomol. Chem, 2015, 13, 5848-5858.
3) Jaroslav Malina, Michael J. Hannon and Viktor Brabec, FEBS J., 2014, 281, 987–997.
4) Siriporn Phongtongpasuk, Susann Paulus, et al., 2013, Angew. Chem. Int. Ed. 52, 11513–11516.
5) Jaroslav Malina, Michael J. Hannon and Viktor Brabec, Scientific Report, 2016, 6, 29674.
6) Lirui Guan and Matthew D. Disney, ACS Chem. Biol., 2012, 7, 73−86

Pt(II)- and Pd(II)-linked metallosupramolecular capsules with improved therapeutic potential for cancer treatment

Anife Ahmedova, [a] Rositsa Mihaylova, [b] Denitsa Momekova, [b] Georgi Momekov [b]

[a] Laboratory of Biocoordination and bioanalytical chemistry, Faculty of Chemistry and Pharmacy, University of Sofia, 1, J. Bourchier blvd.,Sofia 1164, Bulgaria ;[b] Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Street, Sofia 1000, Bulgaria ; email : ahmedova@chem.uni-sofia.bg

Abstract

Metallosupramolecular cages and capsules attract increasing attention due to their potential application as molecular containers and anticancer agents. Herein, we present our recent results on the anticancer activity of anthracene based coordination capsules [1,2] that have recently been designed and synthesised by Yoshizawa and co-workers [3]. The capsules have M_2L_4 composition (where M= Pd or Pt) and provide large hydrophobic cavity, with an average volume of ~580 $Å^3$, which are capable of encapsulating various guest molecules, such as highly reactive radical-initiators [4] or methyl-group rich, biologically active, xanthine derivatives [5]. Beside the demonstrated application of these coordination capsules for safe storage or guest recognition, we have also discovered that they have appropriate stability in presence of small biomolecules and exert very high cytotoxicity against the tested human cancer cells (HL-60, SKW-3, HT-29, and T-24). Moreover, the capsules show high potency to circumvent the multi-drug resistance (up to 125-fold) when compared with cisplatin, and very high selectivity - that is ~10 times more toxic to the cancer cells than to the non-malignant cells (HEK-293). We also demonstrated that the cytotoxicity of the Pt(II) and Pd(II)-linked coordination capsules can be modulated by encapsulation of guest molecules, such as pyrene and caffeine. Mechanistic insights into the observed cytotoxicity profiles were obtained by fluorescence microscopy imaging of tumour cells treated with the capsules, and the overall data suggested the glutathione-triggered disassembly of the capsular structures as a potential activation pathway for the observed cytotoxicity. Our findings illustrate the high potency of the Pt and Pd capsules for improved therapeutic applications and possible combination with their properties for storage and/or delivery.

Acknowledgement: the National Science Fund of Bulgaria (contract: DFNI-B02/24) and the H2020-Twinning project (Materials Networking; 692146) are acknowledged for financial support. Authors are grateful to Dr. Michito Yoshizawa (Laboratory for Chemistry and Life Science, Tokyo Institute of Technology, Japan) for kindly providing the supramolecular capsules.

References

1) A. Ahmedova, D. Momekova, M. Yamashina, P. Shestakova, G. Momekov, M. Akita, M. Yoshizawa, Anticancer Potencies of Pt(II)- and Pd(II)-linked M2L4 Coordination Capsules with Improved Selectivity, Chemistry – As. J., 2016, 11, 474-477.
2) A. Ahmedova, R. Mihaylova, D. Momekova, P. Shestakova, S. Stoykova, J. Zaharieva, M. Yamashina, G. Momekov, M. Akita, M. Yoshizawa, M2L4 coordination capsules with tunable anticancer activity upon guest encapsulation, Dalton Trans., 2016, 45, 13214-13221.
3) N. Kishi, Z. Li, K. Yoza, M. Akita, M. Yoshizawa, An M2L4 molecular capsule with an anthracene shell: encapsulation of large guests up to 1 nm, J. Am. Chem. Soc., 2011, 133, 11438–11441.
4) M. Yamashina, Y. Sei, M. Akita, M. Yoshizawa, Safe storage of radical initiators within a polyaromatic nanocapsule, Nature Commun., 2014, 5, 4662 doi: 10.1038/ncomms5662.
5) M. Yamashina, S. Matsuno, Y. Sei, M. Akita, M. Yoshizawa, Recognition of Multiple Methyl Groups on Aromatic Rings by a Polyaromatic Cavity, Chemistry – Eur. J., 2016, 22, 14147-14150.

New heterobimetallic ferrocene derivatives: evaluation of their potentiality as prospective agents against infectious diseases.

Feriannys Rivas,[a] Esteban Rodríguez,[a] Andrea Medeiros,[b,c] Marcelo Comini,[b] Camila M. Ribeiro,[d] Fernando R. Pavan,[d] Dinorah Gambino,[a]

[a] Química Inorgánica, Facultad de Química, Universidad de la República, Montevideo, Uruguay,' [b] Group Redox Biology of Trypanosomes, Institut Pasteur Montevideo, Montevideo, Uruguay; [c] Departamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; [d] Faculdade de Ciências Farmacêuticas, UNESP, Araraquara, Brazil; email: dgambino@fq.edu.uy

Abstract

Infectious diseases are major causes of human illness worldwide. Tuberculosis is an ancient disease that remains a global public health issue. Diseases caused by genetically related trypanosomatid protozoa, like *Trypanosoma brucei and Leishmania spp*, constitute major health concerns in the developing world. Currently available drugs for the treatment of these diseases are decades old and/or suffer from limited efficacy, undesirable collateral effects and development of resistance.

Bioorganometallic Chemistry offers an innovative approach for the development of new drugs. Our group is currently focused on the development of prospective metal-based drugs mainly based on bioactive ligands, pharmacologically active metals and selected organometallic *cores*.[1-4]

Searching for prospective agents against infectious diseases, four new $[M^{II}(L)(dppf)](PF_6)$ compounds, with dppf = 1,1'-bis (diphenylphosphino) ferrocene, M = Pd or Pt and L = tropolone derivatives, were synthesized and fully characterized in the solid state and in solution. In particular, crystal structure of the compounds was solved by X-ray diffraction methods. The compounds showed good activity (MIC_{90} values in the μM range) against the standard *M. tuberculosis* strain $H_{37}Rv$ ATCC 27294 but were less active against resistant clinical isolates. Low activity and/or low selectivity were obtained against the clinically relevant form of *Leishmania infantum*. In contrast, the compounds displayed fairly good activity and selectivity (tested for J774 macrophages) towards the related trypanosomatid *T. brucei* (IC_{50} values in the low micromolar range). In almost all cases, the inclusion of the {Mdppf} moiety lead to an enhancement of activity compared with the parent tropolone ligands. Pd compounds were more active than the Pt analogues but showed higher unspecific cytotoxicity than Pt ones. Results were compared with those obtained for antitrypanosomal compounds previously developed by us.

These new organometallic compounds could be considered new hits for the development of antitrypanosomal agents.

References

1) B. Demoro, C. Sarniguet, R. Sánchez-Delgado, M. Rossi, D. Liebowitz, F. Caruso, C. Olea-Azar, V. Moreno, A. Medeiros, M. A. Comini, L. Otero, D. Gambino, Dalton Trans. 2012, 41, 1534.

2) E. Rodríguez Arce, C. Sarniguet, T.S. Moraes, M. Vieites, A. I. Tomza, A. Medeiros, M. A. Comini, J. Varela, H. Cerecetto, M. González, F, Marques, M. H. García, L. Otero, D. Gambino, J. Coord. Chem. 2015, 68, 2923.

3) M. Fernández, E. Rodríguez Arce, C. Sarniguet, T. S. Morais, A. I. Tomaz, C. Olea Azar, R. Figueroa, J. Diego Maya, A. Medeiros, M. Comini, M. H. Garcia, L. Otero, D. Gambino, J. Inorg. Biochem. 2015, 153, 306.

4) E. Rodríguez Arce, M. F. Mosquillo, L. Pérez-Díaz, G. A. Echeverría, O. E. Piro, A. Merlino, E. L. Coitiño, C. Maríngolo Ribeiro, C. Q. F. Leite, F. R. Pavan, L. Otero, D. Gambino, *Dalton Trans.*, 2015, 14453.

Photoswitching the DNA-interacting properties of coordination compounds

Andreu Presa, [a] Alyson Megan, [a] Patrick Gamez [a,b]

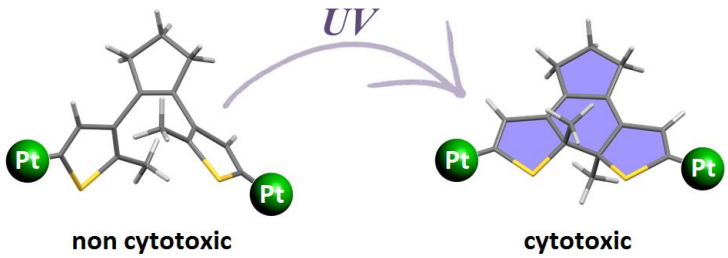
[a] *Department of Inorganic and Organic Chemistry, University of Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain;* [b] *Catalan Institution for Research and Advanced Studies (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain; email: patrick.gamez@qi.ub.es*

Abstract

Photoactivated chemotherapy (PACT) drugs allow to achieve controllable activity with reduced side effects [1]. Regarding coordination compounds, their photoactivation of is commonly based on **metal-centred** processes [2].

Dithienylcyclopentene (DTE) molecules undergo reversible cyclization/ring opening reactions between their colourless (open) and coloured (closed) forms when stimulated with UV and visible light, respectively [3]. The closed/open photoisomers exhibit drastically different physical properties; therefore, such molecules can be used as molecular switches.

In this presentation, a series of photoswitching metal complexes obtained from DTE-based ligands will be presented together with their properties. Besides the anticipated distinct optical behaviours, the open and closed forms of such coordination compounds exhibit different DNA-interacting properties and cytotoxicities [4, 5].



References

1) N. J. Farrer, L. Salassa, P. J. Sadler, Dalton Trans., 2009, 48, 10690-10701.
2) K. Szaciłowski, W. Macyk, A. Drzewiecka-Matuszek, M. Brindell, G. Stochel, Chem. Rev., 2005, 105, 2647-2694.
3) B. L. Feringa. *Molecular Switches*; Wiley-VCH, Weinheim, 2001.
4) A. Presa, L. Barrios, J. Cirera, L. Korrodi-Gregório, R. Pérez-Tomás, S. J. Teat, P. Gamez, Inorg. Chem., 2016, 55, 5356-5364.
5) A. Presa, R. F. Brissos, A. B. Caballero, I. Borilovic, L. Korrodi-Gregório, R. Pérez-Tomás, O. Roubeau, P. Gamez, Angew. Chem. Int. Ed., 2015, 54, 4561-4565.

Acknowledgements

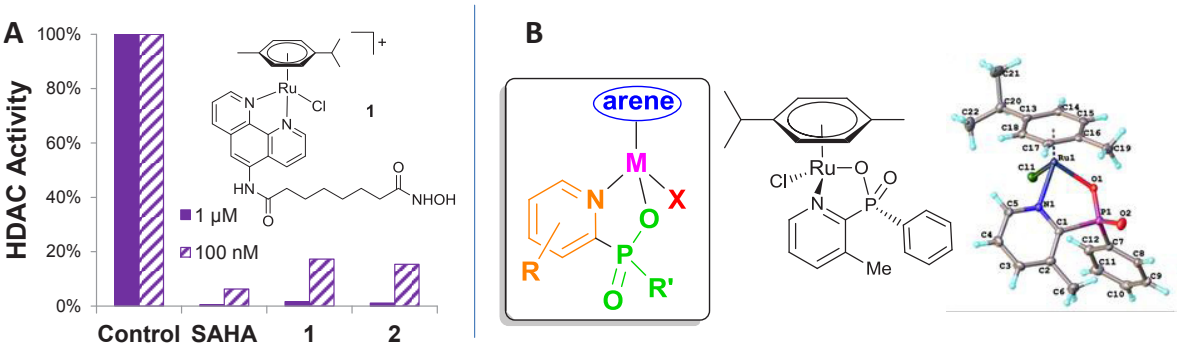
Financial support by the MINECO (projects CTQ2014-55293-P and CTQ2015-70371-REDT) is kindly acknowledged.

Anticancer Metal Complexes: HDAC Enzyme Inhibitors[1] and Pyridylphosphinate Complexes[2]

James W. Walton,[a] Tim R. Blower,[a] Jasmine M. Cross,[a] Jason H. Gill,[b] Dmitry S. Yufit,[a]

[a] *Department of Chemistry, Durham University, UK;* [b] *School of Pharmacy, Durham University, UK;*
e-mail: james.walton@durham.ac.uk

Piano stool metal complexes have been explored for several decades as potential anticancer agents. HDAC enzymes are excellent targets for such therapeutic activity. We present the first examples of Ru(II) and Rh(III) piano stool HDAC inhibitors (Fig A). The novel complexes have anticancer activity comparable to the clinically used HDAC inhibitor SAHA. Strong evidence for HDAC inhibition as a primary mechanism of action is provided. The complexes reported here represent an important step towards the design of highly active and selective HDAC inhibitors. These results have recently been communicated in an Early Career Special Issue of ChemPlusChem.[1]



We also present the synthesis and anticancer properties of a series of 25 new pseudo-octahedral metal complexes (Ru, Os, Rh, Ir).[2] Each complex incorporates a pyridylphosphinate ligand (Fig B), along with a monodentate halide and a capping η^6-bound aromatic ligand. Solid-state structural analyses of two complexes (Fig B) reveal intriguing conformational diastereoselectivity. Aqueous behaviour has been investigated, with respect to hydrolysis of the metal–halide bond and pK_a of the resultant aqua complexes. Binding studies with selected amino acids and nucleobases provide a rationale for the variation in toxicity observed within the series. Finally, an investigation into the ability of the chelating amino acid L-His to displace the phosphinate O–metal bond shows the potential for phosphinate complexes to act as prodrugs that can be activated *in cellulo*.

References

1) J. M. Cross, T. R Blower, N. Gallagher, J. H Gill, K. L Rockley and J. W. Walton, *ChemPlusChem*, 2016, **81**, 1276.
2) J.M. Cross, N. Gallagher, J. H. Gill, M. Jain, A. W. McNeillis, K. L. Rockley, F. H. Tscherny, N. J. Wirszycz, D. S. Yufit and J. W. Walton, *Dalton Trans.*, 2016, **45**, 12807.

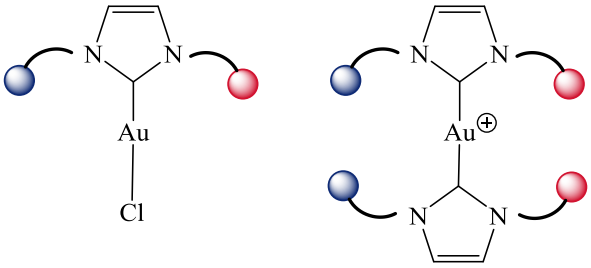
Bioactive NHC-gold complexes

Catherine HEMMERT, Heinz GORNITZKA

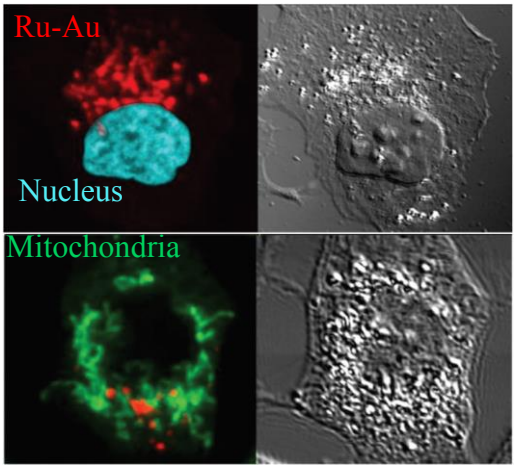
Laboratoire de Chimie de Coordination du CNRS, 205 Route de Narbonne, 31077 Toulouse Cedex 04, France;
email: heinz.gornitzka@lcc-toulouse.fr

Abstract

N-heterocyclic carbene ligands (NHCs) containing functional side-arm have been developed to stabilize gold(I) cations for biomedical applications. These complexes have been tested on a panel of human cancer cell lines, against *Plasmodium falciparum* (malaria) and *Leishmania infantum*.



The correlation between lipophilicity of the gold complexes and biological activities has been evidenced. Moreover, the cationic complexes show higher selectivity than neutral analogues against cancer cell lines in comparison to normal non-cancer cells. Combining a NHC-gold unit with a ruthenium chromophore permitted to localize these complexes in liver cancer cells (Hep3B).



Fluorescence (λ_{exc} = 458 nm, λ_{em} = 620 nm and transmission images of Hep3B cells treated with **Ru-Au** (10 µM, 24 h, in red) and labeled with MitoTracker (in green, bottom) or DAPI (cyan, top).

References

1) C. Hemmert, H. Gornitzka, *Dalton Trans*., 2016, 45, 440–447.
2) C. Hemmert, A. Pramundita Ramadania, L. Boselli, Á. Fernández Álvarez, L. Paloque, J.-M. Augereau, H. Gornitzka, F. Benoit-Vical, *Bioorganic & Medicinal Chemistry*, 2016, 24, 3075–3082.
3) L. Boselli, M. Carraz, S. Mazeres, L. Paloque, G. Gonzalez, F. Benoit-Vical, A. Valentin, C. Hemmert, H. Gornitzka, *Organometallics*, 2015, 34, 1046−1055.
4) C. Hemmert, A. Valentin, H. Gornitzka, L. Paloque, *Eur. J. Med. Chem*., 2015, 94, 22-29.
5) L. Boselli, I. Ader, M. Carraz, C. Hemmert, O. Cuvillier, H. Gornitzka, *Eur. J. Med. Chem*., 2014, 85, 87-94.

Aβ-targeted fluorescent/luminescent probes as potential theranostic agents for Alzheimer's disease

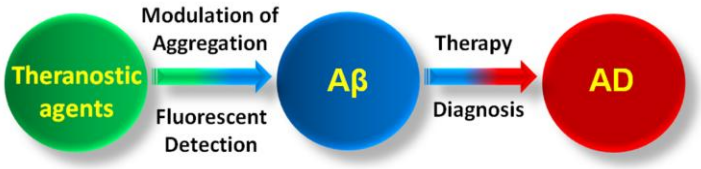
Xiaohui Wang [a,*]

[a] *College of Chemistry and Molecular Engineering, Nanjing Tech University, Nanjing 211816, P. R. China ; email : wangxhui@njtech.edu.cn*

Abstract

Alzheimer's disease (AD) is the most common form of neurodegenerative diseases and is still incurable so far.[1] Traditional therapies use diagnostic and therapeutic drugs separately, which are unfavorable to exploring the pathology of AD and optimizing the efficacy of drugs. Theranostic agents that combine diagnosis and targeted therapy functions into a single platform could overcome the weakness of current approaches.[2] Amyloid-β (Aβ) aggregates, one of the primary pathological hallmarks of AD, have been considered as both biomarkers and therapeutic targets for AD diagnosis and treatment,[3] which provide the possibility of developing theranostic agents able to detect and regulate Aβ aggregation at the same time. Fluorescent/luminescent probes have many advantages to be theranostic agents, including nonradioactive, high biocompatibility, and simple structures, which are easily modified to obtain desired efficacy. In this work, we demonstrate a series of Aβ-targeted fluorescent/luminescent probes and their dual-functional properties toward Aβ aggregation as potential theranostic agents for AD. First, fluorescent chelators, containing metal-chelating group and Aβ-recognizing group, were employed to specifically target metal-associated Aβ aggregates and capture metal ions to attenuate the aggregates, which can be simultaneously self-monitored by fluorescence changes in real-time. Moreover, they were also verified to penetrate the blood brain barrier of mice in vivo and detoxify the neurotoxicity. On the other hand, Aβ-targeted luminescent lanthanide complexes were also explored as potential theranostic agents for AD due to their unique optical properties and ability to modulate metal-free Aβ aggregation under physiological conditions.[4] These findings suggest that the bifunctional Aβ-targeted fluorescent/luminescent probes would provide a promising strategy for AD theranosis.

Financial supports by the National Natural Science Foundation of China (Grant: 21301090) are gratefully acknowledged.



References

1) S. Reardon, *Nature* **2015**, *523*, 509-510.
2) M. L. Bolognesi, A. Gandini, F. Prati, et al., *J. Med. Chem*, **2016**, *59*, 7759-7770.
3) T. Yang, X. H. Wang, C. L. Zhang, et al., *Chem. Commun.* **2016**, *52*, 2245-2248.
4) X. H. Wang, T. Yang, J. Luo, et al., *Chem. Commun.*, **2015**, *51*, 8185-8188.

Medicinal Chemistry of Gold(I) Alkynyl Complexes

Ingo Ott, [a]

[a] *Institute of Medicinal and Pharmaceutical Chemistry, Technische Universität Braunschweig, Beethovenstr. 55, 38106 Braunschweig, Germany, email : ingo.ott@tu-bs.de*

Abstract

Gold complexes are considered as novel anticancer agents due to their strong antiproliferative properties, which are related to antimitochondrial activity, thioredoxin reductase (TrxR) inhibition, reactive oxygen species (ROS) induction and other effects on the cellular level. Several types of gold(I) and gold(III) metallodrugs have been investigated, including organometallic complexes, and confirmed the high potential of gold species in anticancer drug development. [1,2]

Recently, we have reported on organometallic gold(I) complexes with alkynyl ligands as promising candidates for further metallodrug design (see the figure for examples). [3-5] Besides strong cytotoxic activity against several cancer cell lines, potent TrxR inhibition, substantial effects against tumor cell metabolism and cell morphology, induction of the phosphorylation of ERK1, ERK2 and HSP27, as well as strong anti-angiogenic effects have been observed. To further exploit the interesting potential of gold alkynyl organometallics as novel anticancer agents, a careful evaluation of structure-activity-relationships (SAR) together with an optimisation of the pharmacokinetic ADME (absorption, distribution, metabolism, excretion) parameters is of high importance. In this presentation our current findings on these topics will be presented.

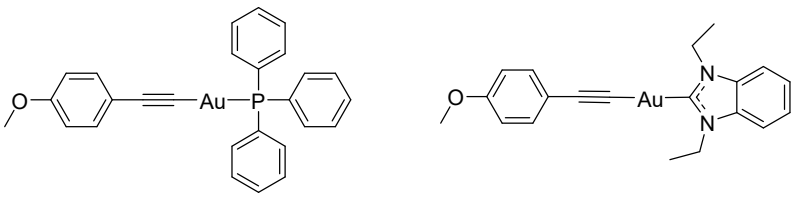


Figure: examples of gold(I) alkynyl complexes

References

1) Ott I., On the medicinal chemistry of gold complexes as anti-cancer drugs. Coord. Chem. Rev. (2009), 253, 1670.

2) Zou T., Lum C. T., Lok C.-N., Zhang J.-J., Che C.-M. Chemical biology of anticancer gold(III) and gold(I) complexes. Chem. Soc. Rev. (2015), 44, 8786.

3) Meyer A., Bagowski C. P., Kokoschka M., Stefanopoulou M., Alborzinia H., Can S., Vlecken D. H., Sheldrick W. S., Wölfl S., Ott I., On the biological properties of alkynyl phosphine gold(I) complexes. Angew. Chem. Int. Ed. (2012), 51, 8895.

4) Andermark V., Göke K., Kokoschka M., Abu el Maaty M. A., Lum C. T., Zou T., Sun R. W.-Y., Aguilo E., Oehninger L., Rodriguez L., Bunjes H., Wölfl S., Che CM, Ott I., Alkynyl gold(I) phosphane complexes: evaluation of structure-activity-relationships for the phosphane ligands, effects on key signaling proteins and preliminary in-vivo studies with a nanoformulated complex. J. Inorg. Biochem. (2016), 160, 140.

5) Vanicek S., Kopacka H., Wurst K., Vergeiner S., Kankowski S., Schur J., Bildstein B. Ott I., Cobaltoceniumethynyl gold(I) as an unusual heterodinuclear bioorganometallic fragment to study the biological properties of alkynylgold complexes. Dalton Trans. (2016), 45, 1345.

Bioconjugated pyrazine-based cyclometalated (C^Npz^C)Au(III) complexes: synthesis and anticancer potential

B. Bertrand,[a] M. Williams,[a] A. Green,[a] J. Fernandez-Cestau,[a] M. Searcey,[b] M. A. O'Connell,[b] M. Bochmann[a]

[a] *School of Chemistry, University of East Anglia, Norwich, NR4 7TJ* *Email: b.bertrand@uea.ac.uk*

[b] *School of Pharmacy, University of East Anglia, Norwich, NR4 7TJ*

Abstract

Gold complexes are widely studied as potential new anti-cancer agents.1 Biscyclometalated Au(III) complexes are particularly known for their stability under physiological conditions and their promising cytotoxic properties.2 We have demonstrated the potential of a pyrazine-based (C^Npz^C)Au(NHC) complex (A figure 1) as anticancer treatment. However, the compound suffered from a certain lack of selectivity for cancer cells.3 We thus explored various strategies to conjugate our known pharmacophore to different biovectors to improve the selectivity of the drug. We have investigated the possibility to attach some amino acid derivatives to the (C^Npz^C)Au(III) scaffold via an open chain carbene ligand. We have also explored the synthesis of a (C^Npz^C)Au(NHC) platform bearing a pentafluorophenyl ester moiety for the conjugation with several vectors including biotin and estradiol derivatives. We measured the cytotoxicity of the complexes against a panel of different human cancer cell lines.

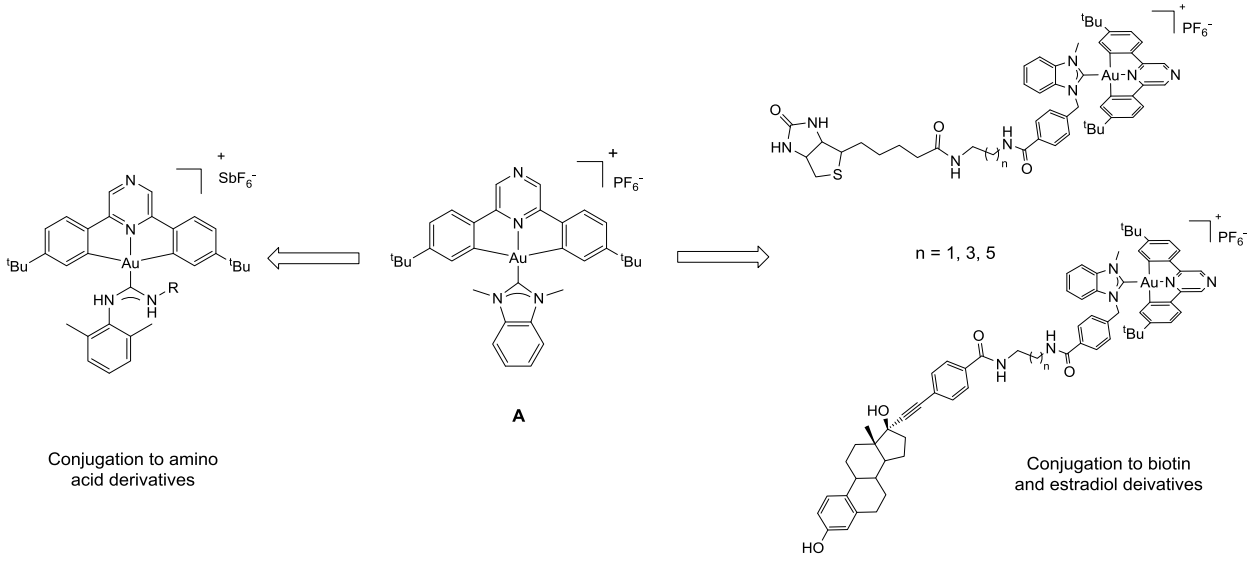


Figure 1: Scheme of the bioconjugated (C^N^{pz}^C)Au(III) carbene complexes.

References

1) Zou, T.; Lum, C. T.; Lok, C.-N.; Zhang,; Che, C.-M. Chemical biology of anticancer gold(III) and gold(I) complexes. Chem. Soc. Rev. 2015, 44, 8786-8801.

2) Bertrand, B.; Casini, A. Dalton Trans. A golden future in medicinal inorganic chemistry: the promise of anticancer gold organometallic compounds. 2014, 43, 4209-4219.

3) Bertrand, B.; Fernandez-Cestau, J.; Angulo, J.; Cominetti, M. M. D.; Waller, Z. A. E.; Searcey, M.; O'Connell, M. A.; Bochmann, M. Inorg.chem. Cytotoxicity of pyrazine-based cyclometalated (C^Npz^C)Au(III) carbene complexes: Impact of the nature of the ancillary ligand on the biological properties. 2017, submitted.

From conception to *in vitro* and *in vivo* evaluations: how a smart probe can be a key tool to develop a novel anti-cancer agent

M. Ali,[a] L. Dondaine,[b] V. Laurens,[b] P. Le Gendre,[a] F. Denat,[a] C. Goze,[a] C. Paul,[b] E. Bodio[a]

[a]*Institut de Chimie Moléculaire de l'Université de Bourgogne, UMR 6302 CNRS, Université de Bourgogne Franche-Comté, 9 avenue A. Savary, BP47870, 21078 Dijon, France,* [b]*EPHE Cancer Immunology Immunotherapy Laboratory; EA7269 EPHE-University of Burgundy; University of Burgundy, Dijon, F-21000*
ewen.bodio@u-bourgogne.fr.

Abstract

Since the pioneer discovery of cisplatin for biological applications by Rosenberg, metal complexes have become the most currently investigated and used class of compounds in cancer chemotherapy. Gold and ruthenium-based derivatives gave very promising results as anticancer agents, although it is still challenging to understand their mechanism of action. To deal with this issue, we have drawn our inspiration from theranostics: we attached a fluorophore on metal-based complexes to be able to track them *in vitro*.

In this study,[1,2] we present several metal complexes involving Au(I), Ru(II), and Os(II) metal ions. A challenging question is whether the metal-phosphine complex is a prodrug that is administered in an inactive precursor form, or rather than the metal remains attached to the phosphine ligand during the treatment. To tackle this issue we choose a phosphine-based smart probe as a ligand, whose strong fluorescence depends on the presence of the gold atom. The *in vitro* biological action of the gold complexes were investigated and studies in healthy zebrafish larvae allowed us to evaluate gold complexes biodistribution and toxicity. The different analyses carried out showed that these gold complexes behaved differently from phosphonium and auranofin, both *in vitro* and *in vivo*.

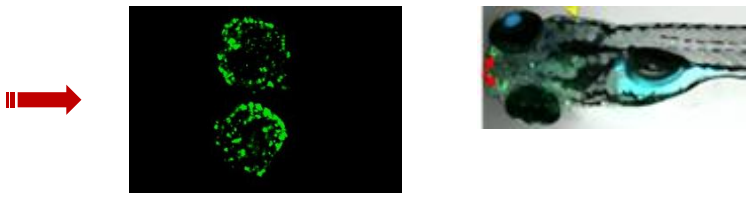


Figure 1: investigated compounds & two-photon microscopy images (MDA, 50 µM, λ_{ex} = 750 nm).

Two-photon microscopy experiments demonstrated that the cellular targets of these gold complexes are not the same as those of the usually reported gold complexes (Figure 1). To finish, a preliminary *in vivo* study on healthy mice and on tumor bearing mice will be presented to highlight the potential of one of the complex as a novel anticancer agent.

References

1. Ali, M., Dondaine, L., Adolle, A., Sampaio, C., Chotard, F., Richard, P., Denat, F., Bettaieb, A., Le Gendre, P., Laurens, V., Goze, C., Paul, C.,* Bodio, E.*, Anticancer Agents: Does a Phosphonium Behave Like a Gold(I) Phosphine Complex? Let a "Smart" Probe Answer! *J. Med. Chem.* **2015**, *58*, 4521-4528.
2. Dondaine, L., Escudero, D., Ali, M., Richard, P., Denat, F., Bettaieb, A., Le Gendre, P., Paul, C., Jacquemin, D.,* Goze, C.,* Bodio, E.*, Coumarin-phosphine-based smart probes for tracking biological relevant metal complexes: from theoretical to biological investigations, *Eur. J. Inorg. Chem.,* **2016**, 545-553.

Structure and function of mammalian Aldehyde Oxidases and their role in the metabolism of drugs and xenobiotics

Maria João Romão[a]

[a] *UCIBIO – Applied Molecular Biosciences Research Unit – Faculty for Sciences and Engineering, NOVA University of Lisbon, 2829-516 Caparica, Portugal*

email : mjr@fct.unl.pt;

Abstract

Mammalian aldehyde oxidases (AOX) belong to the xanthine oxidase (XO) family of Mo-containing enzymes. These enzymes contain Mo bound to an organic molybdopterin cofactor (Moco) with two spectroscopically distinct [2Fe-2S] centres and an FAD site involved in electron transfer [1]. Reactivity appears to be conferred solely by the Mo centre, while specificity is controlled by the amino acid residues in the substrate binding pocket.

Aldehyde Oxidase (AOX) has great toxicological importance since, along with cytochrome P450, it metabolizes different classes of drugs and xenobiotics being an enzyme of emerging significance in phase-I drug metabolism and pharmacokinetics [2]. The crystallization and structure determination of human AOX1 [3] and mouse AOX3 [4] have brought new insights into the structure and the mechanisms underlying substrate/inhibitor binding as well as the catalytic activity of this class of enzymes. Of special relevance was the identification of a novel inhibition site that is common to XO and AOX.

AOX and XO show a high degree of structural similarity but a single XO enzyme is known while several AOX enzymes are present in different animal species. This is the case in rodents, which possess four different AOX isoenzymes while humans express only one (hAOX1) [2]. The reason for this is still unknown and the fact that the different mouse isoforms metabolize different substrates and are localized in a tissue specific manner is a puzzling question and has led to failures in drug development projects.

To clarify the hAOX enzymatic and inhibition mechanisms, several variants were prepared and, kinetic studies in combination with computational and structural studies, allowed identifying the structural determinants for the specific of hAOX1 [5].

The ensemble of the published and novel crystal structures has provided important structural insights into the catalytic and inhibition mechanisms of AOX1 that will be presented in the talk.

References

1) Romão, MJ, *Dalton Transactions*, 2009, 4053-4068
2) Terao, M et al, *Archives of Toxicology*, 2016, 90:4, 753-780
3) Coelho, C et al. *Nature Chemical Biology*, 2015, 11, 779–783
4) Coelho, C et al. *J Biol Chem*, 2012, 287:40690-40702
5) Romao MJ et al, *Current Opinion in Chemical Biology*, 2017, 37, 39-47

Fancy a lift? Glucose and vitamin B_{12} as carriers for the targeted gold-based anticancer chemotherapy

A. Pettenuzzo,[a] E. Fotopoulou,[a] A. Terenzi,[b] I. Ott,[c] L. Ronconi[a]

[a]*School of Chemistry, National University of Ireland Galway, Galway (Ireland);* [b]*Institute of Inorganic Chemistry, University of Vienna, Vienna (Austria);* [c]*Institute of Medicinal and Pharmaceutical Chemistry, Technische Universität Braunschweig, Braunschweig (Germany); email: luca.ronconi@nuigalway.ie*

Abstract

Some gold(III)-dithiocarbamato complexes have shown promising antitumor activity, both *in vitro* and *in vivo*, together with negligible systemic and organ toxicity, although selective tumor targeting is still a major issue.[1]

In order to maximize the impact on cancer cells and minimize side-effects, our latest approaches focus on the conjugation of biologically-active molecules to potential metallodrugs. In particular, we aim at designing "Trojan Horse"-type complexes characterized by an improved selectivity provided by selected coordinated ligands exploiting specific receptors up-regulated in cancer cells, so as to achieve biomolecular recognition and tumor targeting without affecting healthy tissues.

Targeted chemotherapies offer a great potential advantage owing to the expected enhanced specificity of the pharmacological treatment, which would lead to the delivery of higher doses of drugs directly into the target site. This requires the identification of the relevant biomarkers that are over-expressed specifically by diseased cells but not in healthy tissues, so as to achieve such selective targeted delivery.

Rapidly dividing tumor cells require higher amounts of nutrients (*e.g.* methionine, essential to the synthesis of proteins and polyamines) and energy (*e.g.* through the TCA cycle and ATP) for their abnormal growing rate. In this context, it is not surprising that fast-proliferating cancer cells show increased demand and accumulation of both vitamin B_{12} (a vital nutrient characterized by very low bioavailability but involved in hundreds of critical metabolic enzymatic reactions)[2] and glucose (the so-called "Warburg effect")[3] in comparison with healthy ones, thus making them very attractive for specific targeting of tumors. Accordingly, tailored vitamin B_{12} or glucose-like substrates can be conjugated to metal-containing anticancer agents so as to attain the site-specific delivery of drugs into the affected tissues.

Starting from the rationale behind our research work, the main results achieved to date concerning the development of the aforementioned gold-based bioconjugates for the targeted chemotherapy are here illustrated and discussed

Acknowledgements

Financial support by NUI Galway (*College of Science Scholarship* to AP; *Hardiman Research Scholarship* to EF; *Millennium Fund Minor Project 2013* to LR), Irish Research Council (*Postgraduate Scholarship* to AP), and COST Action CM1105 (*STSM Grant*) is gratefully acknowledged.

References

1) E.M. Nagy, L. Ronconi, C. Nardon, D. Fregona, *Mini-Rev. Med. Chem.* **2012**, *12*, 1216
2) A. Pettenuzzo, R. Pigot, L. Ronconi, *Eur. J. Inorg. Chem.*, doi:10.1002/ejic.201601217
3) A. Pettenuzzo, R. Pigot, L. Ronconi *Metallodrugs* **2015**, *1*, 36

Passive and Active Targeting of Kiteplatin to Bone Tumors and Metastases.

Nicola Margiotta,[a] Alessandra Curci,[a] Valentina Gandin,[b] Cristina Marzano,[b] James D. Hoeschele,[c] Giovanni Natile,[a]

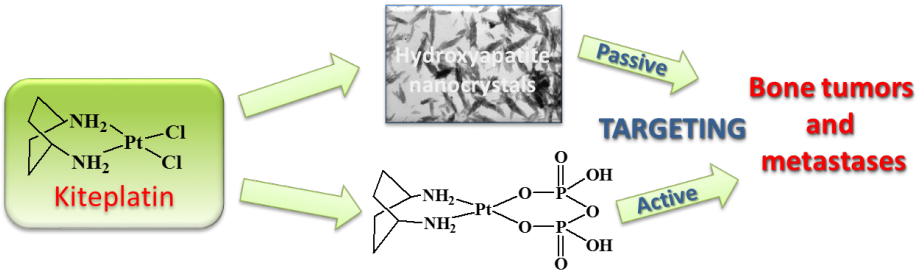
[a)]Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Bari (Italy); [b)]Dipartimento di Scienze del Farmaco, Università di Padova, Padova (Italy) ; [c)]Department of Chemistry, Eastern Michigan University, Ypsilanti, Michigan, (United States). Email: nicola.margiotta@uniba.it

Abstract

Platinum-based anticancer agents, such as cisplatin and oxaliplatin, have poor pharmacokinetic profile and their un-specific distribution in the body leads to systemic toxicity. Therefore, the development of antitumor platinum complexes with ligands specific to target the tumor site is highly desirable and currently actively pursued. In addition, drugs can be preferentially delivered to the tumor site by a nanoparticle formulation that can take advantage of the leaky vasculature surrounding the malignant tissue (enhanced permeability and retention effect).

We have been involved, in the last decade, in the preparation of bone-targeted platinum-bisphosphonate anticancer drugs and their subsequent conjugation with inorganic silica xerogels or hydroxyapatite (HA) nanocrystals with the aim of using these matrices for the local treatment of bone tumors.[1] In the present study, we have investigated the adsorption on and the release from biomimetic HA nanocrystals of kiteplatin [$PtCl_2$(*cis*-1,4-DACH)] (DACH = diaminocyclohexane) and of its 1,1-cyclobutanedicarboxylate derivative [Pt(CBDCA)(*cis*-1,4-DACH)]. The release has been investigated as a function of pH to mimic the different physiological environments of healthy (including blood) and tumor tissues and the *in vitro* cytotoxicity of the releasates from the HA matrices has been assessed against various human cancer cell lines.[2]

Moreover, active targeting of kiteplatin towards bone tumors has been pursued by preparing two new pyrophosphate derivatives that can be activated at acidic pH and hence at the hypoxic and low-pH environment surrounding a tumor mass. The two pyrophosphate derivatives of kiteplatin have also been tested *in vitro* to assess their cytotoxicity against a panel of human tumor cell lines.



Acknowledgments

We acknowledge the University of Bari (Italy), the Italian Ministero dell'Università e della Ricerca, the Inter-University Consortium for Research on the Chemistry of Metal Ions in Biological Systems (C.I.R.C.M.S.B.) for support.

References

[1] a) Iafisco, M.; Margiotta, N. *J. Inorg. Biochem*. **2012**, *117*, 237–247. b) K. Farbod, K. Sariibrahimoglu, A. Curci, A. Hayrapetyan, J.N.W. Hakvoort, J.J.J.P. van den Beucken; M. Iafisco, N. Margiotta, S.C.G. Leeuwenburgh. *Tissue Eng. - Part A* **2016**, *22*, 788-800.

[2] Lelli, M.; Roveri, N.; Marzano, C.; Hoeschele, J. D.; Curci, A.; Margiotta, N.; Gandin, V.; Natile, G. *Dalton Trans.* **2016**, *45*, 13187–13195.

Oxindole ligands as promising therapeutic agents in Alzheimer disease

Camila A. Wegermann,[a] Mauricio T. Oshino, [a]Valentina Pirota,[b] Enrico Monzani,[b] Luigi Casella,[b] Luiz Antônio S. Costa,[c]Ana M. Da Costa Ferreira, [a]

[a] *Instituto de Química, Universidade de São Paulo, São Paulo, 05508-000, SP, Brazil;* [b] *Università di Pavia, Pavia, Italia;* [c]*Universidade Federal de Juiz de Fora, MG, Brazil.*

email : amdcferr@iq.usp.br

Abstract

Oxindole derivatives have been described as promising scaffold for new drugs in the last years. Diverse compounds in this class have already entered clinical tests (phase II and III), especially as potential antitumor agents.[1] Among them, isatin (1*H*-indole-2,3-dione) derivatives exhibit diverse pharmacological activities, as anxiogenic, sedative, anticonvulsant, antibacterial, antifungal, antidiabetic, antiplasmodial, antiviral, and anticancer properties.[2] Particularly, oxindolimines were reported as efficient chelates for copper(II) and zinc(II) ions, forming very stable complexes, capable of entering the cells and targeting DNA and mitochondria. They can cause oxidative damage through the generation of reactive oxygen species (EROs) and induce apoptosis.[3] These complexes can also bind to other biomolecules, including vital proteins responsible for cell cycle as human topoisomerase IB[4] or cyclin-dependent kinases (CDK1/cyclin B and CDK2/cyclin A),[5] and act as efficient inhibitors of their activity. In all these interactions, the nature of the metal, as well as the ligand structure modulate the observed effects in its biological activity.

Recently, we started to investigate possible effects of such ligands toward amyloid peptides, trying to influence their aggregation and subsequent fibril formation, usually associated to Alzheimer disease (AD).[6] Since this sequence of facts seems to be facilitated or enhanced by metal ions as copper or zinc, efficient chelates could have a remarkable inhibition effect. Therefore, hydrazone and imine derivatives from isatin containing additional imidazole or pyridine groups were prepared (*isahim*, *isahpy, isapn*), and characterized by IR and NMR spectroscopy, and ESI-MS spectrometry. Among diverse oxindole derivatives, these were the most active. It was also verified that those ligands bind strongly to amyloid peptides, especially to $A\beta_{1\text{-}16}$, with binding constants in the range 10^9. Interactions occur mostly at His6, His13 and His14 residues. Molecular dynamics simulation studies corroborated the experimental data, indicating efficient interactions between these hydrazones or imines and the peptides. Additionally, these oxindole ligands also compete efficiently for metal ions, forming very stable species, with stability constants 10^4 – 10^5 mol^{-1} L, in the case of copper ions, as determined by UV/Vis spectroscopy. Both effects can explain the observed inhibition of Aβ peptides aggregation, monitored by turbidity assays. The hydrazones exhibit E/Z isomerization, depending on the pH and temperature, and one of the isomers seems to be preferential in interacting with Aβ.These results indicate that oxindole derivatives could be efficient therapeutic agents in AD.

References

1. N. M. Evdokimov, I. V. Magedov, D. McBrayer, A. Kornienko,*Bioorg. Med. Chem. Lett*. 2016, 26, 1558–1560.
2. G. Cerchiaro, A. M. Da Costa Ferreira, *J. Braz. Chem. Soc*. 2006, 17, 1473.
3. G. Filomeni, G. Cerchiaro, et al., *J. Biol. Chem*. 2007, 282, 12010-12021.
4. P. Katkar, S. Castelli, et al., *Metallomics* 2014, 6,117-125.
5. R. B. Miguel, P. D. Petersen, et al., *J. Biol. Inorg. Chem*. 2015, 20, 1205-1217.
6. M. Rowinska-Zyrek, M. Salerno, H. Kozlowski, *Coord. Chem. Rev.*, 2015, 284, 298-312.

Acknowledgments:FAPESP, CNPq, CAPES, NAP-USP, CEPID Redoxoma

Metals in environment and toxicology

Thermodynamics of Pb(II) and Zn(II) binding to MT-3, implications for MT3 structure and function

Rachel Narehood Austin*, [a,c] Dean Wilcox [b], Molly Carpenter [b], Roch de Silva [c], Adnan Shami Shah [c], Michael Stevenson [b], Angela Su [a], Sohee Ki [a], Mary Sever [a], Jamie Bousleiman [a], Alexa Pinsky[a], Irina Morozova[d], Sergey Kalachikov[d], Amen Wiqas[e], Rae Silver[f]

[a] *Barnard College, Columbia University ;* [a,b] *Dartmouth College* [c] *Bates College;* [d] Center for Genome Technology and Biomolecular Engineering, Department of Chemical Engineering; [e] *Department of Biology Barnard College Columbia University ;* [f]*Department of Psychology Columbia University ; *email : rna2113@columbia.edu*

Abstract

The neurochemistry of metallothionein-3 (MT3) remains poorly understood. It is primarily expressed in the central nervous system. We provide evidence that its expression in regulated differently than other metallothioneins. We hypothesize that lead is thermodynamically capable of binding to MT3 and that this interaction might mediate the chemistry of lead *in vivo*. Results from isothermal titration calorimetry (ITC) experiments support the hypothesis that lead binds tightly to MT3. They also show that lead binding to MT3 is thermodynamically preferred over zinc binding to MT3. Triphasic behavior of both lead and zinc binding to MT3 is observed. Efforts to rationalize this behavior and explore its effects on structure are discussed.

Silver subcellular distribution and impact on critical hepatocyte functions consecutive to exposure to non toxic concentrations of silver nanoparticles

Giulia Veronesi,[a,b] Vanessa Tardillo-Suarez,[b] Benoit Gallet,[c] Mireille Chevallet,[a] Peggy Charbonnier,[a] Pierre-Henri Jouneau,[d] Elisabeth Mintz,[a] Isabelle Michaud-Soret,[a] and Aurélien Deniaud,[a]

[a] CEA - CNRS - Univ. Grenoble Alpes, Laboratoire de Chimie et Biologie des Métaux (LCBM) UMR 5249, 17 Rue des Martyrs F-38054 Grenoble, France ; [b] ESRF, The European Synchrotron. 71, Avenue des Martyrs, 38043 Grenoble, France ; [c] CEA – CNRS – Univ. Grenoble Alpes – IBS, 71, Avenue des Martyrs, 38043 Grenoble, France; [d] INAC-SP2M, CEA - Université Grenoble Alpes, 17 Rue des Martyrs F-38054 Grenoble, France ; email : aurelien.deniaud@cea.fr

Abstract

The widespread use of silver nanoparticles (AgNP) in consumer goods raises concerns about their toxicity to humans and their impact on environment[1]. AgNP toxicity in cells and animals has been extensively studied and is at least due to oxidative stress and metal homeostasis disruption. These two processes are strongly interconnected in cells. Furthermore, in animals it has been shown that Ag accumulates in liver following AgNP exposure[2]. At the molecular level, it has been demonstrated that the toxicity depends upon the release of Ag(I) ions from the NP[3,4]. However, the molecular processes enabling AgNP dissolution, the subcellular distribution of Ag(I) species, as well as their impact on hepatocyte metal homeostasis and the related functions remain poorly understood.

In this context, we have studied citrate- and PVP-coated AgNP internalization and fate into the hepatocyte HepG2 cell line as well as the cellular response induced by AgNP exposure. We used a synchrotron nanoprobe to visualize the subcellular distribution of silver. The combined use of X-ray fluorescence (XRF) microscopy on whole cells and electron microscopy allowed the discrimination between the nanoparticulate form located inside endosomes and lysosomes and the ionic species that distribute throughout the cell[5]. Furthermore, the development of a novel correlative electron microscopy – XRF method performed on the same cell section enabled the localization of the subcellular distribution of Ag(I) species under long-term AgNP exposure to non toxic concentration. We thus observed Ag(I) species in the nucleus and we identified crucial nuclear functions altered by AgNP exposure. Using X-ray absorption spectroscopy, we previously showed that Ag(I) recombines with sulphur *in cellulo* in the form of AgS_2 and AgS_3 complexes[5,6]. Altogether, these data are now prompting us towards the identification of the molecular entities affected by Ag(I) in the nucleus.

References

1) S. Chernousova, and M. Epple, *Angewandte Chemie,* 2013, **52**, 1636-1653.
2) M. Van der Zande, et al. *ACS Nano,* 2012, **6**, 7427-7442.
3) F. Herzog, et al. *Particle and Fibre Toxicology,* 2013, **10**, 1-14.
4) V. De Matteis, et al. *Nanomedecine: Nanotechnology, Biol and Med,* 2015, **11**, 731-39.
5) Veronesi, G.*, Deniaud A.* et al., *Nanoscale,* 2016, **8**, 17012-21.
6) Veronesi, G. et al., *Inorg Chem,* 2015, **54**, 11688-96.

A biomimetic approach to rationalize uranyl binding to biological targets

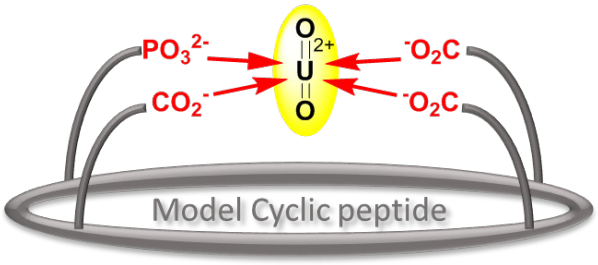
Pascale Delangle,

[a] *INAC/SyMMES, UMR 5819, CEA – CNRS - Université Grenoble Alpes, CIBEST laboratory, 38000 Grenoble (France); email : pascale.delangle@cea.fr*

Abstract

Uranium is a natural element widely found in the environment, due to both natural occurrence in mineral ores or in sea water and industrial applications. The production of nuclear energy uses enriched uranium in ^{235}U for nuclear fission. Despite its ubiquitous distribution, uranium has no essential role in living organisms and presents radiological and chemical toxicities. Despite significant recent advances in the field, there is still a serious lack of knowledge about the molecular interactions responsible for uranium toxicity. The underlying mechanisms need to be unravelled to predict the effect of uranium on living organisms and also to help in designing efficient detoxification agents, to be used in case of dirty bombs or accidental release of uranium in the environment.

At the CIBEST lab, we propose a biomimetic approach to help understanding the interactions of uranium with biological molecules, relevant to uranium toxicity. The most stable uranium ion *in vivo* is the uranyl cation, UO_2^{2+}, which prefers four to six oxygen donors in its equatorial plane, perpendicular to O-U-O bonds. We use short peptide sequences, as simple models of metal-binding sites in proteins to get structural and thermodynamic data, which are not accessible directly with large biological molecules. Cyclic constrained peptide scaffolds that orient metal-coordinating groups found in proteins toward the equatorial plan of the uranyl cation revealed to be highly effective uranyl-binding peptides.[1]



A combination of synthesis, analytical chemistry, X-Absorption Spectroscopy and theoretical calculations allowed us to correlate the amino acid sequence to the stability of the uranyl complexes. Importantly, phosphate groups significantly enhance the affinity of the biomimetic peptides for uranyl, which strongly supports the importance of phosphoamino acids in uranyl binding in proteins. Therefore, our biomimetic approach reinforces the relevance of considering phosphoproteins such as osteopontin as potential uranyl targets in vivo.[2,3]

This work was supported by the toxicology and NRBC programs of CEA.

References

1) C. Lebrun, M. Starck, V. Gathu, Y. Chenavier, P. Delangle, Chem. Eur. J., **2014**, 20, 16566 – 16573
2) M. Starck, N. Sisommay, F. A. Laporte, S. Oros, C. Lebrun, P. Delangle, *Inorg. Chem.*, **2015**, 54, 11557−11562
3) M. Starck, F. A. Laporte, S. Oros, N. Sisommay, V. Gathu, P. L. Solari, G. Creff, J. Roques, C. Den Auwer, C. Lebrun, P. Delangle, *Chem. Eur. J.*, **2017**, DOI: 10.1002/chem.201605481

Advances in mass spectrometry for trace level biological metals speciation analysis

Ryszard Lobinski, Laurent Ouerdane, Joanna Szpunar

CNRS/University of Pau, Institute of Analytical Sciences and Physical Chemistry for the Environment and Materials, IPREM-UMR 5254, 2, av. Pr. Angot, 64053 Pau, FRANCE. email : ryszard.lobinski@@univ-pau.fr

Abstract

Metal species play an essential role in maintaining the structure and the enzymatic activity of biological cells. However, an excess of certain metals can lead to severe toxicity because of metal substitutions in enzymes and oxidative damage. As a consequence, it is crucial to determine the nature of metal species to better comprehend their fate and their role in organisms.

Generally associated with organic molecules, metals are usually present in low concentrations (nM to lM ranges) and occur distributed in a plethora of chemical forms with various properties (covalent or noncovalent species and a wide range of molecular weights). These species are often unstable (labile and prone to oxidation), which makes the analysis cumbersome.

Whereas covalent species can be approached by canonical metabolomic procedures, the identification of noncovalent metal complexes at basal concentrations remains a challenge. X-ray absorption spectroscopy (XAS) and micro-X-ray fluorescence (µXRF) techniques are of particular interest to obtain information on the valence, coordination environment and spatial localization of elements within intact tissues but they have a number of limitations. These include low sensitivity rendering the analysis of biological fluids at basal concentrations virtually impossible, and the critical dependence on a priori knowledge of the nature of the metal complexes present because their signals have to be compared with appropriate standards. *De novo* identification of previously unreported species and detection of minor species are therefore difficult to achieve.

An alternative is liquid chromatography with mass spectrometric (MS) detection which has been developed largely for metabolomics during the last decade. However, successful applications to metal-speciation in biological samples in-vivo have been rare and limited mostly to single elements with few ligands at relatively high concentrations. The reasons are the difficult-to control dissociation of metal complexes during sample preparation and during their exposure to the column stationary phase and electrospray source.

The lecture discusses the recent advances in the development of coupling of chromatographic techniques with dual: elemental (ICP MS) et molecular (electrospray) mass spectrometric detection[1,2], highlighting their advantages and limitations.

References

1) Mounicou, S., Szpunar, J., Lobinski, R., Metallomics: The concept and methodology (2009) Chemical Society Reviews, 38 (4), pp. 1119-1138.
2) Flis, P., Ouerdane, L., Grillet, L., Curie, C., Mari, S., Lobinski, R., Inventory of metal complexes circulating in plant fluids: a reliable method based on HPLC coupled with dual elemental and high-resolution molecular mass spectrometric detection, (2016) New Phytologist, 211 (3), pp. 1129-1141.

A critical look at the merits of metalloproteomic approaches

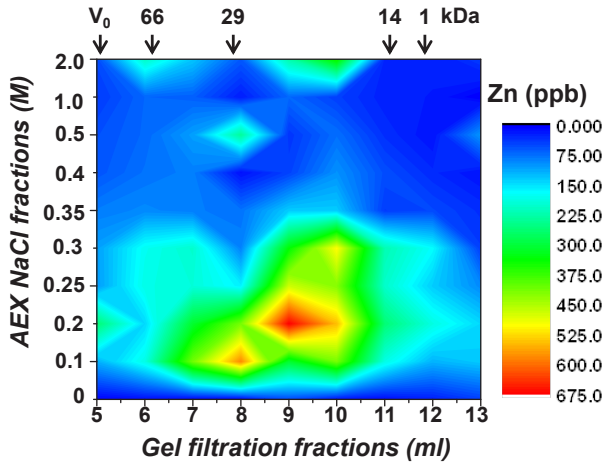
Claudia A. Blindauer

Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

email : c.blindauer@warwick.ac.uk

Abstract

The post-genomic era has seen a proliferation of "-omics" approaches such as transcriptomics and metabolomics, all concerned with the large-scale, high-throughput analysis of a set of (usually) biological molecules or species. The term "metallome" was suggested by the late RJP Williams in 2001, in an article on the chemical selection of elements by cells, where it was defined as the distribution of (metallic) elements in a biological system.[1] An important aspect of this distribution concerns speciation, and it is the latter that is a major focus of Metallomics and its sub-discipline of Metalloproteomics. Technological advances in both inorganic and molecular mass spectrometry were fundamental in developing this new field;[2] nonetheless, it can be argued that now the major challenges in metalloproteomics lie within separation science. To a large extent, limitations in what is currently experimentally possible are owed to the non-covalent nature of interactions between proteins and many metals of interest, amongst them the ubiquitous zinc.



This lecture will give a brief overview of approaches and challenges in metalloproteomics, drawing on work carried out by our team[3] as well as by other groups.

References

1) R.J.P. Williams, Chemical selection of elements by cells. Coord. Chem. Rev. 2001, 216, 583-595.
2) S. Mounicou, J. Szpunar, R. Lobinski, Inductively-coupled plasma mass spectrometry in proteomics, metabolomics and metallomics studies. Eur. J. Mass Spectrom. 2010, 16, 243-253.
3) J. P. Barnett, D.J. Scanlan, C.A. Blindauer, Identification of major zinc-binding proteins from a marine cyanobacterium: Insight into metal uptake in oligotrophic environments. Metallomics 2014, 6, 1254-1268.

Synergistic effects observed in the As^{III}-binding of small ligands possessing multiple thiol groups

L. Szekeres,[a] B. Gyurcsik,[a] T. Kiss,[a,b] S.V. Hoffmann,[c] N.C. Jones,[c] A. Jancsó[a]

[a] *Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary ;* [b] *MTA-SZTE Bioinorganic Chemistry Research Group, Hungary ;* [c] *ISA, Department of Physics and Astronomy, Aarhus University, Denmark ; email : jancso@chem.u-szeged.hu*

Abstract

Arsenic is known to exert severe biological effects on cells and tissues depending on the level and duration of exposure. A few examples for the adverse effects are skin lesions, cardiovascular diseases, gastrointestinal toxicity or the development of various types of cancer.[1] As an interesting contrast arsenic and its derivatives have been applied since the ancient times as therapeutic agents e.g. in the treatment of ulcers, plague or malaria.[2] Most significantly arsenic trioxide (As_2O_3) has been shown to possess important antitumor properties against human acute promyelocytic leukemia (APL).[2] It was suggested that similar mechanisms may operate both in the therapeutic and toxic activities of arsenic,[2] nevertheless, the molecular details of these mechanisms are yet to be explored.

It is well known, that arsenic in its trivalent oxidation state can bind to reduced thiols, especially when more thiol groups are available, and this plays a role in many of its physiological effects.[1,2] Nevertheless, the solution speciation of As^{III} with small ligands bearing multiple thiol groups has been rarely investigated even though understanding the As^{III}-thiol interaction at the level of small models could be essential in unravelling the versatile, sometimes paradoxical biological effects.

With an aim of exploring the possible binding site and coordination environment preferences of As^{III} in biological milieu we have investigated the interactions of arsenous acid (H_3AsO_3 - the typical aqueous form of As^{III}) with several model ligands. Our studies focussed on the antidote 2,3-dimercaptopropanol (**BAL**), a hexapeptide with two Cys residues (Ac-DCSSCY-NH_2 - **DY**) and the As^{III}-binding fragment of an arsenic sensing protein ArsR from *A. ferrooxidans* (Ac-NCCHGTRDCA-NH_2). All of these models bound to H_3AsO_3 efficiently by multiple As-S bonds. Interestingly, bis-ligand species also formed in a significant amount in the As^{III}-**BAL** and As^{III}-**DY** systems by mono-, or bidentate binding mode of the second ligand resulting in a unique {4S} coordination environment for As^{III}. Experiments were also performed with monothiols (e.g. cysteine and glutathione) and in the H_3AsO_3-**BAL**-monothiol ternary systems. The results revealed that the binding of the dithiol to As^{III} could significantly promote the formation of further As^{III}-S bond(s) in spite of the relatively weak affinity of monothiols to H_3AsO_3. The above findings may have significant relevance e.g. in the mechanisms of As^{III}-transfer between biological targets, in the role of As^{III} in modulating the cellular redox status[1,2] or in the explanation of why dithiols increase arsenic toxicity[3] when applied in low concentrations.

References

1) L.C. Platanias, J. Biol. Chem., 2009, 284, 18583-18587.
2) W.H. Miller, Jr., H.M. Schipper, J.S. Lee, J. Singer, S. Waxman, Cancer Res., 2002, 62, 3893-3903.
3) K.-Y. Jan, T.-C. Wang, B. Ramanathan, J.-R. Gurr, Toxicol. Sci. 2006, 90, 432-439.

Acknowledgements: Hungarian National Research, Development and Innovation Office (K_16/120130 and GINOP-2.3.2-15-2016-00038) and COST (CA15126 MOBIEU)

Magnetotactic bacteria and their application

David PIGNOL *et al.*

david.pignol@cea.fr

UMR7265, CEA Cadarache, 13115 Saint Paul lez Durance

Magnetotactic bacteria represent a group of polyphyletic aquatic microorganisms able to synthesize a specialized organelle called the magnetosome. The magnetosome consists of a magnetic nanocrystal of iron oxide (magnetite) or iron sulfide (greigite), surrounded by a biological membrane. The magnetosomes are aligned in chains inside the cytoplasm of the bacteria, forming a true compass needle allowing the cell to passively orient and actively swim in the water column along the earth's magnetic field lines. The synthesis of magnetosomes is genetically controlled by a set of genes common to different species and involved into several processes: invagination of the cytoplasmic membrane to form a nanoreactor, massive iron import and control of its redox state, followed by controlled biomineralization of nano-magnets and finally alignment of the magnetosomes in the cell. Our laboratory develops fundamental approaches to increase our knowledge of the biodiversity of these microorganisms (1) and to identify and characterize the genes (2) and the proteins (3) involved in the molecular mechanisms leading to the synthesis of the magnetosome. This fundamental knowledges feed applied developments that seek to exploit the tremendous potential of these microorganisms in the field of environmental biotechnology and health technologies (4).

References

(1) Lefèvre C.T et al (2011) ***Science***.
(2) Lefèvre C.T. et al. (2013) ***Env. Microb***.
(3) Siponen et al. (2013) ***Nature***.
(4) Boucher et al. (2017) ***Biomaterials***.

Metal partitioning at biointerfaces: going beyond the thermodynamic paradigm for evaluation of biouptake and toxicity

Jérôme F.L. Duval, [a,b]

[a] 1 CNRS, LIEC (Laboratoire Interdisciplinaire des Environnements Continentaux), UMR7360, Vandoeuvre-lès-Nancy F-54501, France. [b] Université de Lorraine, LIEC, UMR7360, Vandoeuvre-lès-Nancy, F-54501, France. email : : jerome.duval@univ-lorraine

Abstract

A mechanistic understanding of the processes governing metal (M) toxicity to- and uptake bymicroorganisms (*e.g.* bacteria, algae) calls for an adequate formulation of metal partitioning at biointerfaces during cell exposure to M.1-6 This includes, in particular, the joint account of metal transport dynamics from bulk solution to biomembrane, the kinetics of metal internalisation, and the dynamics of metal speciation in the vicinity of the active cell surface, which speciation is governed by *e.g.* M complexation by ligands of molecular to nanoparticulate or colloidal size. Popular thermodynamic models such as the Biotic Ligand Model (BLM) and the Free Ion Activity Model (FIAM), while useful for evaluating M uptake and speciation in specific scenarii, do not integrate the complex and generic coupling between the various timescales involved in the problem. In this presentation we shall discuss some basics of (i) metal uptake dynamics by biointerfaces in the absence of significant M complexation in bulk solution, (ii) metal speciation dynamics in suspensions of (nano)particulate ligands,7,8 and (iii) lability of metal complexes in the vicinity of M consuming (bio)interfaces.

References

[1] Duval JFL. *Dynamics of metal uptake by charged biointerphases: bioavailability and bulk depletion.* Physical Chemistry Chemical Physics 15 (2013) 7873.
[2] Duval JFL, Rotureau E. *Dynamics of metal uptake by charged soft biointerphases: impacts of depletion, internalisation, adsorption and excretion.* Physical Chemistry Chemical Physics 16 (2014) 7401.
[3] Rotureau E, Billard P, Duval JFL. *Evaluation of metal biouptake from the analysis of bulk metal depletion kinetics at various cell concentrations: theory and application.* Environmental Science & Technology 49 (2015) 990.
[4] Duval JFL, Paquet N, Lavoie M, Fortin C. *Dynamics of metal partitioning at the cell-solution interface: Implications for toxicity assessment under growth inhibition conditions*. Environmental Science & Technology 49 (2015) 6625.
[5] Duval JFL., Present R. and Rotureau E. *Kinetic and thermodynamic determinants of trace metal partitioning at biointerphases: the role of intracellular speciation dynamics.* Physical Chemistry Chemical Physics 18 (2016) 30415.
[6] Duval JFL. *Coupled metal partitioning dynamics and toxicodynamics at biointerfaces: a theory beyond the biotic ligand model framework*. Physical Chemistry Chemical Physics 18 (2016) 9453.
[7] Van Leeuwen HP, Buffle J, Duval JFL, Town RM. *Understanding the extraordinary ionic reactivity of aqueous nanoparticles*. Langmuir 29 (2013) 10297.
[8] Town RM, Buffle J, Duval JFL, van Leeuwen HP. *Chemodynamics of soft charged nanoparticles in aquatic media: fundamental concepts.* Journal of Physical Chemistry A 117 (2013) 7643.

New methods around metals in biology

Nanosecond metal site dynamics – observed by PAC spectroscopy

L. Hemmingsen[6], S. Chakraborty[1], D.P. Giedroc[2], S. Pallada[3,6], V.L. Pecoraro[4], M. Stachura[5], [6]D. Szunyogh, A.G. Tebo[4]

[1] *Department of Chemistry and Biochemistry, University of Mississippi, MS 38677, USA.*
[2] *Department of Chemistry, Indiana University, Bloomington, IN 47405-7102 USA*
[3] *CERN, 385, Route de Meyrin, Switzerland*
[4] *Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109-1055, USA*
[5] *TRIUMF, 4004 Wesbrook Mall, Vancouver BC, V6T 2A3, Canada.*
[6] *University of Copenhagen, Department of Chemistry, Universitetsparken 5, 2100 København Ø, Denmark, lhe@chem.ku.dk*

Abstract

Dynamics and ligand exchange processes at biomolecular metal sites are essential to enzyme function, transport of metal ions across cell membranes, transfer of metal ions between proteins and in regulatory metallobiochemistry. Many biologically relevant metal ions display nanosecond water exchange dynamics in aqueous solution [1], and it seems plausible that this is also the case at biomolecular binding sites. Perturbed angular correlation (PAC) of γ-rays spectroscopy allows for determination of nanosecond metal site dynamics [2]. Selected examples will be presented including water exchange dynamics at a Cd^{2+} binding site in a *de novo* designed protein, and the effect on the exchange rates by a remote amino acid substitution [3], and possible cysteine exchange dynamics at the Cd^{2+} binding site of CadC [4,5].

References

1. Helm, L.; Merbach, A. E.
Inorganic and Bioinorganic Solvent Exchange Mechanisms
Chem. Rev. 2005, 105, 1923−1960
2. Hemmingsen L., Sas K.N., Danielsen E.
Biological applications of perturbed angular correlation of gamma-rays (PAC) spectroscopy
Chem. Rev. 2004, 104, 4027-4062
3. M. Stachura, S. Chakraborty, A. Gottberg, L. Ruckthong, Pecoraro V.L., Hemmingsen L.
Direct observation of nanosecond water exchange dynamics at protein metal sites
J. Am. Chem. Soc., 2017, 139, 79-82.
4. Tebo A.G., Hemmingsen L., Pecoraro V.L.
Variable primary coordination environments of Cd(II) Binding to Three Helix Bundles Provide a Pathway for Rapid Metal exchange
Metallomics, 2015, 7, 1555-1561
5. S. Pallada, D. Szunyogh, D.P. Giedroc, L. Hemmingsen
Nanosecond ligand exchange dynamics at the Cd^{2+} binding site of CadC
Manuscript in preparation

The use of high-resolution laser ablation-inductively coupled plasma-mass spectrometry imaging for biomedical applications

Thibaut Van Acker, [a] Stijn J. M. Van Malderen[a] and Frank Vanhaecke[a]

[a]*Department of Analytical Chemistry, Ghent University, Campus Sterre, Krijgslaan 281-S12, 9000 Ghent, Belgium*
E-mail corresponding author: Frank.Vanhaecke@UGent.be

Abstract

Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) is a solid sampling micro-analytical technique, capable of performing quantitative spatially resolved elemental analysis with µm lateral resolution. The laser ablation process involves the removal of a tiny amount of mass by focusing a high-energy pulsed laser beam onto the solid sample surface.[1] This plume of ablated material is transported away from the ablation site into the ICP via a He carrier gas flow. In the ICP, the particles are vaporized, the molecules atomized and the atoms thus formed subsequently ionized before entering the mass spectrometer. The ions are separated as a function of their mass-to-charge ratio and the ion beam impacting the detector is subsequently converted into a measurable electrical signal. LA-ICP-MS is characterized by low limits of detection, multi-element capabilities, a wide linear dynamic range and minimal sample preparation. During the last decade, bioimaging of endogenous metals and metal-based drugs via LA-ICP-MS has gained interest due to instrumental improvements which enhance the performance of the system. Recently, the aerosol rapid introduction system (ARIS)[2] was developed at Ghent University and commercialized by Teledyne CETAC Technologies Inc. This device connects the HelEx II laser ablation cell to the ICP-MS unit, hereby minimizing the transfer volume and providing fast washout of the sample aerosol, which speeds up the analysis and boosts the sensitivity. In this way, lower detection limits and/or better spatial resolution images can be achieved and this opens up a new range of applications within the bioanalytical field.

In recent work by Van Acker *et al*.,[3] high-resolution LA-ICP-MS Pt-mapping was performed on 5 µm thick kidney tissue sections of *Macaca fascicularis* which were treated with pharmacological doses of cisplatin in order to investigate the adverse nephrotoxic side effects. Prior to analysis, a histopathological study was performed and the regions of interest (ROIs) were highlighted. The use of the ARIS provided sufficient sensitivity to map the ROIs with laser spot sizes down to 1 µm diameter enabling Pt distribution maps on a (sub)-cellular level to be obtained. These images revealed hot spots with very high Pt signal responses for sloughed necrotic tubular epithelial cells. Another recent application deals with the evaluation of *in vitro* receptor affinity and kinetics of hybrid imaging labels. These hybrid imaging labels consist of an antibody or peptide with both a fluorescent and nuclide tag which enables the cross-combination of confocal fluorescence microscopy and high-resolution LA-ICP-MS imaging.

References

1. Russo, R. E., Mao, X., Gonzalez, J. J., Zorba, V. & Yoo, J. Laser ablation in analytical chemistry. *Anal. Chem.* **85,** 6162–6177 (2013).
2. Teledyne CETAC Technologies. Aerosol rapid introduction system (ARIS). (2016). at <http://www.teledynecetac.com/product/laser-ablation/aris>
3. Van Acker, T. *et al.* High-resolution laser ablation-inductively coupled plasma-mass spectrometry imaging of cisplatin-induced nephrotoxic side effects. *Anal. Chim. Acta* **945,** 23–30 (2016).

Additionally, the authors acknowledge Janssen Janssen Pharmaceutica NV, Teledyne CETAC Technologies, Leiden University Medical Centre and the Flemish Research Foundation for their financial and/or logistic support.

Measurement of Oxygen Concentration Dynamics Inside a Single Cell by PLIM

Toshiaki Kamachi and Hidehiro Ito

Department of Life Science and Technology, Tokyo Institute of Technology; tkamachi@bio.titech.ac.jp

Abstract

Optical methods using phosphorescence quenching by oxygen is suitable for sequential monitoring and non-invasive measurement for oxygen concentration (OC) imaging of a single cell. Phosphorescence intensity measurement is widely used in combination with phosphorescent probes, because triplet state of sensitizer is quenched by oxygen. These probes were usually distributed ubiquitously but heterogeneously inside cell. This distribution is a major disadvantage in phosphorescence intensity measurement. We established the visualization system of OC inside a cell using phosphorescence lifetime measurement (PLIM). This system enabled the direct observation of phosphorescence lifetime inside cell under confocal microscope, so we can visualize the OC inside a whole cell and spheroid under a confocal microscope using a phosphorescent dye. This system uses reversible phosphorescence quenching by oxygen, so it can visualize successive OC change from normoxia to anoxia. Time dependent OC change in an insulin-producing cell line MIN6 by the glucose stimulation was successfully visualized. Assessing detail distribution and dynamics of OC inside cells achieved by the presented system will be useful to understand a physiological as well as pathological oxygen metabolism.

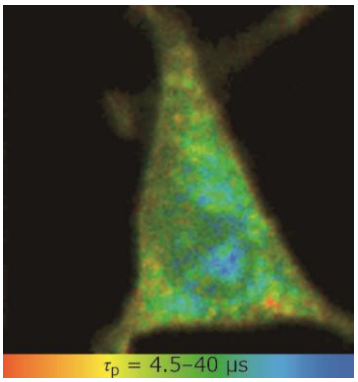


Figure 1 Typical oxygen concentration distribution within a single cell.

References

1) Kurokawa, H.; Kamachi, T. et al., High resolution imaging of intracellular oxygen concentration by phosphorescence lifetime. Sci. Rep. 2015, 5.

Cancer-driven isotopic fractionation

Vincent Balter, [a]

[a] Lyon Lab of Geology. Ecole Normale Supérieure de Lyon. 46, Allée d'Italie. 69342 Lyon Cedex 07 FRANCE; *vincent.balter@ens-lyon.fr*

Abstract

In cancer, copper concentrations increase in the blood and in tumor cells, leading to deleterious side effects. The mechanisms of this copper accumulation and the source of extra copper burden are still poorly understood. In hepatocellular carcinoma (HCC) patients, blood copper is enriched in ^{63}Cu compared to control subjects and this isotopic signature is not compatible with a dietary origin. It rather reflects the massive reallocation in the body of copper immobilized within cysteine-rich proteins such as metallothioneins. I will also show that the blood of HCC patients is enriched in ^{32}S compared to control subjects, an enrichment compatible with the notion that some proportion of blood sulfur originates from tumor-derived sulphides. I will emphasize on the hypoxic conditions of the tumor microenvironment, how they can impair the metabolism of copper and sulfur, notably by changing their redox state and, as a consequence, their ability to bind specific molecules. Finally, isotopic ratios of zinc, carbon and nitrogen, in tumors, in vitro cancer cells and in blood will be discuss.

Beta-detected NMR: Billion-foldincrease in NMR sensitivity

S. Pallada[a],[b],[c], M. Baranowski[d], M. L. Bissell[e], W. Gins[f],L. Hemmingsen[b], F. Hofmann Larsen[b], G. Neyens[f], R. Hardi Thulstrup[b], M. Walczak[h], F. Wienholtz[a], X. F. Yang[a,f], D. Zakoucky[i],M. Kowalska[a]

[a]CERN, Geneva, CH ;[b]University of Copenhagen, Copenhagen, DK ; [c]Democritus University of Thrace, Alexandroupoli, GR ; [d]A Mickiewicz University, Poznan, PL ; [e]University of Manchester, Manchester, UK ; [f]KU Leuven, Leuven, BE ; [g]University of York UK ; [h]Poznan Technical University, Poznan, PL ; [i]Czech Academy of Sciences, Rez, CZ ; speaker email: stavroula.pallada@cerr corresponding author email: magdalena.kowalska@cern.ch

Abstract

In chemistry and biochemistry nuclear magnetic resonance (NMR) is currently the most versatile and powerful spectroscopic technique for characterization of molecular structure and dynamics in solution. However, one dra of the method is its low sensitivity, leading to relatively large amounts of sample (mg or ~10^{17} molecules), which constraints on the systems that may be explored. In addition, not all elements are easily accessible by NMR spectroscopy, as the relevant isotopes display no or poor response.

Our project aims at studying liquid samples with beta-detected NMR (or beta-NMR) [1], which has been success applied in nuclear and solid state physics. This technique relies on the anisotropic emission of beta-particles in t of spin-polarized nuclei, and offers the sensitivity of radiotracer experiments, and this – in principle – for several across the nuclear chart. In addition, the spins are hyperpolarized using lasers, which results in polarizations of 1 The combination of these two features gives beta-NMR over 1billion times more sensitivity than conventional N spectroscopy and makes it applicable to chemical elements which are otherwise difficult to interrogate spectroscopically. Perspectives include chemistry, biochemistry, and material-science applications. A very simila approach – based on hyperpolarization with lasers and on detection of resonances using gamma-ray emission h very recently applied to the Magnetic Resonance Imaging [2].

The project is based at ISOLDE, CERN's facility for the production and research on radioactive nuclei. The goal is t this novel approach to investigate the interaction of essential metal ions, which are spectroscopically silent in m spectroscopic techniques, such as Mg^{2+}, Cu^{+}, and Zn^{2+}, with biological macromolecules, with potential applicatior folding of nucleic acid as well as metalloprotein active sites. The experimental approach follows a successful pro principle experiment [3] in which we recorded ^{31}Mg beta-NMR spectra in an ionic liquid. In 2016 we have design and commissioned a dedicated beam line for producing polarized radioisotopes[4] and in 2017 we aim to perfor first beta-NMR studies.

The contribution will include comparison of beta-NMR with conventional NMR, for the nuclei which have already used (isotopes of Li, Be, Na, Mg), and those envisaged soon (Cu, Zn). The experimental setup and the challenges applying it to liquid samples will be illustrated, and the overarching goal of chemical and biochemical studies wit different metal ions will be presented.

References

[1] W. D. Brewer, Recent developments in radiative detection of nuclear magnetic resonance, 1982, Hyp. Interact. 12, 173

[2] Y. Zheng, G.W. Miller, W.A. Tobias, G.D. Cates, *A method for imaging and spectroscopy using γ-rays and magnetic reson* 2016, Nature 537, 652

[3] A. Gottberg, M. Stachura, M. Kowalska et al., *Billion-Fold Enhancement in Sensitivity of Nuclear Magnetic Resonance Spe for Magnesium Ions in Solution*, 2014, ChemPhysChem 15 (18), 3929

[4] M. Kowalska et al., *New laser polarization line at the ISOLDE facility*, 2016, submitted to J. Phys. G: Nucl. Part. Phys.

Elemental imaging by laser spectrometry: technological advances for biological and medical applications

B. Busser [a,b,c], S. Moncayo[b], F. Trichard[b], F. Pelascini[d], V. Bonneterre[c], N. Pinel[c], J-L. Coll[a], J. Charles[a,c], V. Motto-Ros[b], L. Sancey[a]

[a] *Grenoble Alpes Univ., Institute for Advanced Biosciences, IAB INSERM U1209 CNRS UMR5309 - Grenoble*

[b] *Institute Light and Matter, UMR5306 Univ. Lyon 1-CNRS, 69622 Villeurbanne*

[c] *CHU de Grenoble, 38000 Grenoble ;* [d] *CRITT Matériaux Alsace, 67305 Schiltigheim,*

email : bbusser@chu-grenoble.fr

Introduction: The physiological and pathological roles of exogenous metals, but also endogenous metallic and organic ions are of major interest for the medical community. Elemental imaging of biological tissues is currently a technological challenge, generally requiring complex instruments with restricted accesses. We recently developed an all-optical method, fully compatible with standard microscopy systems, for multi-elemental imaging of biological tissues. Our instrument is based on Laser Induced Breakdown Spectroscopy (LIBS) and allows the *in situ* imaging and quantification of the elements of the periodic table within biological tissues, with ppm-scale sensitivity and a pixel size of up to 10x10 µm². [1]

Methods: A laser is focused on the sample surface, which generates a plasma. Spectrometers analyze the specific optical response from chemical elements (including metals) contained in the biological tissues. Elemental images (maps) are obtained by scanning the surface of the sample. Different spectrometers are used to collect the signal of various metallic elements such as Fe, Ca, Na, P, Mg, Zn, Al, Mn and Cu in the tissue.

Results: The proof-of-concept was obtained by studying the bio-distribution of gadolinium-nanoparticles in tumors or organs (kidneys/liver) after i.v. administration in mice. These experiments helped to describe and understand the kinetic evolution of several metal-based (Gd, Au, Ag, Pt) nanoparticles *in vivo*. We recently upgraded our instrument to work faster and to image the elements contained in **paraffin-embedded samples**, which are the most frequent form of clinical specimens (surgical resections or biopsies). This allowed the identification of different chemical elements in human samples of medical interest. As an example, we were able to identify and map the presence of high levels of aluminium within a post-vaccinal cutaneous granuloma. We also found several metal particles within lung tissues and thoracic lymph nodes from patients with sarcoidosis. We are currently performing exploratory experiments on pathological specimens of medical interest such as brain, lung, skin, or cancer tissues. We already identified elements that are differentially expressed within cancer tissues, but also exogenous metals within exposed tissues. These preliminary results demonstrate the strong potential of this disruptive technology as a complementary tool for biological investigations or medical diagnosis.

Conclusion: This laser spectrometry technique is highly versatile because almost any element, especially metals, can be imaged with high sensitivity. Besides, this technique is complementary to optical microscopy used by medical pathologists for routine diagnostic activity. We describe a panel of recent results obtained with LIBS for the multi-elemental imaging of biological tissues for medical applications, and we highlight the main applications for pre-clinical research and human health.

References

[1] Sancey L., Motto-Ros V., Busser B., *et al*. **Scientific Reports** 2014; 4: 6065

High precision isotopic analysis of U, Cu and Zn in a human neuronal cell model

Eduardo Paredes[a], Emilie Avazeri[b], Véronique Malard[b,c], Claude Vidaud[b], Richard Ortega[d,e], Anthony Nonell[a], Hélène Isnard[a], Frédéric Chartier[f], Carole Bresson[a]

[a] *Den – Service d'Etudes Analytiques et de Réactivité des Surfaces (SEARS), CEA, Université Paris-Saclay, F-91191, Gif sur Yvette, France.*

[b] *CEA, DRF, Biosciences and biotechnologies institute (BIAM), Bagnols-sur-Cèze, France.*

[c] *CEA, CNRS, Aix-Marseille Université, UMR 7265 Biologie Végétale et Microbiologie Environnementales, Laboratoire des Interactions Protéine Métal, 13108 Saint-Paul-lez-Durance, France.*

[d] *University of Bordeaux, CENBG, UMR 5797, F-33170 Gradignan, France.*

[e] *CNRS, IN2P3, CENBG, UMR 5797, F-33170 Gradignan, France.*

[f] *Den – Département de Physico-Chimie (DPC), CEA, Université Paris-Saclay, F-91191, Gif sur Yvette, France.*

email : edes.PAREDES-PAREDES@cea.fr

Abstract

The study of metal isotopic fractionations in biological samples has recently been identified as a potential biomarker of metabolic processes, and as a very promising tool for the early diagnosis and the monitoring of diseases, such as cancer or neurodegenerative disorders. Diseases or the exposure to toxic elements can modulate the cellular homeostasis of essential elements, which may be at the origin of the isotopic fractionations observed *in vivo*. The use of cellular models for *in vitro* isotopic fractionation studies is a promising and under-exploited tool to help in understanding the biological processes governing isotopic fractionation, and further identify major metabolic pathways of metals.

With the aim of investigating uranium cellular pathways and potential toxicity mechanisms following chronic exposure to natural uranium (U_{nat}), human cells differentiated into neuron-like cells were exposed to non-toxic and sub-toxic U_{nat} concentrations for seven days. The isotopic signatures of uranium, copper and zinc in the extracellular and intracellular media were studied. Given the low intracellular Cu and U amounts, efforts were initially focused on the development and validation of a fit-for-purpose analytical procedure for high precision isotope ratio measurements of these elements. An isotopic fractionation was observed for U between the extracellular and intracellular media after cell exposure to 1 and 10 µM U_{nat}, with a preferential incorporation of the lightest isotope ^{235}U by the cells. This isotopic fractionation, however, was not observed for cells exposed to U_{nat} concentrations of 125 and 250 µM. For Cu and Zn, an isotopic fractionation was also found between the extracellular and intracellular media but the incorporation of U by the cells did not induce any change in the isotopic signature of intracellular Cu and Zn.

This work demonstrates for the first time the isotopic fractionation of U by human cells. Our results provided clues for the identification of uranium uptake pathways in human cells, unknown until know. Isotopic fractionation results seem to indicate the presence of two different uranium incorporation pathways, one of which would be active only above a threshold uranium concentration in the exposure solution.

Self-Assembled Monolayers of A-beta peptides: An abiological platform for investigating redox active metals and co-factors invoked in Alzheimer's Disease.

Abhishek Dey

Indian Association for the Cultivation of Science; email : icad@iacs.res.in

Abstract

The water soluble hydrophilic part of human Aβ peptide has been extended to include a C-terminal cysteine residue. Utilizing the thiol functionality of this cysteine residue, self-assembled monolayer (SAM) of these peptides is formed on Au electrodes. AFM imaging confirms formation of small Aβ aggregates on the surface of the electrode. These aggregates bind redox active metals like Cu and cofactors like heme, both of which are proposed to generate toxic reactive oxygen species (ROS) and play a vital role in Alzheimer's disease. The spectroscopic and electrochemical properties of these Cu and heme bound Aβ SAM mirror those reported for the soluble Cu and heme bound Aβ peptide. Large and small aggregates of Aβ peptides, resembling the morphology and dimensions of fibrillar and oligomeric forms of Aβ respectively, relevant to Alzheimer's disease, can also stabilized on electrodes using self-assembly. Both of these forms found to bind redox active Cu and heme resulting in active sites having distinctive biophysical properties. The reduced metal bound Aβ active sites of both the oligomeric and fibrillar forms of Aβ produce detrimental partially reduced oxygen species (PROS). This artificial construct provides a very easy to construct platform for investigating potential drugs affecting aggregation of human Aβ peptides and ROS generation by its complexes with redox active metals and cofactors.

References

1) Kushal Sengupta, Sudipta Chatterjee, Debajyoti Pramanik, Somdatta Ghosh Dey, Abhishek Dey*: *Self-â€‹assembly of stable oligomeric and fibrillar aggregates of Aß peptides relevant to Alzheimer's disease: morphology dependent Cuâ€‹/heme toxicity and inhibition of PROS generation.* Dalton Transactions **2014**, 43(35), 13377-13383.
2) Debajyoti Pramanik, Kushal Sengupta, Soumya Mukherjee, Somdatta Ghosh Dey, Abhishek Dey*: *Self-assembled monolayers of Aß peptides on Au electrodes: an artificial platform for probing the reactivity of redox active metals and cofactors relevant to Alzheimer's disease.* Journal of the American Chemical Society ***2012,*** *134,* 12180-12189.

Metals in imaging and sensing

Elaborating the "pa" and "ox" Ligand Families for Radiopharmaceutical Applications

Chris Orvig

[a] *Medicinal Inorganic Chemistry Gro Medicinal Inorganic Chemistry Group*
Department of Chemistry, University of British Columbia,
2036 Main Mall, Vancouver BC, V6T 1Z1 Canada
orvig@chem.ubc.ca

Abstract

Significant research effort has investigated new bifunctional chelate alternatives to the N_3 and N_4 aminocarboxylate macrocycles NOTA or DOTA, respectively, for the isotopes ^{66,67,68}Ga; however, NOTA and DOTA have become the "gold standard". We report our findings concerning the linear N_4O_2 chelate H_2dedpa and the linear N_4O_4 chelate H_4octapa plus larger analogues for application with a wide variety of radiometal ions useful for imaging, diagnosis and therapy. Concentration dependent labelling after 10 minutes reaction at RT of these ligands with many radiometal ions showed quantitative conversion to the desired products with ligand concentrations as low as 10^{-7} M. With ^{68}Ga and $[dedpa]^{2-}$, specific activities as high as 9.8 mCi nmol^{-1} were obtained without purification. Most notably, in a 2h competition experiment against human *apo*-transferrin, $[^{67}Ga(dedpa)]^+$showed no decomposition. In a direct competition for chelation of ^{67}Ga with equal concentrations of both NOTA and H_2dedpa, over 96% of the gallium isotope was coordinated to $dedpa^{2-}$. Bifunctional analogues of H_2dedpa and H_4octapa all label various M^{3+} at RT within 10 minutes. The stabilities of these building blocks and their modular family of easily accessible ligands, such as H_5decapa, are comparable to, or higher than, that of DOTA; the "pa" family of ligands is under intense current investigation, as are the biodistribution profiles of these platform chelate candidates with a variety of radiometal ions (e.g. $^{64}Cu^{2+}$, $^{111}In^{3+}$, $^{89}Zr^{4+}$, $^{86,90}Y^{3+}$, $^{177}Lu^{3+}$). We will also present our new family of "ox" ligands.

A Disassembly Approach for Imaging Endogenous Pyrophosphate in Living Cells using Metal-Salen Complexes

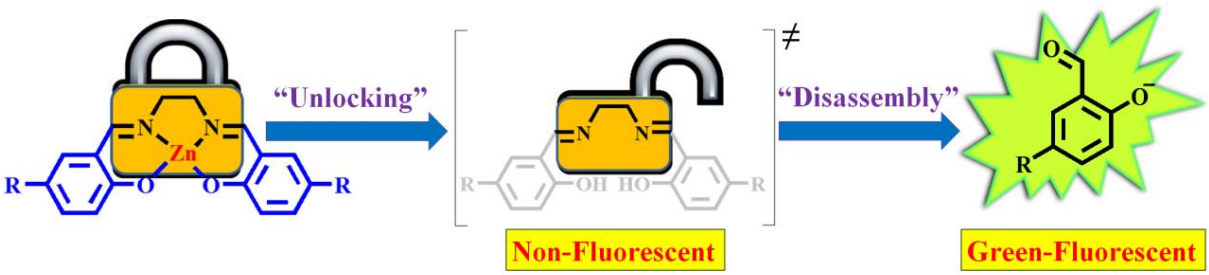
N. Kumari,[a] H. Huang, [b] H. Chao, [b] G. Gasser, [c] and F. Zelder, [a]

[a] Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich (Switzerland);[b] School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275 (P.R. China) [c)] Chimie ParisTech, PSL Research University, 11, Rue Pierre et Marie Curie, F-75005 Paris (France); email : felix.zelder@chem.uzh.ch

Abstract

This talk presents a stimulus-induced disassembly approach in water.[1] In particular, the selective fluorometric detection of polyphosphates in water and biological media is described.

In this method, an analyte sequesters selectively a metal ion from a metal-chelate complex. The "unlocked" ligand hydrolyses subsequently into its molecular subunits. Since the optical properties of the disassembled ligand and its ancestor are distinguishable, the anylate induced depletion of the metal-complex leads to a detectable signal.



The concept is described for the selective detection of pyrophosphate with metal-salen complexes.[1-3] Initially the intrinsic fluorescence of the salicylaldehyde signaling units is suppressed, but is unfolded during the disassembly of the molecule. Unprecedented applications of this strategy for endogenous pyrophosphate detection in the mitochondria of bacterial cells are presented.[4]

References

1) N. Kumari, F. Zelder, *Chem. Commun.*, **2015**, *51*, 17170-17173.
2) S. Lee, K. K. Y. Yuen, K. A. Jolliffe and J. Yoon, *Chem. Soc. Rev.*, **2015**, 44, 1749-1762.
3) S. Bhowmik, B. N. Ghosh, V. Marjomäki, K.Rissanen, *J. Am. Chem. Soc.*, **2014**, *136*, 5543–5546.
4) N. Kumari, H. Huang, H. Chao, G. Gasser, F. Zelder, *ChemBioChem* **2016**, 17, 1211-1215.

Zinc Responsive Contrast Agents

Patrick K. Malikidogo,[a] Fabien Caillé,[a,b] Jean-François Morfin,[a] Franck Suzenet,[b] Eva Toth[a], Célia S. Bonnet,[a]

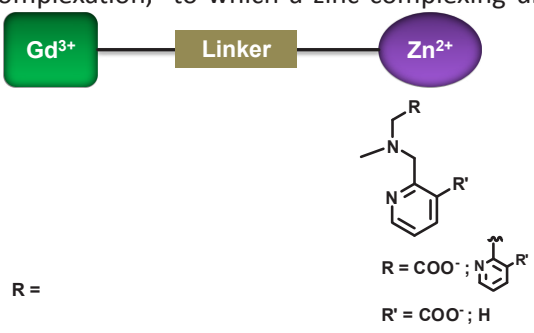
[a] *Centre de Biophysique Moléculaire, UPR 4301 CNRS, rue Charles Sadron, 45071 Orléans, France;* [a,b] *Institut de Chimie Organique et Analytique, UMR 7311 CNRS/Université d'Orléans, rue de Chartres, 45067 Orléans, France*
email : celia.bonnet@cnrs-orleans.fr

Abstract

Magnetic Resonance Imaging (MRI) has been devoted for a long time to obtaining anatomical and functional images. Recently emerging applications in molecular imaging seek information at the molecular level, looking at the biochemical or physiological abnormalities underlying the disease. Unlike anatomic imaging, molecular imaging always requires an imaging probe that is selectively responsive to the parameter to detect. Gd^{3+}-based contrast agents are particularly well-adapted for this purpose and most often the changes on the efficacy (relaxivity) are based on changes of the hydration number and/or rotational dynamics of the complexes; these two parameters being the easiest to be tailored by the chemist.[1]

Zinc is the second most abundant transition metal ion in humans, and it plays a central role in controlling gene transcription and metalloenzyme function. However, its quantitative distribution and its exact role are not well understood. It has also been shown that disturbances in Zn^{2+} homeostasis is implicated in neurodegenerative diseases (Alzheimer, Parkinson), diabetes, and cancers (prostate, pancreas and breast).[2] Therefore, monitoring Zn^{2+} *in vivo* by non-invasive technique such as MRI is important in biomedical research to understand its biological role, and to provide earlier diagnosis for specific pathologies.

We have developed zinc responsive contrast agents based on a pyridine unit already used for Gd^{3+} complexation,[3] to which a zinc complexing unit has been added through a linker (Figure). Detailed potentiometric, relaxometric and ^{17}O studies have given us access to the microscopic parameters controlling the efficacy of the systems.[4] This fundamental understanding has led us to the development of a new generation of contrast agents and their response to Zn^{2+} in the absence and in the presence of Human Serum Albumin will also be discussed.



References

1) Bonnet, C.S., Tei, L., Botta, M., Toth, E. Responsive Probes in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; John Wiley & Sons: Chichester, 2013, 343-385.
2) Dick R. *et al. Am. J. Alzheimers Dis. Other Demen.* 2010, 25, 572 ; Kelleher, S.L. et al., Adv. Nutr., 2011, 2, 101-111.
3) Bonnet, C.S., Buron, F. et al. *Chem. Eur. J.* 2012, *18*, 1419-1431.
4) Bonnet, F. Caillé, *et al.*, *Chem. Eur. J.* 2014, *20*, 10959-10969.

Acknowledgement
Agence Nationale de la Recherche for financial support.

Real-time Intravital Imaging of pH Variation Associated with Osteoclast Activity Using BODIPY Based Two Photon Excitation Probes

Kazuya KIKUCHI, [a,b]

[a] *Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871, Japan,* [b] *Immunology Frontier Research Center, Osaka University, Suita, Osaka 565-0871, Japan ; email : kkikuchi@mls.eng.osaka-u.ac.jp*

Abstract

Intravital imaging by two-photon excitation microscopy (TPEM) has been widely utilized to visualize cell functions. However, small molecular probes (SMPs) commonly used for cell imaging cannot be simply applied to intravital imaging because of the challenge of delivering them into target tissues, as well as their undesirable physicochemical properties for TPEM imaging. Here, we designed and developed a functional SMP with an active-targeting moiety, higher photostability, and fluorescence switch, and imaged target cell activity by injecting the SMP into living animals. The SMPs are based on BODIPY structure which is optimized for photostability and for fluorescence wavelenghth overlap for multicolor imaging. The combination of the rationally designed SMP with a fluorescent protein as a reporter of cell localization enabled quantitation of osteoclast activity and time-lapse imaging of its *in vivo* function associated with changes in cell deformation and membrane fluctuations. Real-time imaging revealed heterogenic behaviors of osteoclasts *in vivo* and provided insights into the mechanism of bone resorption.

References

1) T. Kowada, J. Kikuta, A. Kubo, M. Ishii, H. Maeda, S. Mizukami & K. Kikuchi, "*In Vivo* Fluorescence Imaging of Bone-Resorbing Osteoclasts", *J. Am. Chem. Soc.,* **2011,** *133,* 17772-17776.

2) H. Maeda, T. Kowada, J. Kikuta, M. Furuya, M. Shirazaki, S. Mizukami, M. Ishii & K. Kikuchi, "Real-time Intravital Imaging of pH Variation Associated with Osteoclast Activity", *Nat. Chem. Biol*., **2016,** *12,* 579-585.

Molecular Tools for Detection and Photomodulation of Singlet Oxygen

Youngmin You[a]

[a]*Division of Chemical Engineering and Materials Science, Ewha Womans University, Seoul 03760, Republic of Korea; email : odds2@ewha.ac.kr*

Abstract

Singlet dioxygen ($^1O_2$) belongs to a family of highly reactive oxygen species, because it has very high capacity for oxygenation of a variety of biological substrates, including nucleosides and amino acids containing heteroatoms. Although little is known about the biological process of $^1O_2$ in vivo, growing evidence indicates biological generation, trafficking, and annihilation of the $^1O_2$. To establish the $^1O_2$ biochemistry, molecular tools for $^1O_2$ are essential. This prompted us to develop a series of molecules for detection and photomodulation of biological $^1O_2$. We created isobenzofuran compounds having 1,7-diaryl groups which underwent the ultrafast Diels–Alder reaction with $^1O_2$ to produce fluorescence ratiometric responses.[1] The response was selective to $^1O_2$ over other reactive oxygen species. The ratiometric changes enabled an estimation of accumulated levels of biological $^1O_2$ in RAW 264.7 macrophages. We also found that a coumarin fluorophore having a julolidine unit at the 7 position could generate fluorescence responses to $^1O_2$.[2] Mechanistic studies, including analyses of $^1O_2$ products, revealed an occurrence of $^1O_2$-mediated dehydrogenative oxidation of julolidine into imminium. Taking advantage of this reactivity, we synthesized a molecular dyad capable of sensitization and detection of $^1O_2$. The dyad enabled us to monitor $^1O_2$-mediated induction of apoptosis, followed by necrosis of mammalian cells. This study is suggestive of a signaling action of biological $^1O_2$. To expand photobiological utility of the $^1O_2$ reactivity, 1,7-diphenylisobenzothiophene (DPBS) was prepared.[3] DPBS reacted slowly with photogenerated $^1O_2$ to form unstable endoperoxide, which is subsequently hydrolyzed into 1,2-dikitone. The latter step released hydrogen sulfide (H_2S), an important gasotransmitter. We explored the $^1O_2$-mediated photo-uncaging reaction of H_2S. The studies demonstrated that the reaction can serve a useful strategy for photocontrolled delivery of H_2S.

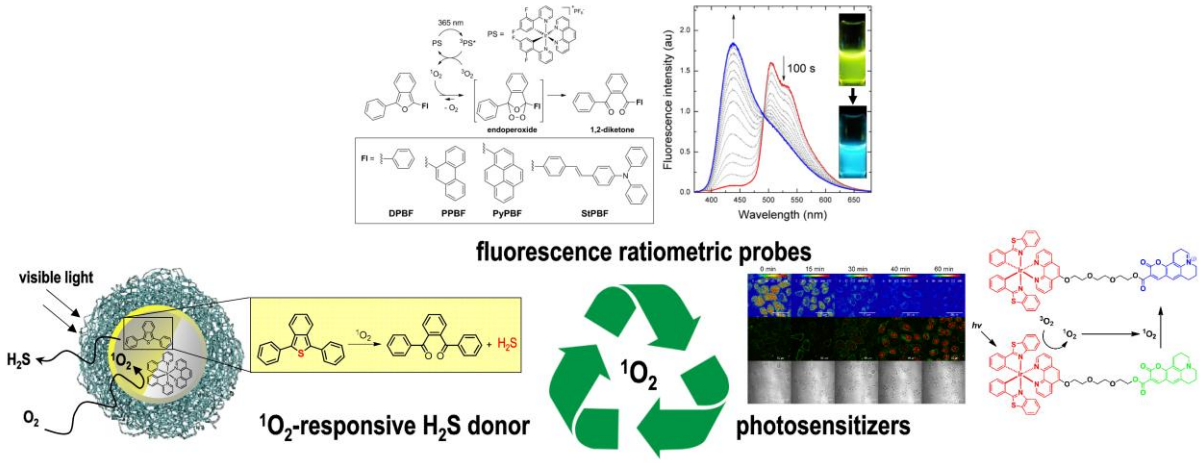


Figure. Molecular tools for biological singlet oxygen ($^1O_2$)

References

1) D. Song, S. Cho, Y. Han, Y. You, W. Nam, *Org. Lett*. **2013**, *15*, 3582–3585.
2) Y. You, E. J. Cho, H. Kwon, J. Hwang, S. E. Lee, *Chem. Commun.* **2016**, *52*, 780–783.
3) S. Y. Yi, Y. K. Moon, Y. You, *unpublished result*.

Seven coordinate lanthanide complexes with a tripodal redox active ligand: Structural, electrochemical and spectroscopic investigations

J. K. Molloy,[a] C Philouze,[a] L. Fedele,[a,b] D. Imbert,[b] O. Jarjayes,[a] F. Thomas[a]

[a] *CIRE, DCM, Univ. Grenoble Alpes - CNRS UMR 5250, F-38000 Grenoble, France,*
[b] *Laboratoire de Reconnaissance Ionique et Chimie de Coordination SCIB, UMR-E3 CEA-UJF, INAC, CEA-Grenoble, 17 rue des Martyrs, 38054 Grenoble Cedex 09 (France).*
email: molloyjk@gmail.com

Abstract

The development of redox active luminescent probes is an exciting field of research as they can been envisaged as useful tools in medical imaging due to their potential for in vivo detection.[1,2] Lanthanide complexes possess fascinating photophysical properties, such as long lived lifetimes and narrow emission bands, which lend them to applications as luminescent and magnetic probes. [3,4] However, the use of lanthanide complexes as redox active probes for biological imaging applications has been hindered due to the difficulty in controlling changeable oxidation states, the lanthanides preferring the +III oxidation state. A novel approach is the incorporation of redox active ligands which can be oxidised independently of the metal ion, which could lead the way in the development of new families of redox probes capable of detection in biological media.[5,6,7] We report two families of radical complexes based on a tris-phenolate scaffold. The selective oxidation of the phenolate moieties induces the switching of the luminescence of the lanthanide complex and thus can function as a redox probe. The properties of these redox active complexes have been studied by electrochemistry, UV-vis absorption and fluorescence spectroscopy and EPR.

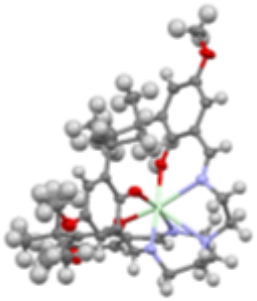


Figure 1: X-Ray Crystal Structure of **1.Nd**

References

1) M. Masarik, J. Gumulec, M Halvna, M. Sztalmoachova, P. Babula, M. Raudenska, M. Pavkova-Goldbergova, N. Cernei, J Sochor, O Zitka, B. Ruttkay-Nedecky, S. Krizkova, V. Adam, R. Kizek, *Integr.Biol*, **2012**, *4*, 672.
2) S. Aime, M. Botta, E. Gianolio, E. Terreno, *Angew. Chem. Int. Ed.*, **2000**, *39*, 747.
3) J.-C. G Bunzli, S. V. Eliseeva, *Chem Sci.*, **2013**, *4*, 1939.
4) S. V. Eliseeva, J.-C. G. Bunzli, *Chem. Soc. Rev.*, **2010**, *39*, 189.
5) M Tropiano, N. L. Kilah, M. Morten, H. Rahman, J. J. Davis, P. D. Beer, S. Faulkner. *J. Am. Chem. Soc.*, **2011**, *133,* 11847.
6) G. Tallec, P.H Fries, D. Imbert, M. Mazzanti, *Inorg Chem.*, **2011**, *50*, 7943.
7) J. K. Molloy, O. Jarjayes, C. Philouze, L. Fedele, D. Imbert and F. Thomas, *Chem Commun*, **2017**, 53, 605.

Development of Monomolecular Multimodal Imaging Probes for Optical/Nuclear Imaging

C. Goze[a], C. Bernhard[a], N. Maindron[a], M. Ipuy[a], D. Lhenry[a], M. Moreau[a], V. Thakare[a], A. Dubois[a], F. Boschetti[b], F. Denat[a]

[a] *ICMUB-UMR CNRS 6302, Dijon, France ;* [b] *Chematech, Dijon, France*

email : christine.goze@u-bourgogne.fr

Abstract

Molecular imaging is a highly promising field of research and innovation with potential in a wide range of applications, including prognostic, diagnostic, drug discovery and development of theranostics.[1] Among the different imaging modalities, there is an increasing interest in combining two different techniques providing an imaging approach that is complementary and highly translational. In particular, combining fluorescence with nuclear imaging techniques (PET or SPECT) can be really advantageous due to their complementarity, both for preclinical and clinical use. [2] For example, fluorescence microscopy and ex vivo optical investigations can provide preclinical in vivo PET or SPECT data, but also optical imaging can be applied clinically for intraoperative image-guided surgery, after a whole body PET or SPECT scan imaging of the patient. In order to elaborate a multimodal imaging agent, labeling the biovector with a single molecule containing both probes, called MOMIP (MonomOlecular Multimodal Imaging Probe), presents numerous advantages compared to the conventional dual-labeling approach (Figure 1). However, as the properties of the MOMIP may strongly affect the behavior of the resulting imaging agent, being able to finely modulate the different components of the bimodal probe is of major importance. The aim of this work is to provide a versatile tool for an easy access to a wide variety of MOMIP. For this purpose, we have chosen a lysine carefully protected with orthogonal protecting groups as a building block, to introduce sequentially the three components of the MOMIP: a fluorescent dye (Cy5, BODIPY, fluorescein), a chelator (DOTA or DOTAGA for ^{111}In labeling, NODAGA for ^{68}Ga or ^{64}Cu labeling), and a bioconjugatable handle (isothiocyanate, azide or tetrazine group, dithiolane ring). Several examples of MOMIP have been synthesized, characterized (including photophysical studies), and conjugated to different biomolecules (full antibodies, antibody fragments, peptides) or nanoparticles. The radiolabeling (^{111}In, ^{68}Ga) of some resulting bimodal bioconjugates has been investigated. Whole-body fluorescence images were recorded after injection in tumor bearing mice. Ex-vivo imaging was performed to provide semi- quantitative information about the tracer biodistribution. This work shows the versatility of a new method for the construction of MOMIP, thus providing access to optimized bimodal imaging agents for a given application.

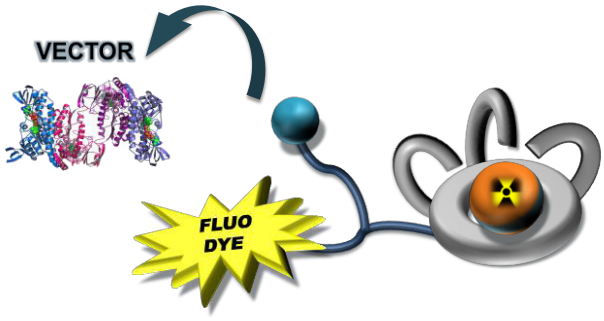


Figure 1: General structure of the MOMIP

References

1) 1. M. L. James, S. S. Gambhir, *Physiol Rev*. 2012, 897-9652.

2) (a) U. Seibold, B. Wängler, R. Schirmacher, C. Wängler. *BioMed. Research International*, **2014**, 1-13. (b) A. Louie, *Chem. Rev*. **2010**, *110*, 3146–3195.

A Customizable Synthetic Approach to Tune Targeting, Bioactivity and Solubility of Theranostic Cp-Re/^{99m}Tc complexes.

Angelo Frei, [a] Roger Alberto, [a]

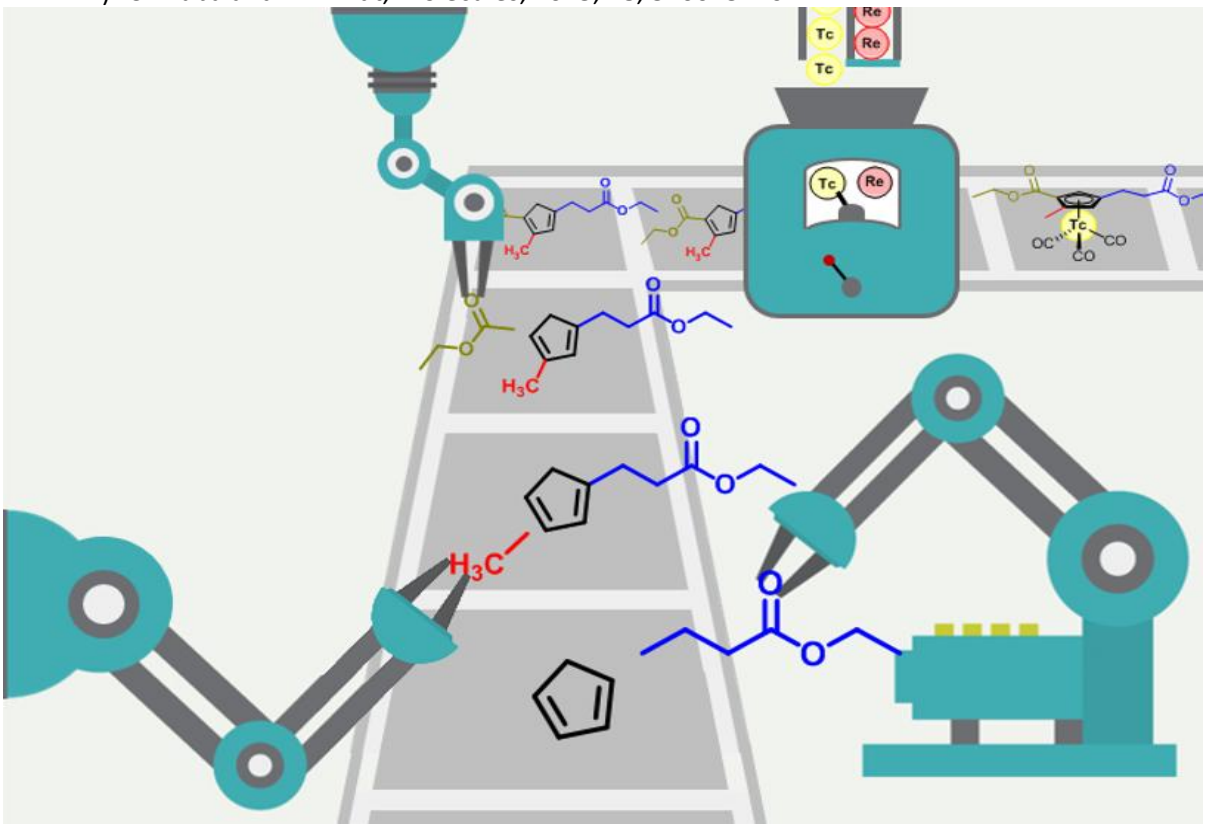
[a] *Dept. of Chemistry, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich Switzerland;*

email : angelo.frei@chem.uzh.ch

The ability to manipulate molecules and their properties at will has been the chemist's dream since the first alchemists attempt to turn lead into gold. Particularly in the field of medicinal chemistry it has been shown again and again, that even very small changes can have dramatic impact on the biological behaviour of a lead compound. ^{99m}Tc is one of the staple radionuclides for SPECT imaging in the clinic, being used in over 80% of all diagnostic nuclear imaging studies.[1] This is mainly due to the artificial elements favourable nuclear properties and its widespread availability at modest cost. However, current ^{99m}Tc tracers lack the high degree of target specificity that is desired in today's clinical applications. Using the cyclopentadiene synthon, we have developed a synthetic approach to multifunctional Cp-Re/^{99m}Tc complexes. The targeting, as well as other physical and chemical properties of the system can be manipulated before coordination to the metal center. We will report on the chemical flexibility of this approach as well as the mono- and bi-functionalization of these Cp-ligands with the synthesis of their respective Re/^{99m}Tc complexes. Furthermore, we are currently exploring the synthesis of new Cp-ligands derivatized with biological functions for *in vitro* and *in vivo* evaluation.

References

1.) I. Amato, Chem. Eng. News, 2009, 87, 58–64.
2.) C. Kluba and T. Mindt, Molecules, 2013, 18, 3206–3226.



Photophysical behavior of carbostyril and coumarin sensitized lanthanide complexes: Energy transfer and deactivation pathways

K. Eszter Borbas, [a]

[a] *Department of Chemistry, Ångström Laboratory, Uppsala University, Box 523, Lägerhyddsvägen 1, 75120 Uppsala, Sweden; email : eszter.borbas@kemi.uu.se*

Abstract

A large group of carbostyril and coumarin-appended lanthanide complexes were prepared based on two chelating frameworks: do3a, to which the antennae were linked *via* azide-alkyne cycloaddition, and dota, to which the antennae were coupled *via* amide bonds. The do3a-ligands carrying coumarin antennae were complexed with Eu and Tb. These species were moderately emissive. The carbostyril-equipped ligands were paired with Eu, Tb, Sm, Dy, and, in some cases, Nd and Yb. Several of the carbostyrils were good sensitizers for not only Eu and Tb, but also for the lesser-studied Sm and Dy. The Nd and Yb species all had sensitized near infrared emission. Non-emissive Gd- and diamagnetic Lu-analogues were also prepared. The former enable the investigation of the ligand photophysics without an emissive lanthanide, while the latter renders ^{1}H NMR characterization of the complexes possible.

The photophysical properties of the ligands and the complexes were studied using steady-state and time-resolved emission spectroscopy. A FRET-type sensitization pathway seems to dominate in architectures wherein coumarin antennae are attached via long linkers to the lanthanide moiety. This pathway also contributes to sensitization in architectures with shorter antenna-lanthanide distances, along with the well-established triplet pathway.

The Eu-complexes had markedly lower residual antenna fluorescence quantum yields than the analogous Gd-complexes. Similar, although less pronounced quenching of the antenna fluorescence was observed for all the other emissive lanthanides. This effect is likely due to a combination of photoinduced electron transfer from the antenna to the lanthanide (especially for the more reducible metal ions), and of a singlet-mediated energy transfer.

Finally, the most emissive complexes were tested for their potential in multiplex detection. Up to six distinct lanthanide emissions could be unambiguously identified in a cocktail of complexes without the need for extensive data processing. A mixture of Eu- and Tb-complexes carrying the same carbostyril antenna was taken up by bacteria and could be detected in the intact cells.

Polymetallic Emitting Lanthanide Dendrimer Complexes and Metal Organic Frameworks as Bright Near-infrared Optical Imaging Agents

Ivana Martinić,[a] Svetlana V. Eliseeva,[a] Alexandra Foucault-Collet,[a] Regis Delatouche,[b] Virginie Placide,[a] Franck Suzenet,[b] Nathaniel L. Rosi,[c] Stéphane Petoud[a,d]

[a] *Centre de Biophysique Moléculaire, CNRS UPR 4301, 45071 Orléans, France*

[b] *Institut de Chimie Organique et Analytique, UMR 7311, 45067 Orléans, Francec*

[a] *Department of Chemistry, University of Pittsburgh, 15260, Pittsburgh, USA*

[a] *Department of Inorganic Chemistry, University of Geneva, CH1211, Geneva 4, Switzerland*

email : stephane.petoud@inserm.fr

Abstract

Fluorescence and luminescence are detection techniques that possess important advantages for bioanalytical applications and biologic imaging: high sensitivity, versatility and low costs of instrumentation. A common characteristic of biologic analytes is their presence in small quantities among complex matrices such as blood, cells, tissue and organs. These matrices emit a significant background fluorescence (autofluorescence), limiting detection sensitivity.

The luminescence of lanthanide cations has several complementary advantages over the fluorescence of organic fluorophores and semiconductor nanocrystals, such as sharp emission bands for spectral discrimination from background emission, long luminescence lifetimes for temporal discrimination and strong resistance to photobleaching. In addition, several lanthanides emit near-infrared (NIR) photons that can cross deeply tissues for non-invasive investigations and that result in improved detection sensitivity due to the absence of native NIR luminescence from tissues and cells (autofluorescence).

The main requirement to generate lanthanide emission is to sensitize them with an appropriate chromophore (“antenna effect”). The choice of this antenna allows the tuning of the excitation wavelength of the resulting complexes.

An innovative concept for such sensitization of NIR-emitting lanthanides is proposed herein; the current limitation of low quantum yields experienced by most mononuclear lanthanide complexes is compensated for by using a large number of lanthanide cations and by maximizing the absorption of each discrete molecule, thereby increasing the number of emitted photons per unit of volume and the overall sensitivity of the measurement. To apply this concept, we have created several metal-organic frameworks and dendrimer macromolecular complexes. We will discuss their designs, synthesis, structures, photophysical properties and their applications for biological imaging in cells with NIR microscopy.

This research is supported through grants from the European Community's Seventh Framework Programmes (ITN Luminet, IEF Dendrimage), l’Agence National de la Recherche and La Ligue Contre le Cancer.

References

1) I. Martinic, S. V. Eliseeva, S. Petoud, Journal of Luminescence. DOI : 10.1016/j.jlumin.2016.09.058.
2) A. Foucault-Collet, K. A. Gogick, K. A. White, S. Villette, A. Pallier, G. Collet, C. Kieda, T. Li, S. J. Geib, N. L. Rosi, S. Petoud, Proc. Natl. Acad. Sci. USA, 2013, 110, 17199.
3) A. Foucault-Collet, C. M. Shade, I. Nazarenko, S. Petoud, S. V. Eliseeva, Angew. Chem., 2014, 53, 2927.

Heterometallic Re(I)/Au(I) complexes as theranostic agents

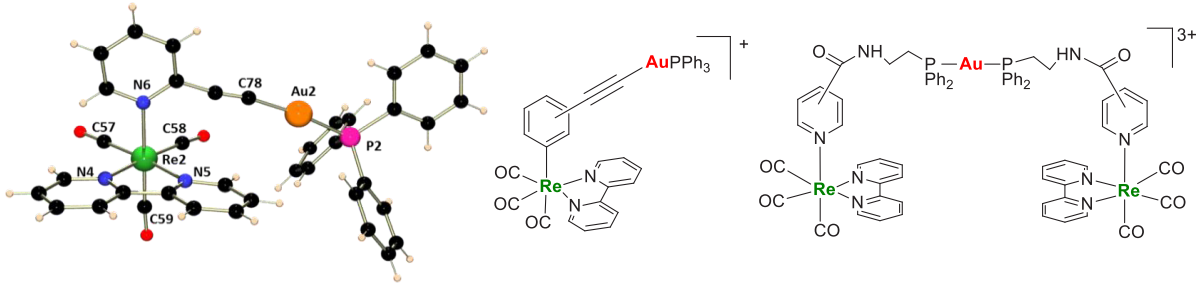
M. Concepción Gimeno,*[a] Andrés J. Luengo,[a] V. Fernández-Moreira,*[a] Isabel Marzo[b]

[a] Departamento de Química Inorgánica, Instituto de Síntesis Química y Catálisis Homogénea (ISQCH), Universidad de Zaragoza-CSIC, E-50009 Zaragoza, Spain; [b] Departamento de Bioquímica y Biología Celular, Universidad de Zaragoza, E-50009 Zaragoza, Spain. E-mail: gimeno@unizar.es

Abstract

Fluorescence microscopy has become one of the most powerful tools in cell biology and related areas of bioscience. Their high sensitive detection that allows to visualize single molecules together with the possibility of monitoring rapidly changing events make this technique as the ideal tool to reveal key information about the mechanism of action of multiple metallodrugs. The idea of using fluorescence microscopy for visualizing biomaterial relays in the availability of having molecules with the suitable photophysical properties that can be used as light-bulbs inside the cells. Unfortunately, gold(I) and gold(III) metallodrugs lack sometimes of such capacity. Therefore, it is imperative to be able to design new species that bear together anticancer activity and emissive properties. A reasonable approach would be the development of bioactive luminescent heterometallic derivatives, being each metallic fragment the responsible of bringing together those features.

As d^6 metal derivatives have been proved to be excellent candidates for cell imaging applications,[1] we have considered the use of bimetallic Re-Au derivatives to be used in diagnosis and therapy.[2] Therefore, a series of luminescent homometallic *fac*-$[Re(bipy)(CO)_3(L)]^+$ and *fac*-$[Re(N^C)(CO)_3(L)]^+$ and heterometallic Re-Au complexes, where L is a functionalized ligand, have been synthesized for the purpose of finding a synergic effect between the excellent photophysical properties of rhenium complexes and the good antiproliferative effects of gold compounds.



References

1) M. P. Coogan, V. Fernández-Moreira, *Chem. Commun.* **2014**, *50*, 384-399
2) V. Fernández-Moreira, I. Marzo, M. C. Gimeno, *Chem. Sci.* **2014**, *5*, 4434-4446.

A lanthanide complex to overcome the two major chokepoints of protein crystallography.

François Riobé,[a] Sebastiano Di Pietro,[a] Sylvain Engilberge,[b] Louise Lassalle,[b] Eric Girard,[b] Olivier Maury.[a]

[a] Univ Lyon, Ens Lyon, CNRS, Université Claude Bernard Lyon 1, UMR 5182, Laboratoire de Chimie, F69342, Lyon, France; [b] Institut de Biologie Structurale, UMR5075 CEA-CNRS-UJF, EPN Campus, 71 avenue des Martyrs, 38044 Grenoble Cedex 9, France; email: francois.riobe@ens-lyon.fr

Protein crystallography, the leading technique for protein structure determination, is limited by two main chokepoints: (a) obtaining protein crystals and (b) solving the phase problem. Since 2000, our team has developed luminescent lanthanide complexes as auxiliary for structure determination of macromolecules, in particular, exploiting the high-phasing power of lanthanide elements.[1]
Our previous works highlighted the supramolecular complexes-protein interactions and the required technical specifications to design compatible agents with high-throughput crystallization platforms.[2] This unprecedented approach lead recently to a new terbium complex, named Crystallophore, which showed exceptional nucleating properties when added to the protein crystallization media.[3] Up to now, this effect was tested on 12 proteins with different molecular weights and different oligomeric states (2 with unknown structure) and compared to the classical screening conditions. It was observed that, in addition to new crystallization conditions, this complex could improves the quality of the crystals obtained compare to native conditions (see picture).
We will present our last results using this new agent for protein crystallography which, in addition to nucleating and phasing properties, also allows an easier detection and centring of the protein crystals thanks to its luminescence. We believe that this all-in-one lanthanide complex is a convenient new additive for biocrystallographer, being both a powerful nucleating agent and providing ready-to-use crystals for diffraction experiments.

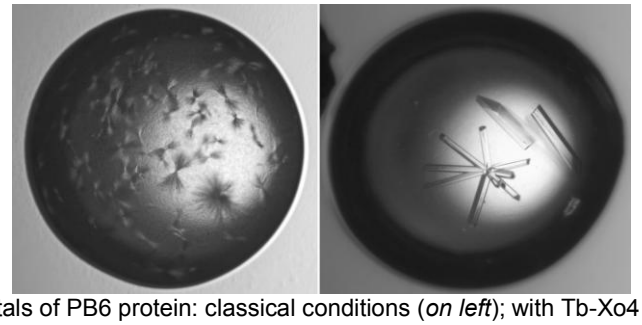


Figure Crystals of PB6 protein: classical conditions (*on left*); with Tb-Xo4 (*on right*).

1) R. Talon, R. Kahn, M. A. Dura, O. Maury, F. M. D. Vellieux, B. Franzetti, E. Girard, *J Synchrotron Rad.*, **2011,** *18*, 74; E. Girard, M. Stelter, J. Vicat, R. Kahn, *Acta Crystallogr D,* **2003,** *59*, 1914.
2) G. Pompidor, A. Daleo, J. Vicat, L. Toupet, N. Giraud, R. Kahn, O. Maury, *Angew. Chem. Int. Ed.* **2008,** *47*, 3388; E. Dumont, G. Pompidor, A. D'Aléo, J. Vicat, L. Toupet, R. Kahn, E. Girard, O. Maury, N. Giraud, *Phys Chem Chem Phys* **2013,** *15*, 18235.
3) S. Engilberge, F. Riobé, S. Di Pietro, L. Lassalle, N. Coquelle, C.-A. Arnaud, D. Pitrat, J.-C. Mulatier, D. Madern, C. Breyton, O. Maury, E. Girard., *submitted*; Patent pending, PCT/FR2016/053539.

Metalloenzymes, inspiration, mimics, function and inhibition

Water splitting catalyzed by the Mn_4CaO_5 cluster in Photosystem II

Johannes Messinger [a,b]

[a] *Uppsala University, Department of Chemistry –Ångström, Molecular Biomimetics, Uppsala, Sweden;* [b] *Umeå University, Chemistry Biology Centre (KBC), Department of Chemistry, Umeå, Sweden;*

email: johannes.messinge@kemi.uu.se

Abstract

The catalytic site for water oxidation in photosynthesis is a simple manganese-calcium-oxide cluster, at first glance not much different to minerals like birnessite ($(Na,Ca,K)_{0.6}(Mn^{4+},Mn^{3+})_2O_4 \cdot 1.5H_2O$). Yet, the water splitting activity per metal center is orders of magnitudes higher for photosystem II. In synthetic systems, similar rates of oxygen evolution can presently only be reached with complexes containing rare and expensive metals such as ruthenium and iridium. Photosystem II is thus a prime example for the activation of base metals for complex reactions involving the well-orchestrated coupling of proton and electron transfer events.

After an introduction into the structure and general reaction pattern of photosystem II, I will present recent results of x-ray free-electron laser experiments on different oxidation states of photosystem II.[1] Subsequently, recent mass spectrometric and advanced EPR data concerning the binding of the two substrate 'water' molecules are reported.[2-3] Finally, the mechanism of water oxidation and possible ways of base metal activation are discussed.

References

1) Young, I. D.; Ibrahim, M.; Chatterjee, R.; Gul, S.; Fuller, F. D.; Koroidov, S.; Brewster, A. S.; Tran, R.; Alonso-Mori, R.; Kroll, T.; Michels-Clark, T.; Laksmono, H.; Sierra, R. G.; Stan, C. A.; Hussein, R.; Zhang, M.; Douthit, L.; Kubin, M.; de Lichtenberg, C.; Pham, L. V.; Nilsson, H.; Cheah, M. H.; Shevela, D.; Saracini, C.; Bean, M. A.; Seuffert, I.; Sokaras, D.; Weng, T. C.; Pastor, E.; Weninger, C.; Fransson, T.; Lassalle, L.; Brauer, P.; Aller, P.; Docker, P. T.; Andi, B.; Orville, A. M.; Glownia, J. M.; Nelson, S.; Sikorski, M.; Zhu, D. L.; Hunter, M. S.; Lane, T. J.; Aquila, A.; Koglin, J. E.; Robinson, J.; Liang, M. N.; Boutet, S.; Lyubimov, A. Y.; Uervirojnangkoorn, M.; Moriarty, N. W.; Liebschner, D.; Afonine, P. V.; Waterman, D. G.; Evans, G.; Wernet, P.; Dobbek, H.; Weis, W. I.; Brunger, A. T.; Zwart, P. H.; Adams, P. D.; Zouni, A.; Messinger, J.; Bergmann, U.; Sauter, N. K.; Kern, J.; Yachandra, V. K.; Yano, J., Structure of photosystem II and substrate binding at room temperature. *Nature* 2016, 540, 453-457.

2) Nilsson, H.; Rappaport, F.; Boussac, A.; Messinger, J., Substrate-water exchange in photosystem II is arrested before dioxygen formation. *Nat Commun* 2014, *5*.

3) Rapatskiy, L.; Cox, N.; Savitsky, A.; Ames, W. M.; Sander, J.; Nowaczyk, M. M.; Rögner, M.; Boussac, A.; Neese, F.; Messinger, J.; Lubitz, W., Detection of the water-binding sites of the oxygen-evolving complex of photosystem II using W-band ^{17}O electron-electron double resonance-detected NMR spectroscopy. *J. Am. Chem. Soc.* 2012, *134*, 16619-16634.

Synthetic biology meets synthetic chemistry
- *In vivo* activation of an apo-hydrogenase using synthetic complexes

N. Khanna[a], C. Esmieu[b], L. S. Mészáros[b], P. Lindblad[a] and G. Berggren[b]

[a] Microbial Chemistry, Department of Chemistry – Ångström Laboratory, Uppsala University, 75120 Uppsala, Sweden; [b] Molecular Biomimetics, Department of Chemistry – Ångström Laboratory, Uppsala University, 75120 Uppsala, Sweden; email : gustav.berggren@kemi.uu.se

Abstract

[FeFe] hydrogenases (HydA) are fascinating enzymes that catalyse the interconversion between H_2 and protons with remarkable efficiency. The reaction occurs at the H-cluster featuring a, in biology unique, dinuclear [2Fe] subsite (Fig. 1).[1] Synthetic chemistry has long been a powerful tool in studies of this cluster, via the preparation of biomimetic model compounds.[2] In 2013 it was shown how such synthetic complexes can be introduced into the enzyme itself under *in vitro* conditions, thus providing a direct link between biomimetic chemistry and biology, and allowing the manipulation of the enzyme using synthetic chemistry.[3, 4]

More recently we discovered how this concept can be extended to *in vivo* conditions, and the apo-enzyme activated using synthetic compounds inside living cells.[5] Here I will present how this can be used to generate both active "native" hydrogenases, as well as "artificial" hydrogenases incorporating non-native cofactors resulting in enzymes with new spectroscopic and catalytic properties, inside living cells.

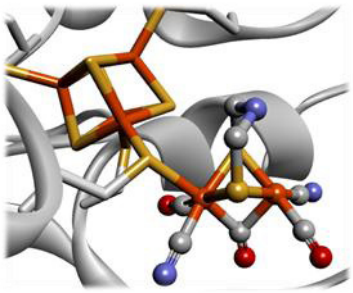


Figure 1: Cartoon representation of the H-cluster (S = yellow; Fe = orange; N = blue; O = red; C = white)

References:

1. Lubitz, W., Ogata, H., Rüdiger, O. & Reijerse, E. Hydrogenases. *Chem. Rev.* (2014) **114**, 4081-4148.
2. Simmons, T.R., Berggren, G., Bacchi, M., Fontecave, M. & Artero, V. Mimicking hydrogenases: From biomimetics to artificial enzymes. *Coord. Chem. Rev.* (2014) **270–271**, 127-150.
3. Berggren, G. et al. Biomimetic assembly and activation of [FeFe]-hydrogenases. *Nature* (2013) **499**, 66-69.
4. Esselborn, J. et al. Spontaneous activation of [FeFe]-hydrogenases by an inorganic [2Fe] active site mimic. *Nat. Chem. Biol.* (2013) **9**, 607-609.
5. Khanna, N.; Esmieu, C.; Mészáros, S., L.; Lindblad, P. and Berggren, G, *manuscript in revision.*

Selective Removal of Endogenous Carbon Monoxide *in vitro* and *in vivo* by Aqueous Hemoprotein Model Complexes

Hiroaki Kitagishi, Saika Minegishi

Department of Molecular Chemistry and Biochemistry, Faculty of Science and Engineering, Doshisha University, Kyotanabe, Kyoto 610-0321, Japan; email : hkitagis@mail.doshisha.ac.jp

Abstract

Carbon monoxide (CO) is endogenously produced in mammalian cells during metabolic degradation of hemin by heme oxygenase (HO) enzymes. It has been believed that endogenous CO functions as a second messenger in the various signalling pathways in the cells. The biological functions of endogenous CO, however, is not well understood because of the difficulty in preparing the cells or animals which lack endogenous CO. In the present study, we demonstrate the selective removal of endogenous CO in cells and in animals using supramolecular hemoprotein model complexes (Figure 1).

HemoCD1, a supramolecular 1:1 inclusion complex of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphinatoiron(II) and per-*O*-methylated β-cyclodextrin dimer having a pyridine ligand, is an aqueous hemoprotein model complex that bind oxygen (O_2) and CO reversibly in aqueous solution at an ambient temperature. The CO affinity of hemoCD1 is much higher than those of native hemoproteins whereas the O_2 affinity is moderate. When hemoCD1 was administered to mice, hemoCD1 captured endogenous CO during its circulation in the blood, and then smoothly excreted in the urine as the CO-bound form. Thus the endogenous CO in the mice was easily removed by hemoCD1. We have recently discovered the homeostatic feedback response of endogenous CO using the mice,[1] i.e., the removal of endogenous CO in mice significantly induced an HO enzyme in the liver to compensate endogenous CO up to the constant level.

The strong CO affinity of hemoCD1 was also applicable for quantification and removal of endogenous CO in the cells. HemoCD1 sensitively detected endogenous CO in the cell lysate (~200 pmol per 10^6 cells). To deliver hemoCD1 into the cells without cell disruption, we synthesized a new hemoCD1 derivative, R8-hemoCD1, which has an octaarginine as a cell-penetrating peptide. We confirmed that R8-hemoCD1 penetrated the cell membrane and captured endogenous CO in the cells. We found that the selective removal of CO in macrophage cells affected the signaling pathway involving inflammation and reactive oxygen species (ROS) levels in macrophage cells.

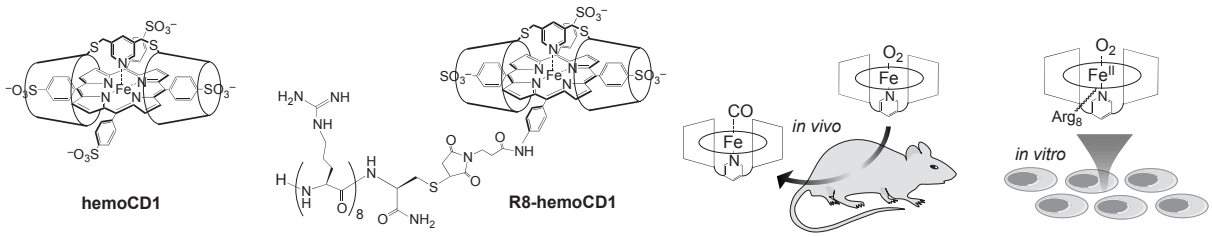


Figure 1. Selective removal of endogenous CO *in vitro* and *in vivo* by aqueous hemoprotein model complexes.

References

1) H. Kitagishi, S. Minegishi et al., *J. Am. Chem. Soc.* **2016**, *138*, 5417.

Why Does Nature Use Manganese for Water Oxidation?

Dimitrios A. Pantazis

Max Planck Institute for Chemical Energy Conversion, Stiftstr. 34-36, 45470 Mülheim an der Ruhr, Germany.

email : dimitrios.pantazis@cec.mpg.de

Abstract

The tetramanganese Mn_4CaO_5 cluster at the active site of the oxygen-evolving complex in Photosystem II is an archetypal example of a complex bioinorganic system comprising interacting open-shell transition metal ions.[1] Structural methods such as X-ray crystallography have considerably enhanced our view of the complex but cannot yet provide unique models of the various catalytic states or even probe all of them. On the other hand, spectroscopic techniques that report on the magnetism and the local or global electronic structure (e.g. electron paramagnetic resonance and X-ray spectroscopies) provide selective information on intermediates and metastable states but without the possibility of direct interpretation in terms of precise geometry. The link between spectroscopy/magnetism and atomistic structure can, however, be established through quantum chemical simulations that focus on the prediction of spectroscopic observables.[2,3] The application of spectroscopy-oriented quantum chemistry to photosynthesis in recent years has revolutionized our understanding of biological water oxidation: analysis of magnetic properties and spectroscopic parameters resulted in the definitive assignment of metal oxidation states,[4,5] in identification of previously unsuspected catalytic intermediates,[5,6] in a functional understanding of the local protein matrix,[7] and in a detailed mapping of atomistic models that rationalize and interconnect disparate experimental observations amassed over decades of experimental work into a coherent mechanistic interpretation. With this new-found insight into functional principles and catalytic intermediates of biological water oxidation, we can begin to understand why nature chose Mn for making oxygen. From a chemical perspective, the answer can be found in two unique properties that make manganese ideal for strictly controlling substrate binding/activation and for prohibiting early onset of oxidative chemistry.

References

1) V. Krewald, M. Retegan and D. A. Pantazis, *Top. Curr. Chem.* **2016**, *371*, 23.
2) D. A. Pantazis, W. Ames, N. Cox, W. Lubitz and F. Neese, *Angew. Chem., Int. Ed.* **2012**, *51*, 9935.
3) N. Cox, D. A. Pantazis, F. Neese and W. Lubitz, *Acc. Chem. Res.* **2013**, *46*, 1588.
4) V. Krewald, M. Retegan, N. Cox, J. Messinger, W. Lubitz, S. DeBeer, F. Neese and D. A. Pantazis, *Chem. Sci.* **2015**, *6*, 1676.
5) N. Cox, M. Retegan, F. Neese, D. A. Pantazis, A. Boussac and W. Lubitz, *Science* **2014**, *345*, 804.
6) M. Retegan, V. Krewald, F. Mamedov, F. Neese, W. Lubitz, N. Cox and D. A. Pantazis, *Chem. Sci.* **2016**, *7*, 72.
7) M. Retegan and D. A. Pantazis, *Chem. Sci.* **2016**, *7*, 6463.

Heme Protein Supramolecules Constructed by Domain Swapping

Shun Hirota

[a] *Graduate School of Materials Science, Nara Institute of Science and Technology ; email : hirota@ms.naist.jp*

Abstract

We have previously shown that horse cytochrome (cyt) *c* forms polymers from monomers by 3D domain swapping its C-terminal α-helix successively (runaway domain swapping).[1] The C-terminal α-helix of dimeric horse cyt *c* was displaced from its original position in the monomer, and the Met80–heme coordination was perturbed significantly in the dimer, causing higher cyanide ion binding affinity and peroxidase activity compared to those in the monomer. Cyt *c* domain swapping occurred regardless of Met80 mutation to Ala, and the domain-swapped dimer stability was less affected by the Met80–heme coordination.[2] Cyt *c* formed domain-swapped oligomers by the interaction between the N- and C-terminal α-helices at the early stage of folding from its unfolded state,[3] and the interaction important for domain swapping was also detected in the monomer molten globule state.[4] Domain-swapped oligomeric cyt *c* interacted more strongly with the anionic phospholipid-containing vesicle and the HeLa cell outer membrane compared to the monomer.[5] *Psudomonas aeruginosa* cyt c_{551} and *Hydrogenobacter thermophilus* (HT) cyt c_{552} formed oligomers by domain swapping the N-terminal region containing the heme.[6] By elongation of the hinge loop in HT cyt c_{552}, the domain swapping of the C-terminal region was also observed.[7] High-order domain-swapped oligomers of HT cyt c_{552} were produced during *E. coli* culture, whereas the domain-swapped protein amount decreased when the protein stability was decreased by mutation.[8] The domain-swapped dimers of *Allochromatium vinosum* cyt *c*′ formed high-order oligomers in the absence of CO. The cyt *c*′ oligomers dissociated to domain-swapped dimers in the presence of CO, and re-associated by CO readdition.[9] Cyt cb_{562} also domain swapped and formed a dimer, where the two helices in the N-terminal region of one protomer interacted with the other two helices in the C-terminal region of the other protomer. In the crystal, three domain-swapped cyt cb_{562} dimers formed a unique cage structure with a Zn-SO_4 cluster inside the cavity.[10] We have previously shown that myoglobin (Mb) forms a domain-swapped dimer with two extended α-helices. An artificial heterodimeric protein with two different heme active sites (a bis-histidine-coordinated heme and a H_2O/histidine-coordinated heme) was constructed by domain swapping two Mb surface mutants with opposite charges.[11]

References

1) S. Hirota, Y. Hattori, S. Nagao, et al., Proc. Natl. Acad. Sci. USA, 2010, 107, 12854–12859.
2) S. Hirota, N. Yamashiro, Z. Wang, S. Nagao, J. Biol. Inorg. Chem., in press, DOI: 10.1007/s00775-017-1446-3.
3) P. P. Parui, M. S. Deshpande, S. Nagao, et al., Biochemistry, 2013, 52, 8732–8744.
4) M. S. Deshpande, P. P. Parui, H. Kamikubo, et al., Biochemistry, 2014, 53, 4696–4703.
5) S. Junedi, K. Yasuhara, S. Nagao, et al., ChemBioChem, 2014, 15, 517–521.
6) S. Nagao, M. Ueda, H. Osuka, et al., PLoS One, 2015, 10, e0123653.
7) C. Ren, S. Nagao, M. Yamanaka, et al., Mol. BioSyst., 2015 11, 3218–3221.
8) Y. Hayashi, M. Yamanaka, S. Nagao, et al., Sci. Rep., 2016, 6, 19334 (8 pages).
9) M. Yamanaka, M. Hoshizumi, S. Nagao, et al., Protein Sci., in press, DOI: 10.1002/pro.3090.
10) T. Miyamoto, M. Kuribayashi, S. Nagao, et al., Chem. Sci., 2015, 6, 7336–7342.
11) Y.-W. Lin, S. Nagao, M. Zhang, et al., Angew. Chem. Int. Ed., 2015, 54, 511–515.

Combining electrochemistry, site-directed mutagenesis and theoretical chemistry to study the reactivity of metalloenzymes: FeFe hydrogenase as an example

A Kubas, M Sensi, C Orain, D De Sancho, L Saujet, C Gauquelin, I Meynial-Salles, P Soucaille, H Bottin, M Fontecave, S Roy, V Artero, C Baffert, V Fourmond, R B. Best, J Blumberger, L de Gioia, L Bertini & C Léger[a]

Aix Marseille Univ, CNRS, Laboratoire de Bioénergétique et Ingénierie des Protéines, Institut de Microbiologie de la Méditerranée, Marseille, France

leger@imm.cnrs.fr http://bip.cnrs-mrs.fr/bip06

Abstract

FeFe hydrogenases catalyze H_2 oxidation and formation at an inorganic active site (the "H-cluster"), which consists of a $Fe_2(CO)_3(CN)_2$ (dithiomethylamine)] subcluster covalently attached to a Fe_4S_4 subcluster (fig 1). This active site is sensitive to light and small ligands such as O_2. Studying the effect of inhibitors is a common approach for learning about the reactivity of enzymes' active sites; in the case of hydrogenase, understanding these reactions is particularly important because they may negatively impact the use of hydrogenase for the photoproduction of H_2. Here we show that combining theoretical methods, site-directed mutagenesis and electrochemical kinetics[1] is a powerful approach for understanding at a molecular level how oxygen[2] and light[3] affect the active site and the activity of hydrogenase.

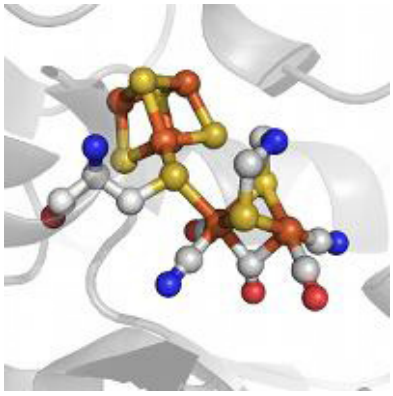


Figure 1. The $[Fe_2(CO)_3(CN)_2$(dithiomethylamine)] active site of FeFe hydrogenase.

References

1) (a) V Fourmond and C. Leger, "Modelling the voltammetry of adsorbed enzymes and molecular catalysts", **Curr. Op. Electrochem** (2017) (b) V. Fourmond and C. Leger "Protein Electrochemistry: Questions and Answers" **Adv Biochem Eng Biotechnol**. (2016)

2) (a) A Kubas, C Orain, D De Sancho, L Saujet, M Sensi, C Gauquelin, I Meynial-Salles, P Soucaille, H Bottin, C Baffert, V Fourmond, R Best, J Blumberger, C Leger, "Mechanism of O2 diffusion and reduction in FeFe hydrogenase", **Nature Chemistry** 9, 88–95 (2017). (b) C Orain, L Saujet, C Gauquelin, P Soucaille, I Meynial-Salles, C Baffert, V Fourmond, H Bottin, and C Leger "Electrochemical measurements of the kinetics of inhibition of two FeFe hydrogenases by O2 demonstrate that the reaction is partly reversible", **J. Am. Chem. Soc.** 137, 12580-12587 (2015)

3) M Sensi, C Baffert, C Greco, G Caserta, C Gauquelin, L Saujet, M Fontecave, S Roy, V Artero, P Soucaille, I Meynial-Salles, H Bottin, L de Gioia, V Fourmond, Christophe Leger and L Bertini, "Reactivity of the excited states of the H-cluster of FeFe hydrogenases," **J. Am. Chem. Soc.** 138 13162-13618 (2016)

Protein crystal as mesoporous material for catalysis.

Stéphane Ménage, [a]Sarah Lopez, [a]Christine Cavazza,[b] Caroline Marchi-Delapierre. [a]

[a]BioCE and [b] BioCat teams, Laboratory Chemistry and Biology of Metals, CNRS-CEA-UGA,Grenoble F38000 ; email : stephane.menage@cea.fr

Abstract

The search for new catalysts represents still a great challenge for sustainable chemistry. While heterogeneous catalysis has been very successful, the heterogeneization of enzymes remains a great opportunity since enzymeshave reached highest efficiencies and selectivities but suffers from low stability. In the 90's, a new technology has emerged to solve this drawback, based on the cross linking methodology applied to enzyme crystals (CLEC), using the mesoporous properties of protein crystals.[1]While CLECs were successfully used for hydrolytic or reduction catalysis, none were used in the domain of oxidation. In our teamLCBM/BioCE, we combine inorganic and bio-catalyisto develop artificial enzymes, issued of the insertion of an inorganic complex into a host protein.[2] Here, we will combine heterogeneous and bio-catalysis by developingCLECs of artificial enzymes. Few years ago, O_2 reductive activation by an iron complex embeddedin a crystal of NikA, a Ni transport protein, wasobservedbut only the iron ligand was oxidized.[3] Today, by modifying the artificial active site iron complex, we have mastered a catalytic version for alkene oxidative cleavage. Furthermore, the use of other oxidants allowed us to reach selective and efficient catalysis for the oxychlorination of alkenes (6000 TONs, 1000 $TON.h^{-1}$ with high stereospecificity). During the presentation, the two catalytic systems will be described, the role of the embedded iron complex revealed and insights on reactions mechanism proposed thanks to protein crystallography. We will also demonstrate the high stability of protein crystals upon exposition to organic solvent, oxidizing conditions and high temperatures. These two examples of "*in cristallo*" catalysis represent a new development in the search for green catalysis, combining robustness, selectivity, easy recovery and the use of less hazardous substances.

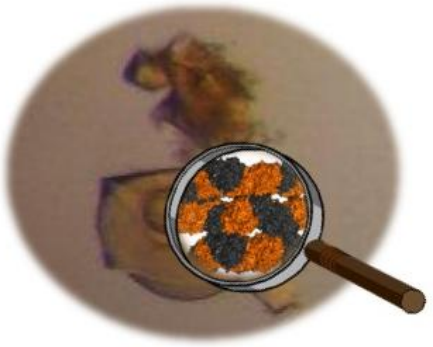
We thank ARCANE Labex and ANR for financial support.

References

1) L. Z. Vilenchik, J. P. Griffith, N. St. Clair,M. A. Navia, A. L. Margolin *J.Am. Chem..Soc,* 4290-94. b) N. St. Clair, Y.-F. Wang, A. L. Margolin, *Angew. Chem. Int. Ed.*, 2000, 39, 380-383.

2) a)C. Esmieu, M.V. Cherrier; P. Amara, E. Girgenti, C. Marchi-Delapierre, F.Oddon, M. Iannello, A. Jorge-Robin, C. Cavazza, S. Ménage, S.*Angew. Chem. Int Ed.* 2013, 52, 3922-3925. b) P. Rousselot-Pailley, C. Bochot, C. Marchi-Delapierre, A. Jorge-Robin, L. Martin,J. C. Fontecilla-Camps, C. Cavazza, S. Ménage *ChemBioChem*2009, *10*, 545-552.

3) C. Cavazza, C. Bochot, P. Rousselot-Pailley, P. Carpentier, M. V. Cherrier, L. Martin, C. Marchi-Delapierre, J. C. Fontecilla-Camps, S. Ménage*Nature Chemistry*2010, 2, 1069-1076.



Biomimetic Hydroxylation Catalysis mediated by new Tyrosinase Model Complexes

Patricia Liebhäuser, [a] Lena Ebert, [a] Alexander Hoffmann, [a] Sonja Herres-Pawlis [a]

[a] *RWTH Aachen University, Institute for Inorganic Chemistry, Landoltweg 1, 52074 Aachen ;*
email : sonja.herres-pawlis@ac.rwth-aachen.de

Abstract

The majority of copper enzymes in biological systems directly activate dioxygen to perform a myriad of essential chemical transformations almost exclusively performed with copper. Tyrosinases (Ty), ubiquitously found in both eukaryotes and prokaryotes, are well recognized examples that perform the first committed step in the synthesis of melanine from tyrosine by catalytic hydroxylation of the phenol to a catechol by activating O_2. Chemical model complexes extract the chemical reactivity of Ty to small molecules which allows a better investigation of structural variations and their influences on reactivity as well as the translation to industrially useful processes.

As ligand systems, we use guanidines[1-3] and bis(pyrazolyl)methanes[4-7] in order to stabilize bis(μ-oxo) and peroxo complexes which are both discussed as active Ty species. In our ongoing studies, we developed versatile new ligands to explore new ways to the catalytic hydroxylation of various phenolates. We found that subtle changes in the ligand sphere have a dramatic influence on the stability of the Cu_2O_2 species as well as on its catalytic reactivity. Especially the donor difference between pyrazolyl and pyridinyl units is crucial to obtain the catalytic activity (Figure 1).[7]

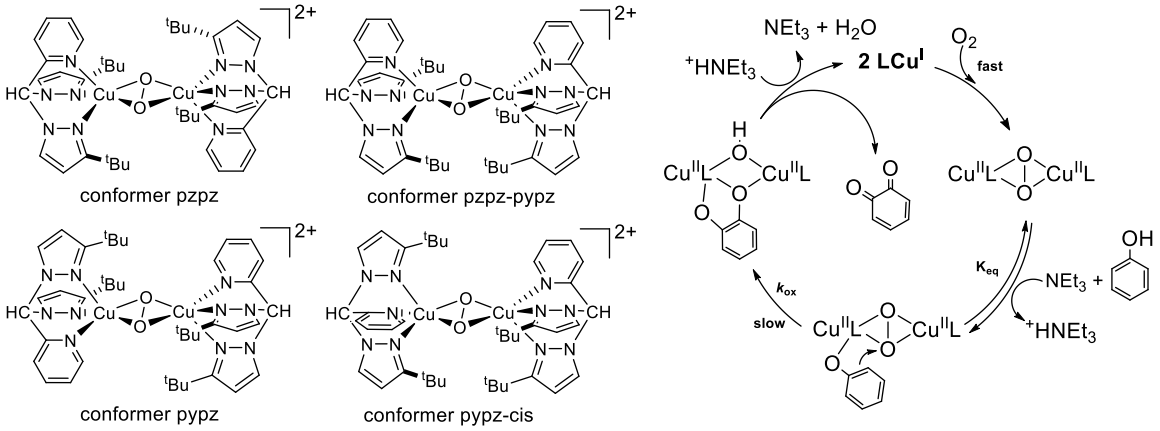


Figure 1. Four possible conformers of $[Cu_2O_2\{HC(3^tBuPz)_2(Py)\}_2]^{2+}$ and catalytic cycle

References

1) S. Herres-Pawlis, R. Haase, P. Verma, A. Hoffmann, P. Kang, T. D. P. Stack, Eur. J. Inorg. Chem. 2015, 5426–5436.
2) M. Rohrmüller, A. Hoffmann, C. Thierfelder, S. Herres-Pawlis, W. G. Schmidt, J. Comput. Chem. 2015, 36, 1672–1685.
3) A. Hoffmann, M. Wern, T. Hoppe, M. Witte, R. Haase, P. Liebhäuser, J. Glatthaar, S. Herres-Pawlis, S. Schindler, Eur. J. Inorg. Chem. 2016, 4744–4751.
4) A. Hoffmann, C. Citek, S. Binder, A. Goos, M. Rübhausen, O. Troeppner, I. Ivanović-Burmazović, E. C. Wasinger, T. D. P. Stack, S. Herres-Pawlis, Angew. Chem. Int. Ed. 2013,52, 5398-5401.
5) A. Hoffmann, S. Herres-Pawlis, Chem. Commun. 2014, 50, 403-405.
6) C. Wilfer, P. Liebhäuser, A. Hoffmann, H. Erdmann, O. Grossmann, L. Runtsch, E. Paffenholz, R. Schepper, R. Dick, M. Bauer, M. Dürr, I. Ivanovic-Burmazovic, S. Herres-Pawlis, Chem. Eur. J. 2015, 21, 17639 – 17649.
7) A. Hoffmann, S. Herres-Pawlis, Phys. Chem. Chem. Phys. 2016, 18, 6430—6440.

Influence of Cysteinate Protonation on the Electronic Structure and Function of Nickel Containing Metalloenzymes

Jason Shearer and Brenna Keegan

Department of Chemistry, University of Nevada, Reno, NV 89557, USA; email : shearer@unr.edu

Abstract

Nickel containing metalloenzymes are found in a wide variety of microorganisms. All but one of the redox active nickel containing metalloenzymes that have been discovered to date possess cysteinate ligation. Two of these cysteinate-ligated metalloenzymes are nickel superoxide dismutase (NiSOD), which catalyses superoxide disproportionation, and nickel iron hydrogenase ([NiFe]H_2ase), which catalyses proton reduction to H_2. Recent spectroscopic and structural analyses of these two enzymes has revealed that at least one of the cysteinate sulphur atoms coordinated to their nickel centres is protonated, forming the Ni^{II}-$S(H^+)$-Cys moiety.[1,2] The function(s) of this moiety in both NiSOD and [NiFe]H_2ase is not known. The present study discloses a nickel-containing metallopeptide-based system, {Ni(m1S_3)} (m1S_3 = T^aCDLP-CGVYD-PA; T^a = thioacetamide), which contains Ni^{2+} in a square planar S_3N coordination environment. We will provide evidence that this system undergoes cysteinate sulphur atom protonation events at physiological pH, forming two Ni^{II}-$S(H^+)$-Cys moieties. Formation of these Ni^{II}-$S(H^+)$-Cys moieties dramatically alters the peptide's nickel-binding affinity and the resulting metallopeptide's stability towards reactive oxygen species. A detailed spectroscopic (optical, X-ray absorption, and X-ray emission spectroscopy) and computational investigation of the protonated and unprotonated forms of {Ni(m1S_3)} will show that cysteinate protonation dramatically influences the electronic structure of the metal-centre. The differential behaviour of the protonated and unprotonated forms of {Ni(m1S_3)} can be readily rationalized by the changes in electronic structure. Based on this study it will be suggested that, among other functions, the M-$S(H^+)$-Cys moiety is involved in the protection of metal-sites from oxidative damage and the promotion of metal-ion transfer in metal transporter proteins.

References

1) Ogata, H.; Nishikawa, K.; Lubitz W. *Nature* **2015**, *520*, 571
2) Szilagyi, R.K.; Bryngelson, P.A.; Maroney, M.J.; Hedman, B.; Hodgson, K.O.; Solomon, E.I. *J. Am. Chem. Soc.* **2004**, *126*, 3018.

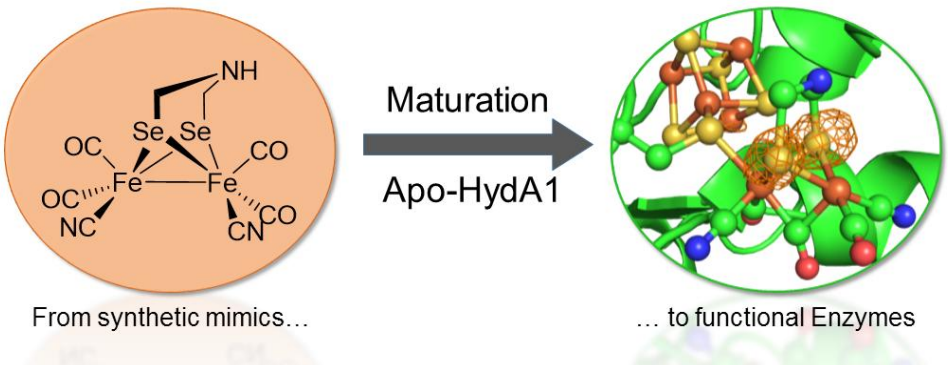
A Fully Active Semiartificial [FeFe]-Hydrogenase

F. Wittkamp[a], L. Kertess[b], C. Sommer[c], J. Esselborn, E. Reijerse[c], W. Lubitz[c], T. Happe[b], M. Winkler[b], U.-P. Apfel[a]

[a)] Ruhr-Universität Bochum, Anorganische Chemie I / Bioanorganische Chemie, Universitätsstraße 150, 44801 Bochum; [b)] Ruhr-Universität Bochum, Biochemie der Pflanzen, Universitätsstraße 150, 44801 Bochum; [c)] Max-Planck-Institut für chemische Energiekonversion, Stiftstraße 34-36, 45470 Mülheim an der Ruhr ; email : ulf.apfel@rub.de

Abstract

The recent finding that fully active [FeFe]-Hydrogenases can be obtained via maturation of the apo-protein with the synthetic [2Fe-2S] cofactor enables the targeted alteration of the native enzymes and allows for more in-depth mechanistic studies.[1,2] Recently, different cofactor mimics of the type $[Fe_2(CO)_4(CN)_2\{(SCHR)_2X\}]^{2-}$ (R = H, CH_3, X = NH, CH_2, O, S) were tested regarding their catalytic activity inside HydA1 of *C. reinhardtii*. However, solely $[Fe_2(CO)_4(CN)_2\{(SCH_2)_2NH\}]^{2-}$, the synthetic precursor of the natural cofactor, revealed full wild type activity and allowed for proton reduction and hydrogen oxidation.[3] This observation clearly highlights the importance of the proton-shuffling amine-group. We recently reported on the S/Se exchange in the [4Fe-4S] cluster attached to the di-iron active site. This assembly revealed wild-type like activity and suggested high tolerance of the active site towards electronic manipulations.[4] The next straightforward step was to alter the chemistry of the [2Fe2S] cofactor by S/Se exchange. Herein, we report on the synthesis of $[Fe_2(CO)_4(CN)_2\{(SeCH_2)_2NH\}]^{2-}$ and its incorporation as well as reactivity in the natural binding pocket of HydA1 of *C. reinhardtii* and Cp1 of *C. pasteurianum*. These semiartificial enzymes are the first fully active [FeFe]-hydrogenases containing a non-natural cofactor and revealing a wildtype like activity.



References

1) G. Berggren, A. Adamska, C. Lambertz, T. R. Simmons, J. Esselborn, M. Atta, S. Gambarelli, J.-M. Mouesca, E. Reijerse, W. Lubitz, T. Happe, V. Artero, M. Fontecave, *Nature* **2013**, *499*, 66.

2) J. Esselborn, C. Lambertz, A. Adamska-Venkatesh, T. Simmons, G. Berggren, J. Noth, J. Siebel, A. Hemschemeier, V. Artero, E. Reijerse, M. Fontecave, W. Lubitz, T. Happe, *Nat. Chem. Biol.* **2013**, *9*, 607.

3) J. F. Siebel, A. Adamska-Venkatesh, K. Weber, S. Rumpel, E. Reijerse, W. Lubitz, *Biochemistry* **2015**, *54*, 1474.

4) J. Noth, J. Esselborn, J. Güldenhaupt, A. Brünje, A. Sawyer, U.-P. Apfel, K. Gerwert, E. Hofmann, M. Winkler, T. Happe, *Angew. Chem. Int. Ed.* **2016**, *55*, 8396.

Biosynthesis of carbon monoxide dehydrogenase, a key enzyme of the water-gas shift reaction: toward the biological upgrading of syngas

Christine Cavazza [a], Marila Alfano[a], Julien Perard[a], Christian Basset[a], Barbara Zambelli[b], Stefano Ciurli[b]

[a] *Laboratoire de Chimie et Biologie des Métaux-Biocatalysis, UMR 5249, CEA-UGA-CNRS, Grenoble, France;* [b] *Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy. email : christine.cavazza@cea.fr*

Abstract

The gasification of biomass results in the production of syngas, mainly composed of CO, CO_2 and H_2. Syngas is considered to be an inexpensive and versatile substrate for the production of a variety of renewable fuels and chemicals with different H_2/CO ratios through the Fischer-Tropsch process. The Water Gas Shift reaction (WGSR, $CO + H_2O \rightarrow CO_2 + H_2$) is one of the most important reactions used to balance H2/CO ratios. In the perspective of a green economy, the development of biomass gasification requires the development of biological WGSR. In biological systems, CODH and [NiFe]-hydrogenase are key components of WGSR. CODH is a complex metalloenzyme with a unique [$NiFe_3S_4$] active site[1,2] (Figure 1). Although the mechanism is well studied[3], not much is known about the biosynthesis of the C-cluster. A better understanding of its biosynthesis is a starting point for its use as a potential tool in biological syngas purification processes.

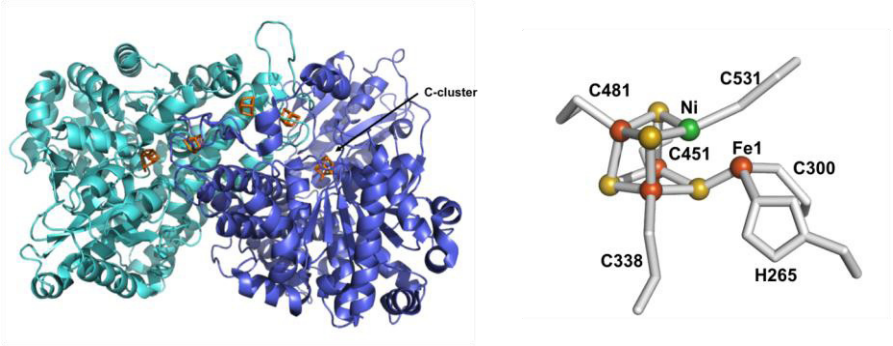


Figure 1: Structure of *R. rubrum* CODH at 2.8 Å (PDB code: 1jqk) and its active site

CooC, CooT and CooJ from *Rhodospirillum rubrum* are accessory proteins, essential for nickel insertion into CODH. CooC is proposed to carry out the ATP-dependent insertion of nickel into the C-cluster but the mechanism of nickel insertion remains elusive. The roles of CooT and CooJ are even less well understood. Recently, we structurally and biochemically characterized CooT and highlighted the existence of a novel Ni-binding protein family. Here, we report the development of in vivo and vitro maturation protocols of CODH in the easy-to-grow bacterium *Escherichia coli* as well as the functional study of the Coo proteins.

References

1) H. Dobbek; V. Svetlitchny; L. Gremer; R. Huber; O. Meyer **(2001)**, Science, 293, 1281.
2) C.L. Drennan; J. Heo; M.D. Sintchak; E. Schreiter; P.W. Ludden **(2001)** Proc. Nati. Acad Sci. USA, 98, 11973
3) M.W. Ribbe **(2015)**, Angew. Chem. Int. Ed., 54, 8337.

Hemoprotein reconstituted with a manganese porphycene complex toward catalytic hydroxylation of inert C–H bonds

Koji Oohora,[a,b] Kosuke Nitta,[a] Natsuno Chiba,[a] Hiroyuki Meichin,[a] Takashi Hayashi[a]

[a] *Graduate School of Engineering, Osaka University,* [b] *PRESTO JST; email : oohora@chem.eng.osaka-u.ac.jp*

Abstract

Hemoprotein is one of the promising scaffolds to create artificial metalloenzyemes because of the diversity of structures and the specific reaction fields in the intrinsic cofactor binding sites. Whereas metalloenzymes generally have large molecular weight (over 40 kDa), myoglobin (Mb), an oxygen storage hemoprotein, has relatively smaller molecular weight (17 kDa), indicating a suitable protein to be engineered. However, Mb shows very low peroxidase and monooxygenase activities and does not catalyze alkane hydroxylation despite including the same cofactor, heme b, as heme-dependent enzymes such as horseradish peroxidase and cytochrome P450. In this decade, our group has reported artificial metalloproteins by hemoprotein reconstitution with artificial cofactors (Figure 1). Recently, we focus on Mb reconstituted with a manganese porphycene complex (MnPc) to catalyze alkane hydroxylation.[1]

Mb reconstituted with MnPc (rMb) was successfully obtained by the conventional method. The obtained protein was identified with UV-vis absorption spectroscopy and mass spectrometry. Furthermore, the X-ray structure reveals the incorporation of MnPc into the intrinsic heme binding site and the axial ligation by His93, which is the axial ligand of heme in native Mb. The reaction of rMb with mCPBA produced the spectroscopically detectable intermediate ($t_{1/2}$ > 40 s) at 10 °C and pH 8.5. Since no characteristic near-infrared absorption for radical cation of the porphycene framework and no signal in X-band EPR spectra obtained with both of perpendicular and parallel modes was observed, the intermediate was assigned as a Mn(V)-oxo species. In addition, we carried out the H_2O_2-dependent hydroxylation of ethylbenzene by rMb. From the GC analysis, it is found that rMb catalyzes the hydroxylation to provide 1-phenylethanol with the turnover number of 13 at 25 °C and pH 8.5 without any by-product such as ketone, whereas native Mb and other reconstituted proteins show no product under the same condition. In addition, kinetic data, log k_{obs} versus BDE(C(sp^3)–H) for ethylbenzene, toluene, and cyclohexane present a linear relationship with negative slope, showing that the reaction occurs via a hydrogen-atom abstraction by a Mn(V)-oxo species as a rate-determining step.

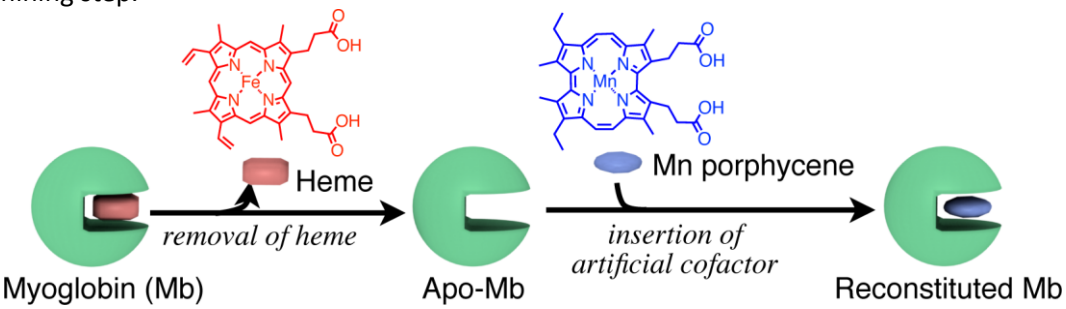


Figure 1. Reconstitution of myoglobin with an artificial cofactor.

References

1) K. Oohora, Y. Kihira, E. Mizohata, T. Inoue, T. Hayashi, *J. Am. Chem. Soc.* **2013**, *135*, 17282–17285.

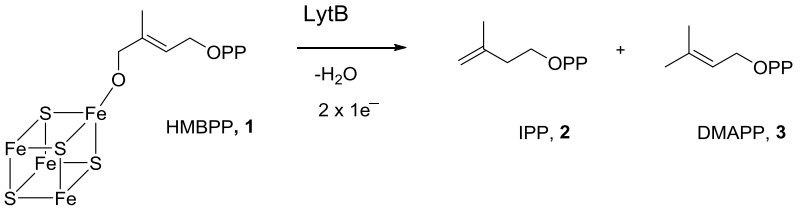
LYTB, A METALLOENZYME TARGET FOR THE DEVELOPMENT OF NEW ANTIMICROBIALS

P. Chaignon,[a] C. S. Müller,[c] M. Parisse,[a] C. D. Poulter,[b] V. Schünemann,[c] M. Seemann[a]

[a] Université de Strasbourg, CNRS, CHIMIE UMR 7177, F-67000 Strasbourg, France; [b] Department of Chemistry, University of Utah, 315 South 1400 East RM 2020, Salt Lake City, Utah 84112, United States; [c] *Fachbereich Physik, TU Kaiserslautern, Erwin-Schrödinger-Strasse 46, D-67653 Kaiserslautern, Germany;*
email :mseemann@unistra.fr

Abstract

LytB (also called IspH) is an essential enzyme for the survival of most bacteria including *Escherichia coli, Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Vibrio cholerea* but also of the parasite *Plasmodium falciparum* (responsible for malaria). LytB does not exist in humans and is therefore a valuable target for the development of new specific antibacterial and antiparasitic drugs. This enzyme catalyzes the conversion of (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate **1** into isopentenyl diphosphate (IPP, **2**) and dimethylallyl diphosphate (DMAPP, **3**), the two building blocks of all isoprenoids. This reaction is unprecedented and involves two one-electron transfers and a water elimination. LytB is an oxygen-sensitive protein harboring *in vivo* a peculiar $[4Fe\text{-}4S]^{2+}$ center, with one iron linked to three inorganic sulfur atoms from the cluster and to two or three non-sulfur ligands (N and/or O) as shown by Mössbauer spectroscopy investigations [1]. Very recently, synchrotron-based nuclear resonance vibrational spectroscopy (NRVS) experiments, a technique that senses specifically vibrations involving iron, led to the identification of these unknown ligands as three water molecules [2].



Mössbauer investigations of LytB in presence of the HMBPP **1**, revealed also that the first step in the catalytic mechanism of LytB is the binding of HMBPP *via* its OH group to the $[4Fe\text{-}4S]^{2+}$ [1,3]. This feature was used to design new extremely potent inhibitors of LytB [3,4].

References

1) M. Seemann, K. Janthawornpong, J. Schweizer, L.H, Böttger, A. Janoschka, A. Ahrens-Botzong, E. Ngouamegne Tambou, O. Rotthaus, A.X. Trautwein, M. Rohmer, V. Schünemann. *J. Am. Chem. Soc.* **2009**, *131*, 13184

2) I. Faus, A. Reinhard, S. Rackwitz, J. A. Wolny, K. Schlage, H.-C. Wille, A. Chumakov, S. Krasutsky, P. Chaignon, C. D. Poulter, M. Seemann, V. Schünemann. *Angew. Chem. Int. Ed.* **2015**, *54*, 12584

3) A. Ahrens-Botzong, K. Janthawornpong, J. A. Wolny, E. Ngouamegne Tambou, M. Rohmer, S. Krasutsky, C.D. Poulter, V. Schünemann, M. Seemann. *Angew. Chem. Int. Ed*. **2011**, *50*, 11976

4) K. Janthawornpong, S. Krasutsky, P. Chaignon, M. Rohmer, C.D. Poulter, M. Seemann. *J. Am. Chem. Soc.* **2013**, *135*, 1816

Inorganic Complexes for Applications in Biology: Mn-Complexes as SOD mimics from Design to Evaluation in Cells

Clotilde Policar,[a] Emilie Mathieu,[a] Anne-Sophie Bernard,[a] Elodie Quévrain,[a] Nicolas Delsuc,[a] Barry Lai,[c] Philippe Seksik, [a, b] Joelle Masliah [a, b]

[a] Departement de Chimie, Ecole Normale Superieure, PSL Research University, UPMC Univ Paris 06, CNRS, Laboratoire des Biomolecules (LBM), 24 rue Lhomond, 75005 Paris, France ; [b] CNRS, INSERM, APHP, INRA, Laboratoire des Biomolecules (LBM), 27 rue de Chaligny, 75012 Paris,:[c] X-ray Science Division, Argonne National Laboratory, Argonne, Illinois 60439, United States.

clotilde.policar@ens.fr

Superoxide dismutases are redox active metalloproteins that protect the cell against oxidative stress. These enzymes are highly efficient in catalyzing the dismutation of superoxide, with several physico-chemical parameters that have been evolutionarily optimized.[1] In this talk, the bio-inspired strategy that we use to design Mn-based SOD mimics will be presented. A wide range of SOD-mimics have been studied in the last decade,[2-4] that show intrinsic SOD-activity in an *in vitro* context,[5] some of which also demonstrate anti-oxidant properties in cells or in animals models.[3] From these studies we have learned that an SOD-mimic can be active outside a cellular context and be inactive within cells or a whole organism. Successful implementation into cellular and biological environments is thus key to biomedical applications.[6] We will present two cellular models in which we have assayed the activity of an SOD-mimic (**1**). Activated macrophages produce ROS and RNS fluxes under appropriate activation, and they constitute a relevant model in which to test the cellular efficacy of a biologically-relevant antioxidant. In this system, compound **1** demonstrated the ability to diminish the flux of superoxide.[5] In the second model, we used intestinal epithelial cells HT29 that have been modified to be very sensitive to bacterial lipopolysaccharide with a high inflammatory response. Compound **1** was studied in this cell line using an *inorganic cellular chemistry* approach that combined an investigation of its intracellular speciation using mass spectrometry, its quantification and distribution using electron paramagnetic resonance, and spatially-resolved X-ray fluorescence with evaluation of its biological activity. Interestingly, **1** shows intracellular anti-inflammatory activity, remains at least partially coordinated, has a diffuse distribution over the whole cell, and functionally complements mitochondrial MnSOD.[6] This is promising in the context of oxidative stress induced diseases inflammatory diseases.

References

1. Policar, C., Mimicking SODs, Why and How: Bio-Inspired Manganese Complexes as SOD Mimics. In *Redox Active Therapeutics*, Reboucas, J. S.; Batinic-Haberle, I.; Spasojevic, I.; Warner, D. S.; St. Clair, D., Eds. Springer: 2016; pp 125-164.
2. Iranzo, O., Manganese complexes displaying superoxide dismutase activity: A balance between different factors. *Bioorg. Chem.* **2011,** *39*, 73-87.
3. Miriyala, S.; Spasojevic, I.; Tovmasyan, A.; Salvemini, D.; Vujaskovic, Z.; St. Clair, D.; Batinic-Haberle, I., MnSOD and its mimics. *Biochim. Biophys. Acta* **2012**, 794-814.
4. Durot, S.; Policar, C.; Cisnetti, F.; Lambert, F.; Renault, J.-P.; Pelosi, G.; Blain, G.; Korri-Youssoufi, H.; Mahy, J.-P., Series of Mn Complexes Based on N-Centered Ligands and Superoxide – Reactivity in an Anhydrous Medium and SOD-Like Activity in an Aqueous Medium Correlated to MnII/MnIII Redox Potentials. *Eur. J. Inorg. Chem.* **2005**, 3513-3523.
5. Bernard, A.-S.; Giroud, C.; Ching, H. Y. V.; Meunier, A.; Ambike, V.; Amatore, C.; Guille Collignon, M.; Lemaître, F.; Policar, C., Evaluation of the Anti-Oxidant Properties of a SOD-mimic Mn-Complex in Activated Macrophages. *Dalton Trans.* **2012,** *41*, 6399-6403.
6. Mathieu, E.; Bernard, A.-S.; Delsuc, N.; Quevrain, E.; Gazzah, G.; Lai, B.; Chain, F.; Langella, P.; Bachelet, M.; Masliah, J.; Seksik, P.; Policar, C., A cell penetrant manganese SOD-mimic is able to complement MnSOD and exerts an anti-inflammatory effect on cellular and animal models of inflammatory bowel diseases. *Inorg. Chem.* **2017,** *56*, 2545-2555.

Substrate specificity and regioselectivity of polysaccharide monooxygenases

Van V. Vu[a]

[a]*NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam ; email : vanvu@ntt.edu.vn*

Abstract

Polysaccharide monooxygenases (PMOs) were recently discovered enzymes that degrade recalcitrant polysaccharides in an unprecedented oxidative mechanism. PMOs activate oxygen with a Type-2 monocopper active site on an open protein surface and hydroxylate the C-H bonds of the glycosidic linkage. The resulting hydroxylated intermediates likely spontaneously undergo elimination, which breaks the glycosidic linkage. The oxidative cleavage highly likely occurs on the surface of the substrates without the need to separate the polysaccharide chains from insoluble matrix as observed in canonical glycoside hydrolases (GHs). The newly created chain ends can be further processed by GHs. Some cellulose-active PMOs exhibit remarkable synergy with GHs, which could reduce significantly the cost of enzymatic technology for biomass conversion. The regioselectivity and substrate specificity of PMOs are important aspects in developing optimal synergistic mixtures of PMOs and GHs. We employed rigorous bioinformatics and biochemical analyses to classify cellulose-active PMOs in to three subgroups with distinct regioselectivity, as well as determined some protein motifs governing their regioselectivity [1]. We recently discovered a novel PMO family that acts on α(1→4) glycosidic linkages of starch rather than the β(1→4) glycosidic linkages of cellulose and chitin as observed in the well-studied cellulose-active PMOs and chitin-active PMOs. This finding showed that PMOs are diverse and have a broad range of activities [2]. Further studies involving spectroscopic analysis, biochemical assays, and molecular dynamic simulations are underway to gain further insights in to the substrate specificity and regioselectivity of PMOs.

References

1. Vu, V.V.; Beeson, W.T.; Phillips, C.M.; Cate, J.D.H; Marletta, M.A., Determinants of Regioselective Hydroxylation in the Fungal Polysaccharide Monooxygenases, *J Am Chem Soc,* **2014***, 136*, 562-5.

2. Vu, V.V.; Beeson, W.T.; Span, E.A.; Farquhar, E.R.; Marletta, M.A., A family of starch-active polysaccharide monooxygenases, *Proc Natl Acad Sci USA,* **2014***, 111*, 13822-27.

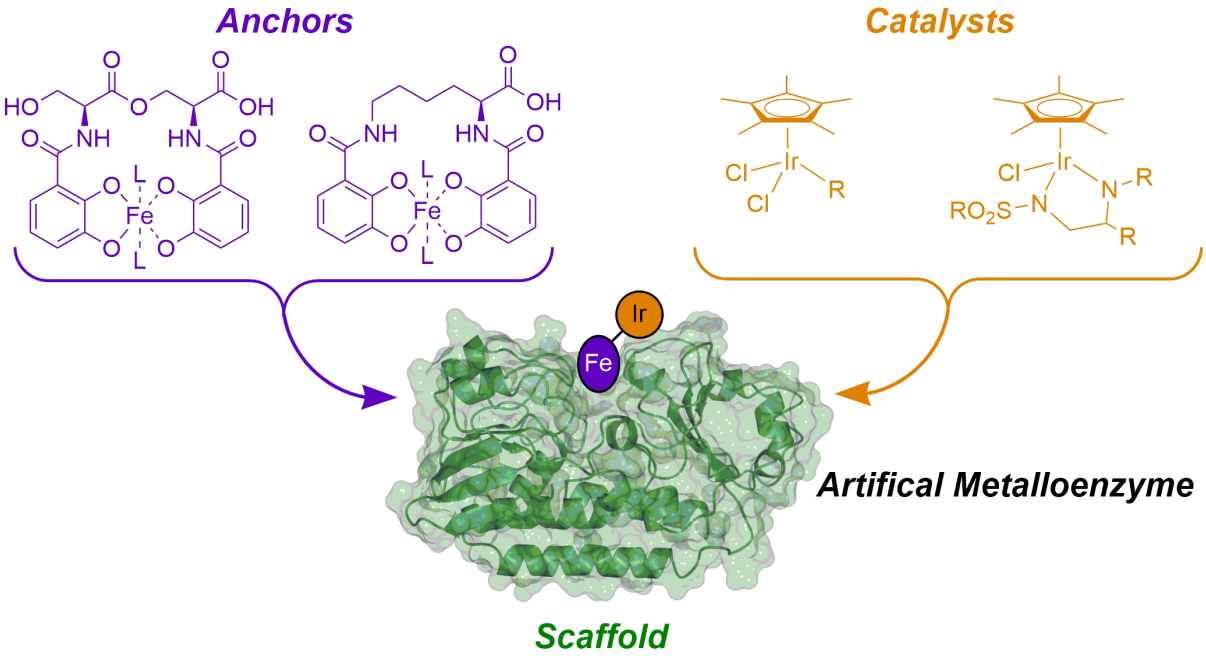
Artificial metalloenzyme design: Exploring siderophore-catalyst systems

Daniel J. Raines,[a] Elena V. Blagova,[b] Justin Clarke,[a,b] Kevin Hohl,[a] Olga V. Moroz,[b] Johan P. Turkenburg,[b] Ellis J. Wilde,[a,b] Keith S. Wilson,[b] and Anne-Kathrin Duhme-Klair [a]

[a] *Department of Chemistry, University of York, Heslington, York YO10 5DD, UK;* [b] *York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, UK: email:daniel.raines@york.ac.uk*

Abstract

Research towards novel artificial enzymes has drawn much attention in recent years with several strategies existing for their development. Incorporation of organometallic centres within a protein scaffold lends itself to an attractive solution to this problem. Key to this is the identification of new protein-catalyst hybrid systems which lend themselves towards development of systems able to catalyse synthetically relevant reactions with rates and selectivities of enzymes.[1]



Here we present the development of such an artificial metalloenzyme using the siderophore binding protein CeuE (*C. jejuni*),which binds tetradentate siderophores with high affinity,[2-4]and the exploration of different siderophore anchoring, and catalyst moieties, designed to optimize the first generation of artificial enzymes based on this system.

References

1) M. Hoarau, C. Hureau, E. Gras, P. Faller, *Coord. Chem. Rev.*, **2016**, *308*, 445-459.
2) D. J. Raines, O. V. Moroz, E. V. Blagova, J. P. Turkenburg, K. S. Wilson, A. K. Duhme-Klair, *Proc. Nat. Acad. Sci.*, **2016**, *113*, 5850-5855.
3) D. J. Raines, O. V. Moroz, K. S. Wilson, A. K. Duhme-Klair *Angew. Chem. Int. Ed.*, **2013**, *52*, 4595-4598
4) E. J. Wilde, A. Hughes, E. V. Blagova, O. V. Moroz, R. P. Thomas, J. P. Turkenburg, D. J. Raines, A. K. Duhme-Klair, K. S. Wilson, *Sci. Rep.,* **2017**, Accepted.

Photoelectrochemistry of the water oxidation enzyme Photosystem II: From basic understanding to semi-artificial photosynthesis

Erwin Reisner

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK; email : reisner@ch.cam.ac.uk, Web: http://www-reisner.ch.cam.ac.uk.

Abstract

In natural photosynthesis, light is used for the production of chemical energy carriers to fuel biological activity and the first protein in the photosynthetic chain is the water oxidation enzyme Photosystem II. This presentation will summarise our progress in the development of protein film photoelectrochemistry as a technique for the light-dependent activity of this enzyme adsorbed onto an electrode surface to be studied.[1] Materials design enabled us to develop 'tailor-made' 3D electrode scaffolds for optimised integration of the 'wired' enzyme and these investigations yielded valuable insights into the performance of Photosystem II and interfacial charge transfer pathways. Examples are the identification of unnatural electron escape routes to the electrode and a recently elucidated O_2 reduction pathway that short-circuits the known water-oxidation process.[2]

The integration of Photosystem II in a photoelectrochemical circuit has also enabled the *in vitro* re-engineering of natural photosynthetic pathways. We succeeded in assembling an efficient enzyme-based full water splitting cell driven by light through the rational wiring of Photosystem II to a [NiFeSe]-hydrogenase.[3] This hydrogenase displays unique properties for water splitting applications as it displays good H_2 evolution activity, little product (H_2) inhibition and some tolerance towards O_2.[4] The semi-artificial water splitting cell shows how we can harvest and utilise electrons generated during water oxidation at Photosystem II electrodes for the generation of renewable H_2 with a wired hydrogenase through a direct pathway unavailable to biology. This work is currently supported by an ERC Consolidator Grant 'MatEnSAP' (682833).

References

[1] Kato, Zhang, Paul & Reisner, *Chem. Soc. Rev.*, **2014**, *43*, 6485–6497.

[2] Zhang, Sokol, Paul, Romero, van Grondelle, & Reisner, *Nature Chem. Biol.*, **2016**, *12*, 1046–1052.

[3] Mersch, Lee, Zhang, Brinkert, Fontecilla-Camps, Rutherford & Reisner *J. Am. Chem. Soc.*, **2015**, *137*, 8541–8549.

[4] Wombwell, Caputo & Reisner, *Acc. Chem. Res.*, **2015**, *48*, 2858–2865.

Metals and biomolecules

Siderophores as anchors in artificial metalloenzymes

Daniel J. Raines[1], Justin E. Clarke,[1,2] Ellis J. Wilde[1,2], Elena V. Blagova[2], Keith S. Wilson[2] and

Anne-Kathrin Duhme-Klair[1]

[1]*Department of Chemistry, University of York, Heslington, York YO10 5DD, UK; email: akd1@york.ac.uk*

[2]*Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, UK*

Abstract

Artificial metalloenzymes that consist of a synthetic catalyst that is linked *via* an anchor to a protein scaffold allow the bio-orthogonal reactivity of organometallic catalysts to be combined with the selectivity of enzymes. A particular strength of this approach lies in the synthesis of enantiomerically pure compounds and the potential use of artificial enzymes *in vivo*, for example within the periplasm of a Gram-negative bacterial cell.[1] To complement the commonly used biotin-(streptavidin) affinity pair, we have identified ferric siderophore-binding protein pairs that allow organometallic catalysts to be anchored with suitably high affinities. Siderophores are ligands that are produced by bacteria for the uptake of essential iron and hence, ferric siderophore complexes are bound both strongly and enantioselectively by their cognate periplasmic binding proteins.[2, 3] By linking iridium-based transfer hydrogenation catalysts to selected siderophore-binding protein systems (Figure 1) the reduction of prochiral imines to chiral amines was achieved with encouraging levels of enantioselectivity.

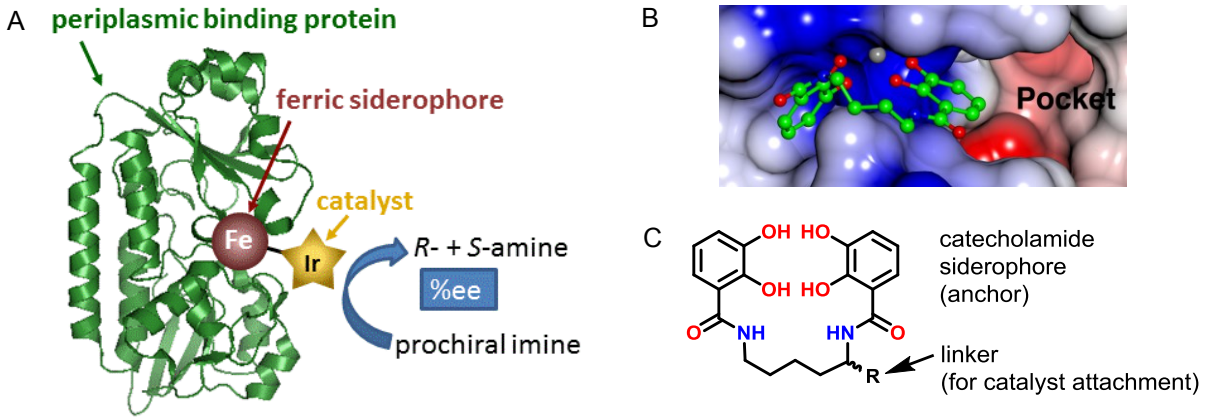


Figure 1: A) Schematic representation of an artificial metalloenzyme that consists of a periplasmic binding protein (PBP) scaffold (green) with bound ferric siderophore anchor (red) and attached transfer hydrogenation catalyst (orange). B) [Fe(siderophore)]⊂PBP crystal structure that highlights a chiral pocket that is available for the accommodation of a catalyst (PBP: electrostatic surface representation; Fe grey; siderophore: C green, O red, N blue). C) Example of a catecholamide siderophore derivative.

References

1) M. Jeschek, R. Reuter, T. Heinisch, C. Trindler, J. Klehr, S. Panke, T. R. Ward, *Nature*, **2016**, *537*, 661.
2) D. J. Raines, O. V. Moroz, K. S. Wilson and A. K. Duhme-Klair, *Angew. Chem. Int. Ed.*, **2013**, *52*, 4595.
3) D. J. Raines, O. V. Moroz, E. Blagova, J. P. Turkenburg, K. S. Wilson, A.-K. Duhme-Klair, *PNAS*, **2016**, *113*, 5850.

Bio(macro)molecule functionalization using iClick reactions: New tools for Inorganic Chemical Biology

Ulrich Schatzschneider[a]

[a] *Institut für Anorganische Chemie, Julius-Maximilians-Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany; email : ulrich.schatzschneider@uni-wuerzburg.de*

Abstract

For the regiospecific introduction of novel functionalities to bio(macro)molecules, bio-orthogonal reactions are required which do not show cross-reactivity with the normal constituents of biological systems and exhibit reaction kinetics faster than the biological processes of interest.[1,2] So far mostly a domain of bioorganic chemistry, the introduction of metal-based functionalities also holds great promise for applications in *Inorganic Chemical Biology* by exploration of metal-inherent properties such as interesting photophysical and photochemical characteristics, electrochemical bistability, tunable ligand-exchange kinetics, and availability of stable as well as radioactive isotopes.

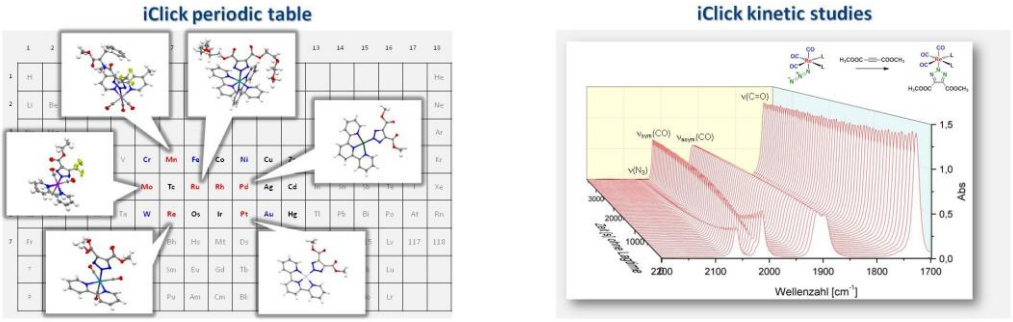


Figure 1. (left) "iClick" periodic table with compounds already prepared by room-temperature catalyst-free cycloadditions and (right) kinetics of the iClick reaction monitored by IR spectroscopy.

In that context, our group develops novel chemistry to quickly generate molecular diversity in metal complex-bio(macro)molecule conjugates.[3] Based on an innovative and flexible reaction between a metal-azide complex and an alkyne coupling partner in a [3+2] cycloaddition reaction, a particular highlight of our procedure is the formation of very robust triazolate linkers directly in the inner metal coordination sphere under retention of the stereochemistry, without the need for a catalyst, and in most cases at room temperature.

In this presentation, we will give a systematic insight in the structural and electronic preferences of the reaction together with kinetic data, including the trapping of intermediates at low temperature (-20 °C), which revealed 2nd order rate constants comparable to the Staudinger ligation reaction well-established in bioorganic chemistry.[1,2] Very recent results will also be presented on the application of the methodology to lyszoyme functionalization serving as a model protein.

References

1) C.R. Bertozzi, E.M. Sletten, Angew. Chem. Int. Ed. 2009, 48, 6974.
2) C.P. Ramil, Q. Lin, Chem. Commun. 2013, 49, 1107.
3) L. Henry, U. Schatzschneider, B. Mützel, P.V. Simpson, C. Nagel, K. Fucke, U. Schatzschneider, Chem. Commun. 2014, 50, 15692.

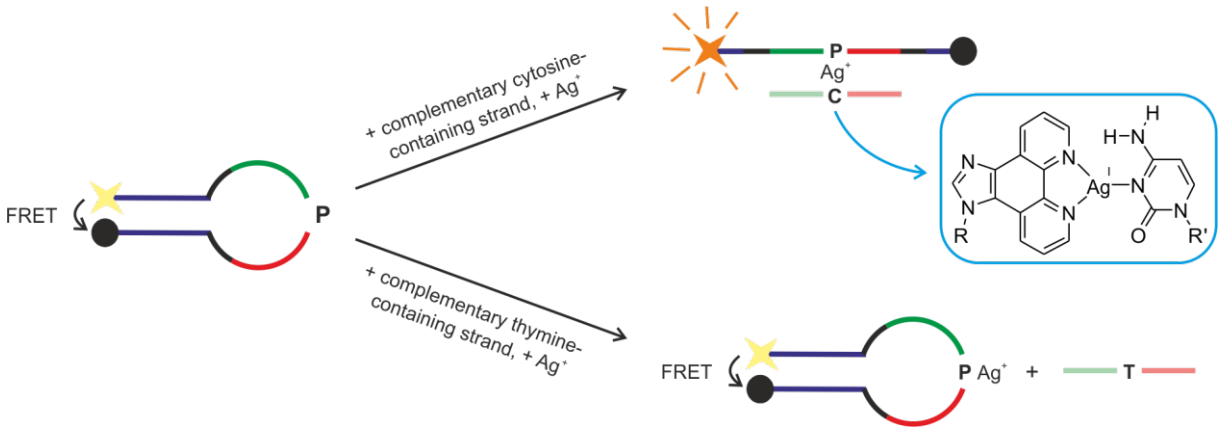
A metal-mediated base pair that discriminates between the canonical pyrimidine nucleobases

Biswarup Jash, [a] Philipp Scharf, [a] Célia Fonseca Guerra, [b] Dominik A. Megger, [c] Jens Müller [a]

[a] Institut für Anorganische und Analytische Chemie, Westfälische Wilhelms-Universität Münster, Corrensstraße 30, 48149 Münster, Germany; [b] Department of Theoretical Chemistry, Amsterdam Center for Multiscale Modeling (ACMM), VU Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands ; [c)] Medizinisches Proteom-Center, Ruhr-Universität Bochum, Universitätsstraße 150, 44801 Bochum, Germany. email: mueller.j@uni-muenster.de

The introduction of metal-based functionality into nucleic acids via artificial metal-meditated base pairing is a topical area of research that has attracted a lot of interest recently. Numerous applications are possible for the resulting conjugates, ranging from sensors to an exploitation of the modified charge transfer capabilities of nucleic acids.[1-3]

We report here the first successful application of metal-mediated base pairing in the sensing of natural oligonucleotide sequences.[4] Towards this end, 1*H*-imidazo[4,5-*f*][1,10]phenanthroline (**P**) was used as an artificial nucleobase. DNA oligonucleotides with a **P** nucleoside can be applied for the discrimination of the canonical nucleobases thymine and cytosine, as the respective silver(I)-mediated base pairs exhibit significantly different stabilities. A molecular beacon based on this approach has been developed.[5] Such a beacon can principally be applied for the detection of single-nucleotide polymorphisms in sequences with a medicinally relevant T→C or C→T transition.



References

1) Y. Takezawa, J. Müller, M. Shionoya, *Chem. Lett.* **2017**, *46*, in press (doi: 10.1246/cl.160985).
2) Y. Tanaka, J. Kondo, V. Sychrovský, J. Šebera, T. Dairaku, H. Saneyoshi, H. Urata, H. Torigoe, A. Ono, *Chem. Commun.* **2015**, *51*, 17343–17360.
3) G. H. Clever, C. Kaul, T. Carell, *Angew. Chem. Int. Ed.* **2007**, *46*, 6226–6236.
4) S. Taherpour, O. Golubev, T. Lönnberg, *Inorg. Chim. Acta* **2016**, *452*, 43–49.
5) B. Jash, P. Scharf, N. Sandmann, C. Fonseca Guerra, D. A. Megger, J. Müller, *Chem. Sci.* **2017**, *8*, 1337–1343.

Artificial siderophores: how can we exploit them?

Elzbieta Gumienna-Kontecka,[a] Evgenia Olshvang, [b] Jenny Besserglick,[b] and Abraham Shanzer[b]

[a]Faculty of Chemistry, University of Wroclaw, Wroclaw, Poland; [b] Weizmann Institute of Science, Rehovot, Israel;
email: elzbieta.gumienna-kontecka@chem.uni.wroc.pl

The difficulties in synthesis of structurally complicated natural siderophores (low molecular weight molecules produced by microorganisms to acquire iron) has directed the siderophore research towards biomimetic chemistry, aiming at mimicking or reproducing the function of the natural product rather than its detailed structure. This approach allowed diversifying the arsenal of biologically active siderophore-type molecules, introducing additional desired chemical and/or physical properties, and providing means to identify general motifs governing interplay between structure and function in biological activity.

Following these principles, we have been working on characterization of novel biomimetic compounds, artificial siderophores, in terms of iron complex formation and stability, for the construction of structural probes of microbial iron uptake processes [1, 2] and iron sensors.[3]

Over the years, we were able to couple iron binding or its release with a signaling component in order to elicit spectrophotometric signals. Attaching a fluorescent 6-aminonaphthalimide group at the carboxyl terminus of desferrioxamine biomimetic analogue provided a tool to track the path of the iron from the environment to the cells of Yersinia enterocolitica.[2] Moreover, inspired by L-vulnibactin - microbial siderophore from Vibrio vulnificus, we fused the iron(III) binding site with a fluorescent probe into a single functionality, and studied tripodal phenoloxazoline-based ligands. Due to their fluorescent properties, the Fe(III) coordination event could be easily monitored, with detection limits in the low ng/mL range.[3]

Here we will present Fe(III) binding properties of novel artificial siderophores in the perspective of using the compounds as tools for investigating iron uptake by siderophore-utilizing organisms. We will also discuss abilities of these biomimetics to coordinate other metal ions in order to use them for imaging and detection purposes.

Acknowledgements

Financial support by Polish National Science Centre (2015/19/B/ST5/00413) is gratefully acknowledged.

References

1) Olshvang et al, *Dalton Trans* 2015, 44, 20850.
2) Kornreich-Leshem et al, *J Am Chem Soc*, 2005, 127, 1137.
3) Kikkeri et al, *Inorg Chem*, 2007, 46, 7, 2485.

"on-off"
Metal ion binding and release in metallothioneins

Eva Freisinger

Department of Chemistry, University of Zurich, 8057 Zurich, Switzerland ; email : freisinger@chem.uzh.ch

Abstract

Metallothioneins (MTs) are ubiquitous proteins generally characterized by a low molecular weight, a large Cys content and, as a result, a high affinity for metal ions with d^{10} electron configuration that are organized in cluster structures. MTs are among others involved in the regulation of physiologically important Zn^{II} and Cu^{I} ions, the protection against xenobiotic heavy metal ions, e.g. Cd^{II} and Hg^{II}, and are linked to oxidative stress conditions due to the redox activity of the many Cys residues. While the thermodynamic constants for metal ion binding to MTs are high (e.g. $K = 3.1 \cdot 10^{11}$ M^{-1} for rabbit Zn_7MT2),[1] the kinetics of metal ion release are still fast enough (e.g. $k = 5.8 \cdot 10^{-4}$ s^{-1} for human Zn_7MT2)[2] to easily allow metal ion release and exchange processes to occur.

While the vertebrate isoforms, which strictly contain 20 Cys as sole ligands, have been in the focus of studies for 60 years now, MTs from other phyla add to the great variety of amino acid sequences for MTs we know today, showing different lengths, diverse amounts and distribution pattern of Cys residues, as well as sometimes even His residues as additional metal ion binding ligands. It will be the aim of this talk to provide a closer look at the stability of metal ion binding and the possibility of metal exchange processes in non-vertebrate MTs.

References

1) D. W. Hasler, L. T. Jensen, O. Zerbe, D. R. Winge, M. Vašák, Biochemistry, 2000, 39, 14567-14575.
2) E. A. Peroza, A. Cabral, X. Wan, E. Freisinger, Metallomics, 2013, 5, 1204-1214.

Artificial proteases based on amphiphilic metal complexes

S. Hinojosa, C. Perera-Bobusch, N. Kulak

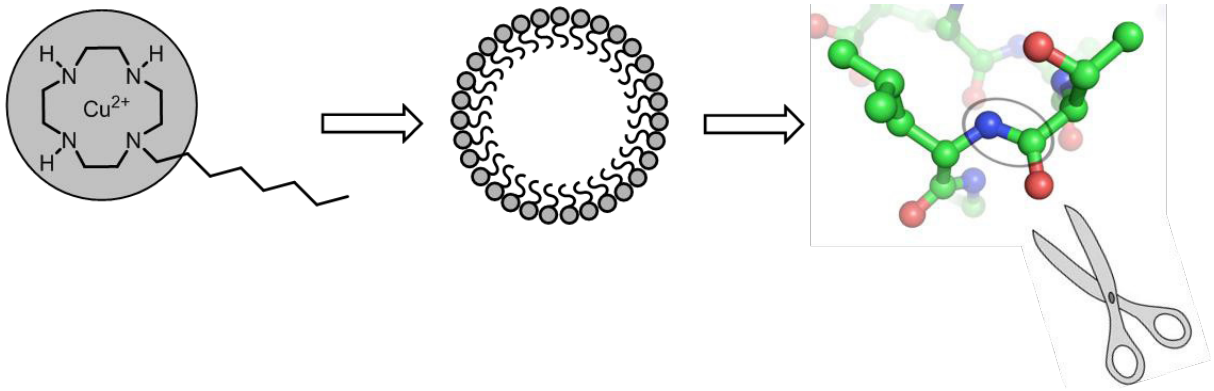
Freie Universität Berlin, Institut für Chemie und Biochemie; email : nora.kulak@fu-berlin.de

Abstract

In the Kulak group we develop metal complexes for the interaction with biomolecules. Our motivation is that manipulation of disease-related nucleic acids or proteins bears a potential for combating various diseases.

The degradation of pathogenic structures like amyloidogenic peptides is of high importance for therapeutic purposes.[1] Such a cleavage can be carried out by so-called proteases. As synthetic chemists we seek for small metal complexes that do the same job like natural enzymes, but come with some advantages concerning stability, prize and accessibility to rational design. Some of the artificial proteases developed so far are based on metal complexes of the macrocyclic ligand cyclen (1,4,7,10-tetraazacyclododecane) and its oxygen analog oxacyclen (1-oxa-4,7,10-triazacyclododecane).[2] Their proteolytic activity, which has been known in the literature for a couple of years already, is relatively low though when compared to natural enzymes.[3]

In this presentation, the application of cyclen-based metal complexes as artificial metalloproteases for the cleavage of model proteins will be discussed. Approaches for increasing their efficiency will be presented: heteroatom exchange and the mono N-alkylation resulting in micelle formation. The proteolytic activity of these derivatives is comparable to that of the most efficient artificial systems known so far, which require covalent bond formation with the protein to be cleaved and immobilization on solid support, respectively.[4]



References

[1] J. S. Derrick, J. Lee, S. J. C. Lee, Y. Kim, E. Nam, H. Tak, J. Kang, M. Lee, S. H. Kim, K. Park, J. Cho, M. H. Lim J. Am. Chem. Soc., 2017, 139, 2234.

[2] C. Perera-Bobusch, J. Hormann, C. Weise, S. Wedepohl, J. Dernedde, N. Kulak, Dalton Trans. 2016, 45, 10500.

[3] S. Jang, J. Suh, Org. Lett. 2008, 10, 481.

[4] S. H. Yoo, B. J. Lee, H. Kim, J. Suh, J. Am. Chem. Soc. 2005, 127, 9593.

Zr(IV)-Substituted Polyoxometalates as a novel class of artificial proteases: Catalytic and molecular interaction studies

H. G. T. Ly,[a] T. N. Parac-Vogt[a]

[a] *Laboratory of Bioinorganic Chemistry, Molecular Design and Synthesis, Department of Chemistry, KU Leuven, Belgium; email : honggiang.lythi@chem.kuleuven.be*

Abstract

Selective cleavage of proteins is one of the most important procedures in analytical biochemistry and biotechnology applications, frequently used for protein structure/function/folding analysis, protein engineering, and target-specific protein-cleaving drug design.[1] This is, however, a challenging task. Commonly used natural proteases are expensive, operate only in a narrow temperature and pH range, often suffer from self-digestion and often have limited selectivity. Therefore, new, efficient, and selective cleaving agents that are sufficiently active at non-denaturing pH and temperature conditions are highly needed.

In the search for new artificial peptidases, Zr(IV) was incorporated into lacunary polyoxometalate frameworks (POMs) to form metal-substituted polyoxometalates, which have been intensively investigated in our research group for the hydrolysis of peptides and proteins.[2] In this study, reactivity of a series of Zr(IV)-substituted POMs towards the hydrolysis of peptide bonds in dipepties and proteins as well as the interaction between POMs and the substrates were investigated. The cleavage was purely hydrolytic, occurred under mildly acidic or physiological pH, and was selective. The interaction was evidenced by several spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, circular dichroism (CD), tryptophan fluorescence, and UV-Vis spectroscopies.

References

1) K. B. Grant and M. Kassai, *Curr. Org. Chem.* **2006,** *10*, 1035.

2) (a) H. G. T. Ly, G. Absillis, S. R. Bajpe, J. A. Martens, and T. N. Parac-Vogt, *Eur. J. Inorg. Chem.* **2013,** *2013*, 4601; (b) H. G. T. Ly, G. Absillis, and T. N. Parac-Vogt, *Dalton Trans.* **2013,** *42*, 10929; (c) H. G. T. Ly, G. Absillis, and T. N. Parac-Vogt, *New J. Chem.* **2015,** DOI: 10.1039/C5NJ00561B; (d) H. G. T. Ly, G. Absillis, and T. N. Parac-Vogt, *Eur. J. Inorg. Chem.* **2015,** *2015*, 2206; (e) H. G. T. Ly, G. Absillis, R. Janssens, P. Proost, and T. N. Parac-Vogt, *Angew. Chem. -Int. Ed.* **2015,** *54*, 7391; (f) H.G.T.Ly, T. Mihaylov, G. Absillis, K. Pierloot, and T. N. Parac-Vogt, *Inorg.Chem.,* **2015**, 54,11477

RNA G-quadruplex structures and binding to Pt(II) complexes

Roland K. O. Sigel

University of Zurich, Department of Chemistry, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland;
email: roland.sigel@chem.uzh.ch

Abstract

RNAs fulfil multiple functions in a living cell, including regulation of gene expression on either the transcriptional or the translational level. In any of these functions, metal ions, and in some instances also metal ion complexes are a crucial part of their regulatory mechanism.[1]

An example that has been known only for a few years are RNA G-quadruplexes, which occur in the 5'-UTR of mRNAs.[2] Similar to their DNA counterparts, RNA quadruplex formation is highly dependent on the kind of metal ion,[3] but they are always of parallel stranded topology. We focus on three different RNA G-quadruplexes, namely TERRA (telomeric repeat-containing RNA), NRAS (neuroblastoma rat sarcoma viral oncogene homolog), and BCL2 (B-Cell Lymphoma/Leukemia-2). The BCL2 RNA takes up several co-existing structures in solution, depending on which of the excess Gs present in the linkers is involved in the tetrads. We subsequently systematically restricted the intrinsic folding dynamics of this G4-forming sequence to yield the formation of only two different intramolecular loop-isomer G-quadruplex structures in the native 5'-UTR context and finally the fold of a single G4 structure suitable for NMR studies. By NMR and fluorescence lifetime and anisotropy spectroscopy we characterize in addition the interaction with a highly charged Pt(II) complex, designed to specifically target such structures and modulate their regulatory function.[4] Due to their regulatory function, RNA G quadruplex sequences serve as potential targets for drugs, e.g. by stabilizing the quadruplex fold.[2]

Financial support by the Swiss National Science Foundation, the UZH Forschungskredit, the European Research Council, the China Scholarship Council, and the University of Zurich is gratefully acknowledged.

References

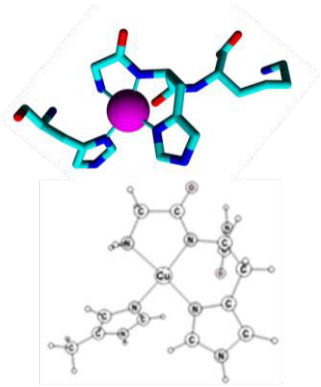
1) "Structural and Catalytic Roles of Metal Ions in RNA" *Met. Ions Life Sci.* **2011**, *9*, pp 391.
2) S. Balasubramanian, L. H. Hurley, S. Neidle, *Nature Rev. Drug Discov.* **2011**, *10*, 261.
3) H. Guiset-Miserachs, D. Donghi, R. Börner, S. Johannsen, R. K. O. Sigel, *J. Biol. Inorg. Chem.* **2016**, *21*, 975.
4) A. Dominguez-Martin, Z. Wang, F. Steffen, R. Börner, Q. Cao, Z.-W. Mao, R. K. O. Sigel, *in preparation*.

Xaa-His peptides – candidate cupric chaperones and metallohormones

Wojciech Bal[a], Arkadiusz Bonna[a,b], Karolina Bossak[a], Simon C. Drew[c], Kathryn M. Haas[d], Radosław Kotuniak[a], Mariusz Mital[a,e], Dawid Płonka[a], Jarosław Poznański[a], M. Jake Pushie[f]

[a] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warsaw, Poland; [b] Department of Biochemistry, University of Cambridge, Tennis Court Road, Downing Site, Cambridge CB2 1QW, United Kingdom; [c] Department of Medicine (Royal Melbourne Hospital), The University of Melbourne, Victoria 3010, Australia; [d] Department of Chemistry and Physics, Saint Mary's College, Notre Dame, IN 46556, USA; [e] Florey Department of Neuroscience and Mental Health, The University of Melbourne, Victoria 3010, Australia; [f] Department of Surgery, University of Saskatchewan, Saskatoon, SK S7N 5E5, Canada; email: wbal@ibb.waw.pl

Abstract



Cu(II) complexes of peptides containing a His2 residue have been a subject of chemical research for decades, but the only instance of such a complex in biochemistry is Cu(GHK), a liver cell proliferation and wound healing agent [1]. Interestingly, the stability of this complex measured in vitro, ca. 100 fM at pH 7.4 is not sufficient to support its existence in the bloodstream in the presence of a vast excess of albumin, which binds Cu(II) with a 1 pM affinity. We identified other His2 bioactive peptides: GHTD-amide released by pancreas, α-factor of yeast (WHWLQLKPGQPMY) and Aβ5-x peptide family (with the RH N-terminal sequence). We found that all bind Cu(II) with affinities similar to those of GHK and albumin [2]. Moreover, the 3N chelate ring of His2 peptide enables the formation of ternary complexes, and our investigations revealed that imidazole rings are their favored ternary ligands (Fig). Such (3+1N) ternary complexes are readily formed between GHK and albumin, the latter unexpectedly serving solely as the imidazole ring donor. We hypothesize that this complex serves as chaperone, facilitating Cu(II) exchange among its targets in the bloodstream and on cell surfaces. As structural and thermodynamic properties described above are shared by all His2 peptides, we propose that their biological activity is, at least in part, Cu(II)-dependent (thus these peptides act as metallohormones) and, conversely, the His2 peptides participate in extracellular copper physiology.

References

1) Pickart, L.; Vasquez-Soltero, J.M.; Margolina, A, GHK Peptide as a Natural Modulator of Multiple Cellular Pathways in Skin Regeneration. Cosmetics 2015, 2, 236-247.

2. Bossak, K.; Mital, M.; Poznański, J.; Bonna, A.; Drew, S.; Bal, W, Interactions of α-Factor-1, a Yeast Pheromone, and Its Analogue with Copper(II) Ions and Low-Molecular-Weight Ligands Yield Very Stable Complexes, Inorg. Chem. 2016, 55, 7829−7831.

Turned electron flow in an uptake hydrogenase

Lívia S. Mészáros[a], Namita Khanna[a], Hélder H. Miranda[a], Henning Krassen[a], Natalia Battchikova[b], Eva-Mari Aro[b], Peter Lindblad[a], Stenbjörn Styring[a], Ann Magnuson[a] & Patrícia Raleiras[a]

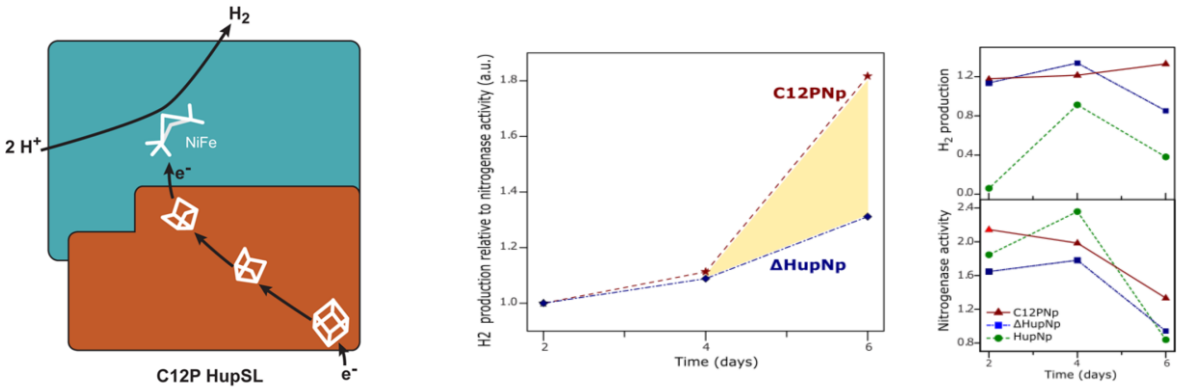
[a] Uppsala University, Department of Chemistry, Uppsala, Sweden ; [b] University of Turku, Department of Biotechnology, Turku, Finland ; email : livia.meszaros@kemi.uu.se

The small subunit of [NiFe] hydrogenases (HupS) contain three iron-sulfur clusters: a proximal 4Fe-4S cluster, a medial 3Fe-4S cluster and a distal 4Fe-4S cluster [1]. In the uptake hydrogenases, which oxidize molecular hydrogen, these clusters are responsible for the electron flow from the active site to the surface of the enzyme.

The iron-sulfur clusters are usually coordinated by four cysteines residues. The cyanobacterial uptake hydrogenases show unusual FeS binding motifs, an asparagine and three cysteines in the proximal cluster, and one glutamine and three cysteines in the distal cluster [1].

Amino acid substitution mutants were made in the HupS protein from *Nostoc punctiforme* affecting the coordination of the proximal 4Fe-4S cluster. The amino acid substitution mutants were characterized with EPR spectroscopy and hydrogenase activity measurements. Changing one of the coordinating cysteines to proline leaded a shift in its catalytic bias from hydrogen oxidation to hydrogen production [2].

Examination the role of the unusual coordination of the proximal FeS cluster showed that the asparagine has important role in the coordination of the cluster.



References

1) Raleiras, P.; Kellers, P.; Lindblad, P.; Styring, S.; Magnuson, A. Isolation and characterization of the small subunit of the uptake hydrogenase from the cyanobacterium *Nostoc punctiforme*. J Biol Chem., 2013, 288(25), 18345-52.

2) Raleiras, P.; Khanna. N.; Miranda HH.; Mészáros LS.; Krassen H.; Battchikova N.; Aro E-M.; Ho F.; Magnuson A.; Lindblad P.; Styring S. Turning around the electron flow in an uptake hydrogenase. EPR spectroscopy and in vivo activity of a designed mutant in HupSL from *Nostoc punctiforme*. Energy and environmental science 2016,9, 581-594

Understanding the structural features of amyloidogenic proteins: the role played by metal ions and membrane interactions

Daniela Valensin

Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy

email : daniela.valensin@unisi.it

Abstract

Amyloidogenic proteins are generally associated with severe neurodegenerative disorders like, Alzhemier's Disease (AD), Transmissible spongiform encephalopathies (TSEs) and Parkinson's Disease (PD). These disorders afflict millions of people worldwide and their main hallmarks are the presence of amyloid plaques in the brain, primarily formed by fibrils of misfolded cellular proteins. Several different factors are able to influence the morphology and kinetics of amyloids formation [1-4]. Among them, copper-protein and membrane-protein interactions have been taken into account. In presence of membrane environments, amyloidogenic proteins usually form stable α helix structures. The regions involved in the helicoidal rearrangements correspond to regions critical for protein aggregation as well. The conformational rearrangements strongly affect copper binding features, in terms of donor atoms and complex stability. This behavior is explained by considering that metal binding domains are often located nearby regions experiencing transitions from random-coil to α helix. We have investigated the structural details of membrane interaction and copper binding in PrP, Aβ and αS with the main aim to highlight how α-helix structure, induced by membrane interaction, affects copper binding domains of amyloidogenic proteins [5-8].

Acknowledgment

We thank PRIN (Programmi di Ricerca di Rilevante Interesse Nazionale) (2010M2JARJ_004 and 2015T778JW), CIRMMP (Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine Paramagnetiche), CIRCMSB (Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici) for financial support.

References

1) Jiang D, Rauda I, Han S, Chen S, Zhou F. Langmuir, 2012, 28, 12711.
2) Faller P, Hureau C, Berthoumieu O, Inorg. Chem., 2013, 52, 12193.
3) Faller P, Hureau C, La Penna G. Acc. Chem. Res., 2014, 47, 2252.
4) Curtain CC, Kirby NM, Mertens HD, Barnham KJ, Knott RB, Masters CL, Cappai R, Rekas A, Kenche VB, Ryan T, Mol. Biosyst., 2015, 11, 190.
5) Hecel A, De Ricco R, Valensin D. Coord. Chem. Rev., 2016, 327-328, 8-19.
6) Valensin D, Dell'Acqua S, Kozlowski H, Casella L. J Inorg Biochem., 2016, 163, 292-300.
7) Dell'Acqua S, Pirota V, Monzani E, Camponeschi F, De Ricco R, Valensin D, Casella L. Inorg Chem. 2016, 55, 6100-6.
8) Hecel A, Migliorini C, Valensin D, Luczkowski M, Kozlowski H. Dalton Trans. 2015, 44, 13125-32

Heme- DNA Complexes

Yasuhiko Yamamoto,[a,b] Tomokazu Shibata,[a] Yuya Katahira,[a] Kentaro Ochi,[a] Yusaku Nakayama,[a] Hulin Tai,[c] Akari Watanabe,[d] Tomomi Nakao,[d] Sachiko Yanagisawa,[d] Takashi Ogura[d], Hikaru Hemmi,[e] Masaki Hagihara,[f] Akihiro Suzuki,[g] and Saburo Neya[h]

[a] Dept. of Chem., Univ. of Tsukuba; [b] TARA Center, Univ. of Tsukuba; [c] Grad. Sch. of Mater. Sci., NAIST; [d] Grad. Sch. of Life Sci., Univ. of Hyogo; [e] Food Res. Inst., NARO; [f] Grad. Sch. Of Sci. and Tech.; Hirosaki Univ.; [g] Dept. of Mater. Eng., Nat'l. Inst. of Tech., Nagaoka Coll.; [h] Grad. Sch. of Pharm. Sci., Chiba Univ.
email : yamamoto@chem.tsukuba.ac.jp

Abstract

Aside from the importance of the G-quartet (*Figure 1*A) as a therapeutic target, Sen and co-workers [1] demonstrated the possibility of utilizing the G-quartet as a scaffold for deoxyribozymes that exhibit catalytic activities similar to those of hemoproteins. A complex between heme (*Figure 1*B) and G-quadruplex DNA has been shown to exhibit peroxidase activity [1]. This finding opened up new research fields for exploring the biological versatility of heme as the prosthetic group of DNAzymes. The size and planarity of a G-quartet are well-suited for interaction with a porphyrin ring through π-π stacking interaction. We are characterizing complexes between hemes and parallel G-quadruplex DNAs from a single repeat sequence of the human telomere, d(TTAGGG), (*Figure 1*C) and its related ones [2]. Heme binds specifically to the 3'-terminal G-quartet of the DNA to form a stable "heme-DNA complex". In a heme(Fe^{2+})-DNA complex, carbon monoxide (CO) is coordinated to the heme Fe atom on the side of the heme opposite the G-quartet, and a water molecule (H_2O) is also coordinated as another axial ligand *trans* to CO (*Figure 1D*) [3]. In this study, the heme environment in the heme-DNA complexes has been characterized using NMR and resonance Raman. The study revealed the influence of the DNA sequence on the heme environment in the complex, which provides valuable clues to elucidate the catalytic activities of the complexes.

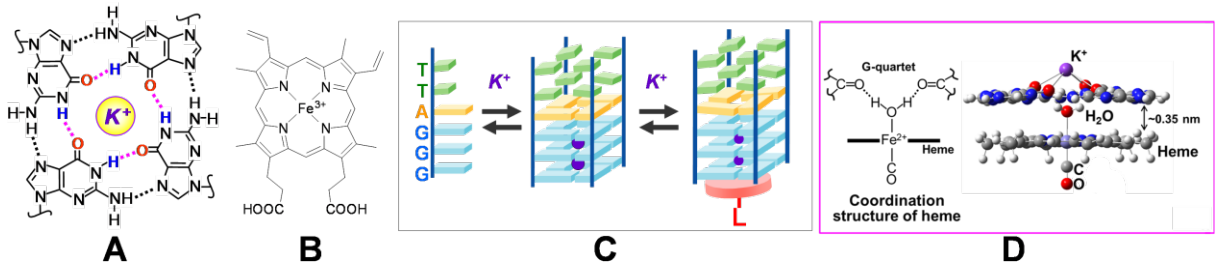


Figure 1. Molecular structures of G-quartet (*A*), heme(Fe^{3+}) (*B*), formation of G-quadruplex DNA formed from d(TTAGGG), $(d(TTAGGG))_4$, (*C*), and a CO adduct of heme(Fe^{2+})-$(d(TTAGGG))_4$ complex and coordination of CO and H_2O to heme(Fe^{2+}) of the complex (D).

Acknowledgements This work was supported by Grants-in-Aid for Scientific Research 16KT0048 (Y. Y.) and 16K17926 (T. S.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The NMR spectra were partly recorded with Bruker spectrometers at the Chemical Analysis Center, University of Tsukuba.

References

1) P. Travascio *et al.*, J. Am. Chem. Soc. **2001**, *123*, 1337-1348.
2) H. Shimizu *et al.*, Bull. Chem. Soc. Jpn. **2015**, *88*, 644-652.
3) Y. Yamamoto *et al.*, Biochemistry **2015**, *54*, 7168-7177.

Architectural Control of Molecular Self-Assembly of Bioorganometallic Conjugates with Nucleobases

Toshiyuki Moriuchi, Xiangtai Meng, Yuki Sakamoto, and Toshikazu Hirao

Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Yamada-oka, Suita, Osaka 565-0871, Japan; email: moriuchi@chem.eng.osaka-u.ac.jp

Abstract

Recently, the research field of bioorganometallic chemistry, which is a hybrid area between organometallic chemistry and biology, has attracted much attention. Conjugation of organometallic compounds with biomolecules such as nucleobases, amino acids, and peptides is envisioned to provide novel bioorganometallic systems depending on the properties of both components. Nucleobases of DNA possess donors and acceptors for hydrogen bonding, which permits self-association into various structurally defined molecular assemblies. A variety of nucleobase derivatives with fluorescent, electrical, magnetic, and metal ion binding properties have been reported to expand the scope of their applications. Herein, we report the architectural control of molecular self-assembly of bioorganometallic conjugates composed of organometallic compounds and nucleobases.[1-4]

A guanosine-based Au(I) isonitrile complex **GAu(I)** was demonstrated to form a G-octamer aggregate via self-assembly, wherein the quartet or octamer was formed in the absence or presence of a potassium ion, respectively, exhibiting a switchable emission based on Au(I)-Au(I) interaction as shown in Figure 1.[1] The formation of the quartet, octamer, and polymeric columnar aggregate was found to be controlled by the amount of a potassium ion.[2]

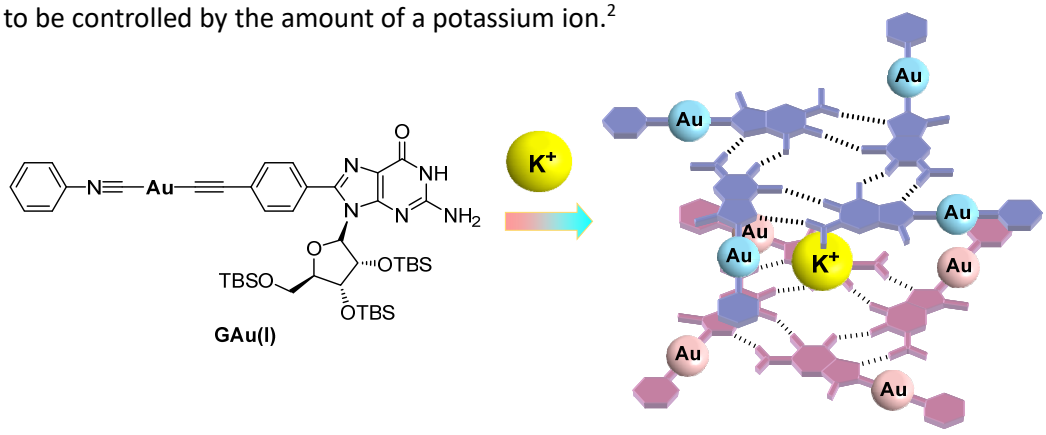


Figure 1 Formation of G-octamer of **GAu(I)** to induce Au(I)-Au(I) interaction upon addition of KPF_6.

The tuning of the emission properties of the bioorganometallic platinum(II) conjugates with a uracil moiety was also performed by changing the direction of hydrogen bonding sites and the molecular scaffold having complementary hydrogen bonding sites for the uracil moiety, which depends on the control of the aggregated structures.[3]

References

1) X. Meng, T. Moriuchi, M. Kawahata, K. Yamaguchi, T. Hirao, *Chem. Commun*. **2011**, *47*, 4682-4684.
2) X. Meng, T. Moriuchi, N. Tohnai, M. Miyata, M. Kawahata, K. Yamaguchi, T. Hirao, *Org. Biomol. Chem.* **2011**, *9*, 5633-5636.
3) T. Moriuchi, Y. Sakamoto, S. Noguchi, T. Fujiwara, S. Akine, T. Nabeshima, T. Hirao, *Dalton Trans.* **2012**, *41*, 8524-8531.
4) X. Meng, T. Moriuchi, Y. Sakamoto, M. Kawahata, K. Yamaguchi, T. Hirao, *RSC Adv.* **2012**, *2*, 4359-4363.

How proteins match Zn(II) affinity with their biological function?

Anna Kocyła, Adam Pomorski, Agnieszka Drozd, Justyna Adamczyk, Tomasz Kochańczyk, Artur Krężel

[a] *Department of Chemical Biology, Faculty of Biotechnology, University of Wrocław, Joliot-Curie 14a, 50-383 Wrocław, Poland : artur.krezel@uwr.edu.pl*

Abstract

The mechanism by which zinc proteins associate and dissociate their Zn(II) ions and the factors that modulate its affinity and stability remain incompletely understood [1]. The question whether the protein is saturated with Zn(II) in cell environment is linked to its biological function, redox status and zinc availability, which depends on both zinc buffering and muffling [2]. These is mostly based on both thermodynamic and kinetic properties of zinc binding sites in proteins. We focus our attention on intra- and intermolecular interactions in zinc proteins that are crucial for protein stability and cell functionality. Careful characterization of the metallothionein metalation pathway and its weak binding site reveals the importance of the interdomain interaction as well as Cys21 residue. Thorough examination of Zn(II)-mediated protein interaction of Rad50 protein, CD4-Lck interface, and several zinc finger domains revealed that hydrogen bond network around coordinating cysteine residues highly modulates Zn(II)-to-protein affinity adjusting it to cellular function and localization [3-5]. We show that the presence or lack of metal-dependent protein folding is the most important factor that modulates zinc proteins stability and reactivity.

Acknowledgements

Financial support by National Science Center (grants 2012/07/E/NZ1/01894 and 2014/13/B/NZ1/00935) is gratefully acknowledged.

References

[1] T. Kochańczyk, et al. Relationship between the architecture of zinc coordination and zinc binding affinity in proteins–insights into zinc regulation. 2015, *Metallomics*, 7, 244.

[2] A. Krężel, W. Maret, Zinc-buffering capacity of a eukaryotic cell at physiological pZn. 2006, *J. Biol. Inorg. Chem.*, 11, 1049.

[3] T. Kochańczyk , et al. Metal-coupled folding as the driving force for the extreme stability of Rad50 zinc hook dimer assembly. 2016, *Sci. Rep.* 6, 36346.

[4] Y.B. Park et al. Eukaryotic Rad50 functions as a rod-shaped dimer. 2017, *Nat. Sctruct. Mol. Biol.*, doi: 10.1038/nsmb.3369.

[5] A. Miłoch, A. Krężel, Metal binding properties of the zinc finger metallome – insights into variations in stability. 2014, *Metallomics*, 6, 2015.

Ruthenation of non-stacked guanines in DNA G-quadruplex structures.Enhancement of the*c-MYC* gene expression

Jessica Rodriguez,[a] M. Eugenio Vázquez,[b] José Luis Mascareñas,[b]

[a]UPS, LaboratoireHétérochimieFondamentaleAppliquée, Université de Toulouse and CNRS, LHFA UMR 5069, 118 route de Narbonne, Toulouse F-31062, France; [b] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), and Departamento de Química Orgánica. Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.; email: jessica.rodriguez.villar@gmail.com

Abstract

Guanine quadruplexes (GQs) are compact four-stranded DNA structures that play a key role in the control of a variety of processes, including gene transcription.[1]Herein we demonstrate that bulky ruthenium complexes of type $[Ru(terpy)(bpy)X]^{n+}$ (X = Cl, RSR´, Met), metalatesterically-accessible guanines in the GQ of the *c-MYC* promoter region, and increase the expression levels of this oncogene, most probably by disrupting the parallel GQ structure present in its gen promoter site (Figure 1). We also show that exchangeable tioether ligands (X = RSR', Met) allow the regulation of their metalating activity with visible light.[2]Importantly, the complexes exhibit very low toxicity.

Given that the increase of c-*MYC* transcriptional levels has been pointed out as an important step in several research lines in cancer therapy, and in particular in the renewal ability of cancer stem cells,[3] our discovery might provide new powerful tools for biological applications.

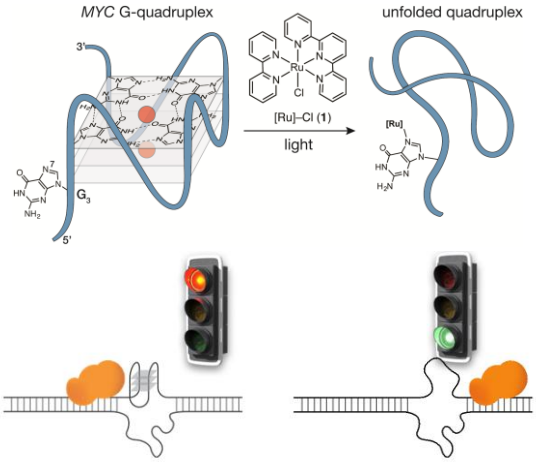


Figure 1. Top) Cartoon representing thestructure of the GQ of c-MYC highlighting the metalation selectivity and the disruption of the G-quadruplex.Bottom)Representation of the plausible mechanism for the enhancement of the *c-MYC* expression.

References

1a) E. Henderson, C. C. Hardin, S. K. Walk, I. Tinoco Jr., E. H. Blackburn, *Cell***1987**, *51*, 899; b) P. Murat, S. Balasabrumanian, *Curr. Opin. Gen. Develop*. **2014**, *25*, 22; c) L. H. Hurley, *Biochem.Soc. Trans*. **2001**, *29*, 692; d) H. Bertrand, S. Bombard, D. Monchaud, M.-P.Teulade-Fichou, *J. Biol. Inorg.Chem*. **2007**, *12*, 1003.

2J. Rodríguez, J. Mosquera, J. R. Couceiro, M. E. Vázquez, J. L. Mascareñas, *Angew. Chem. Int. Ed.*, **2016**, *55*, 15615-15618.

3 K. Takahashi, S. Yamanaka, *Cell***2006**, *126*, 663.

Flash presentations

New Designing of Organometallic Iridium(III) Complexes as Improved Photocytotoxic and Cytotoxic Agents: A structure-activity Relationship Study

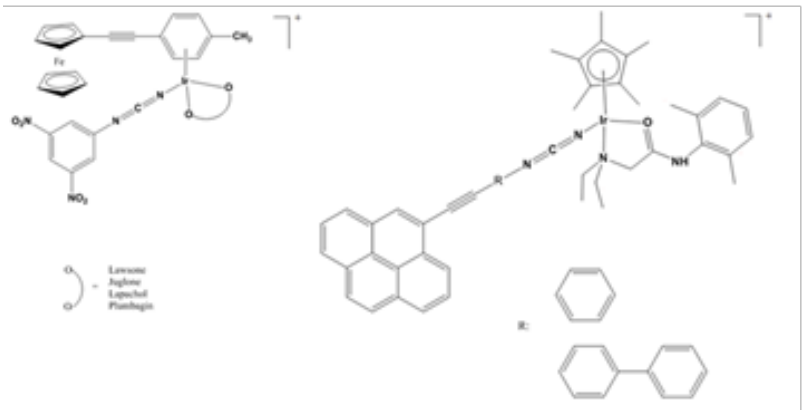
Leila Tabrizi [a]

[a] *School of Chemistry, National University of Ireland, Galway, University Road, Galway, Ireland; email : LEILA.TABRIZI@nuigalway.ie*

Abstract

Organometallic Ir(III) complexes of the type [IrIII(η6-arene-C≡CFc)(L)(3,5-(NO2)2pcyd)](PF6), [Cp*IrIII(LC)(PECA)](PF6), [Cp*IrIII(LC)(PECB)](PF6) (L: lawsone, juglone, lapachol, plumbagin, PECA: 4-(Pyren-10-yl)ethynyl-phenylcyanamide , PECB: 4'-(Pyren-10-yl)ethynyl-4-cyanamidobiphenyl, LC: lidocaine, 3,5-(NO2)2pcyd, 3,5-dinitro phenylcyanamide, FcC≡CH: ferrocenyl acetylene, C≡CFcIP: 1'-(phenanthro[9,10-d]imidazole) ferrocenyl -1-acetylene) have been synthesized and fully characterized. For the first time, in these complexes a ferroence molecule has been attached to an arene, which is then fixed to an Iridium (III) centre. Remarkably, these complexes with ferrocene exhibit significant cytotoxic effects, which emerged as the most cytotoxic derivative in comparison with other complexes. The complexes increase the production of reactive oxygen species (ROS) in MCF-7 cells. The new compounds also inhibit the enzyme thioredoxin reductase activity at nanomolar concentrations. Furthermore, the complexes induce major levels of cancer cell death by apoptosis that is in correlation with activity in cytotoxicity studies.

The PECA or PECB moiety decreased the inherent dark-cytotoxicity and increased the photo-toxicity simultaneously, both of which contributed to the greatly improved photodynamic therapy (PDT) efficacy, ultimately resulting in cancer cell apoptosis. The main contributor for such a greatly enhanced PDT efficacy was the effect of the PECA or PECB moiety on the cellular uptake and intracellular ROS levels. We therefore demonstrate that the combination of PECA or PECB with organometallic Iridium(III) complexes may be an emerging strategy to develop novel complexes as potential photosensitizers (PSs) in photodynamic therapy (PDT).



References

1) Leila Tabrizi*, Hossein Chiniforoshan, Designing new iridium(III) arene complexes of naphthoquinone derivatives as anticancer agents: a structure–activity relationship study, 2017, Dalton Transactions, DOI: 10.1039/c6dt04339a

Influence of copper and membrane-mimicking environment on the structure of the Prion Protein N-terminal domain

Aleksandra Hecel,[a] Daniela Valensin,[b] Henryk Kozłowski,[a]

[a] *Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50383 Wroclaw, Poland;*
[b] *Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via A. Moro 2, 53100 Siena, Italy;*
email : aleksandra.hecel@chem.uni.wroc.pl

Abstract

Undoubtedly, Neurodegenerative Diseases (ND) are one of the most serious diseases in modern society. Understanding the molecular mechanisms underlying these disorders is a challenge for global science. ND are still based on huge number of hypothesis, therefore it is important to find out the mechanism which leads to the pathogenic process.

Prion disorders, are associated with protein infectious agents called prions that cause the conversion of the normal cellular prion protein isoform (PrP^{C}) into the abnormal scrapie prion protein isoform (PrP^{Sc})[1)]. PrP^{Sc} is highly insoluble and prone to aggregation. Elevated β-sheet content of PrP^{Sc} is essential for protein aggregation and formation of pathological deposits[2)].

The human Prion Protein ($hPrP^{C}$) is able to bind up to six Cu^{2+} ions. Four of them are distributed in the octarepeat domain, containing four tandem-repetitions of the sequence PHGGGWGQ. Immediately outside the octarepeat domain, in so called PrP amyloidogenic region, two additional and independent Cu^{2+} binding sites (His96 and His111) are present. Furthermore, the amyloidogenic fragment of PrP contains a two methionine residues (Met109 and Met112) known to act as Cu^{+} binding site[3)].

The amyloidogenic region of hPrP contains an N-terminal polar head and a long C-terminal hydrophobic tail. The presence of the PrP hydrophobic region, highly conserved among different species, is essential for PrP^{C} - PrP^{Sc} conversion in the phospholipid membrane. The PrP is bound to the cell membrane via its C-terminal GPI anchor, so it is reasonable to assume that it may interact with the lipid bilayer. In the last years, a huge number of studies have suggested that the misfolding process is influenced by the interactions of these proteins with copper ions and membranes[4)]. The amyloidogenic human prion protein fragment ($hPrP_{91-127}$) undergoes to large conformational changes in presence of detergent micelles and forms stable α-helical structure. The α-helix structuring of amyloidogenic proteins, upon membrane interaction, also strongly affect Cu^{2+} and Cu^{+} binding, in terms of donor atoms and complex stability[5,6)].

References

1) S.B. Prusiner, Science, 1982, 216, 136-144.
2) S.B. Prusiner, N. Engl. J. Med. 2001, 344, 1516-1526.
3) H. Kozlowski, M. Luczkowski, M. Remelli, D. Valensin, Coord. Chem. Rev; 2012, 256, 2129-2141.
4) A. Hecel, R. De Ricco, D. Valensin, Coord. Chem. Rev; 2016, 327-328, 8-19.
5) A. Hecel, C. Migliorini, D. Valensin, M. Luczkowski, H. Kozlowski, Dalton Trans. 2015, 44, 13125-13132.
6) D. Valensin. E. Padula. A. Hecel, M. Luczkowski, H. Kozlowski, J. Inorg. Biochem. 2016, 155, 26-35.

Acknowledgements: financial support MNiSW (Ministerstwo Nauki i Szkolnictwa Wyższego) project 0420/1859/16.

Oxidative reactivity promoted by copper-prion peptide complexes

Simone Dell'Acqua,[a] Chiara Bacchella,[a] Enrico Monzani,[a] Stefania Nicolis,[a] Giuseppe Di Natale,[b] Enrico Rizzarelli,[b] Luigi Casella [a]

[a] *Dipartimento di Chimica, Università di Pavia, Via Taramelli 12, 27100, Pavia, Italy;* [b] *Istituto di Biostrutture e Bioimmagini, Consiglio Nazionale delle Ricerche, Via P. Gaifami 18, Catania, Italy*

email: simone.dellacqua@unipv.it

Abstract

Prion diseases are a group of neurodegenerative diseases caused by prion protein (PrP) conformational changes.[1] The key molecular event in the pathogenesis of such diseases is the conformational conversion of prion protein, PrP^{C}, into a misfolded form rich in β-sheet structure, PrP^{Sc}, but the detailed mechanistic aspects of prion protein conversion remain enigmatic. There is uncertainty on the precise physiological function of PrP^{C}, but several evidences support the notion of its role in copper homeostasis. Despite the large number of investigations on the structural properties of copper-prion complexes, little is known regarding the related reactivity.

We focused on the redox reactivity of Cu^{2+} bound to three N-acetylated and C-amidated PrP fragments, $PrP_{106-114}$, PrP_{84-114} and PrP_{76-114}. These peptides include the fragment outside the octarepeats (92-114), with the high affinity Cu site involving H96 and H111, and the larger fragments also containing one (84-114) or two (76-114) of the Cu sites within the four octarepeats.[2] We found that copper(II) binding to prion peptides does not prevent Cu redox cycling in the presence of reducing agents and the formation of reactive oxygen species (ROS). The toxic effects of these species are exacerbated in the presence of catecholamines, indicating that dysfunction of catecholamine vesicular sequestration or recovery after synaptic release is a dangerous amplifier of Cu induced oxidative stress. Cu bound to prion peptides including the high affinity site involving histidines adjacent to the octarepeats exhibits marked catalytic activity toward dopamine and 4-methylcatechol. The resulting quinone oxidation products undergo parallel oligomerization and endogenous peptide modification yielding catechol adducts at the histidine binding ligands. These modifications add to the more common oxidation of Met and His residues produced by ROS. Compared to previous studies, oxidative degradation of Cu-prion peptides is much faster than that undergone by Cu-β-amyloid[3,4] and Cu-α-synuclein[5,6] complexes in the same conditions.

References

1) J. Collinge, "Mammalian prions and their wider relevance in neurodegenerative diseases". Nature, 2016, 539, 217-226.
2) G. Di Natale, I. Turi, G. Pappalardo, I. Sóvágó, E. Rizzarelli, "Cross-Talk Between the Octarepeat Domain and the Fifth Binding Site of Prion Protein Driven by the Interaction of Copper(II) with the N-terminus". Chem. Eur. J. 2015, 21, 4071-4084.
3) D. Ciregna, E. Monzani, G. Thiabaud, S. Pizzocaro, L. Casella, "Copper-β-amyloid peptides exhibit neither monooxygenase nor superoxide dismutase activities." Chem. Commun. 2013, 49, 4027-9.
4) V. Pirota, S. Dell'Acqua, E. Monzani, S. Nicolis, L. Casella, "Copper-Aβ peptides and the oxidation of catecholic substrates: Reactivity and endogenous peptide damage." Chem. Eur. J. 2016, 22, 16964-16973.
5) S. Dell'Acqua, V. Pirota, C. Anzani, M. M. Rocco, S. Nicolis, D. Valensin, E. Monzani, L. Casella, "Reactivity of copper-α-synuclein peptide complexes relevant to Parkinson's disease". Metallomics 2015, 7, 1091-1102.
6) S. Dell'Acqua, V. Pirota, E. Monzani, F. Camponeschi, R. De Ricco, D. Valensin, L. Casella, "Copper(I) forms a redox stable 1:2 complex with α-Synuclein N-Terminal peptide in a membrane-like environment". Inorg. Chem. 2016, 55, 6100-6106.

Combatting antimicrobial resistance with novel photoactivatable metal complexes

Huaiyi Huang, [a] Peter J. Sadler [a]

[a] *University of Warwick; H. Huang.17@warwick.ac.uk*

Abstract

The widespread and inappropriate use of antimicrobials have led to life-threatening antimicrobial resistance. Considering the time-consuming separation of effective antimicrobial agents from bacterial metabolites and the high mutation rate of bacterial chromosomal and protein expression, the investigation of novel mechanisms for bacterial treatment is warranted.[1] Antimicrobial photodynamic therapy (aPDT) is a novel method that combine photosensitizers and suitable light source to treat microbial infections.[2] The aPDT relies on the generation of highly toxic reactive oxygen species (ROS) to kill bacteria.

One of the central problems in antibacterial therapy is to develop targeting strategies. Most current antimicrobials target the bacterial cell wall and cannot access the bacterial intracellular volume.[3] Maltohexaose is a maltooligosaccharide consisting of six glucose units and functions as the major glucose source for bacteria which can reach millimolar within bacteria. Different from bacteria, mammalian cells do not express maltodextrin transporter system and thus cannot internalize compounds conjugated to maltohexaose.[4] In recent years, ruthenium(II) polypyridyl complexes have been widely used as photosensitizers in PDT.[5] With these in mind, we have design and synthesis maltohexaose conjugated luminescent ruthenium(II) antimicrobial complexes promising for bacteria targeting, imaging and photodynamic therapy. Upon light irradiation, we can identify the microbial infection area by fluorescent microscopy. The photosensitizers ruthenium(II) complexes will generate ROS during irradiation and induce irreversible oxidative damage to multiple bacteria components including cell membranes, organelles, intracellular proteins or DNA. Our work provides a novel strategy for treating antimicrobial resistant bacteria with photoactivatable ruthenium complex.

References

1) World Health Organization, WHO global strategy for containment of antimicrobial resistance. 2001.
2) Braun, C. Dehn, F. Krause, S. Jepsen, Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. *J. Clin. Periodontol.*, 2008, 35(10), 877-884.
3) R. Leclercq, P. Courvalin, Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. *Antimicrob. Agents. Chemother.*, 1991, 35(7), 1267.
4) X. Ning, S. Lee, Z. Wang, D. Kim, B. Stubblefield, E. Gilbert, N. Murthy, Maltodextrin-based imaging probes detect bacteria in vivo with high sensitivity and specificity. *Nat. Mater.*, 2011, 10(8), 602-607.
5) N. A. Smith, P. Zhang, S. E. Greenough, M. D. Horbury, G. J. Clarkson, D. McFeely, P. J. Sadler, Combatting AMR: photoactivatable ruthenium (ii)-isoniazid complex exhibits rapid selective antimycobacterial activity. *Chem. Sci.*, 2017, 8(1), 395-404.

Metal-catalyzed oxidation of Aβ, ROS production and aggregation: an Alzheimer's Disease story

Clémence Cheignon, [a,b,c] Peter Faller,[a,b,d] Fabrice Collin, [a,b,c] Christelle Hureau [a,b]

[a] LCC (Laboratoire de Chimie de Coordination), CNRS UPR 8241, 205 route de Narbonne, 31062 Toulouse Cedex 09, (France),[b] Université de Toulouse ; UPS, INPT, 31077 Toulouse (France), [c] UMR 152 Pharma Dev, Université de Toulouse, IRD, UPS (France), [d] Institut de Chimie (UMR 7177), 4 rue B. Pascal, F-67000 Strasbourg (France) ; email : clemence.cheignon@lcc-toulouse.fr

Abstract

Alzheimer's disease is the most frequent form of dementia in the elderly. One of the features of AD is the formation of senile plaques in brain, mainly composed of the 40/42-residue Amyloid-β peptide (Aβ) on aggregated form. Metal ions such as copper and zinc are also present in high levels of concentration in senile plaques, and form complexes with Aβ. In the presence of a reducing agent, Cu-Aβ complexes can catalyze the production of Reactive Oxygen Species (ROS), including the hydroxyl radical ($HO^{\bullet}$).[1,2] This reaction proceeds through a low-populated Cu-Aβ state,[3,4] called In-Between State, which is an elusive state little described so far.

The hydroxyl radicals released during the Cu-Aβ mediated ROS production are very reactive and they can cause oxidative damages to surrounding neuronal biomolecules and to the Aβ peptide itself. Indeed, it has been shown that Aβ is the first target for ROS during this reaction.[5]

We present here key structural features of this elusive In-Between State responsible for ROS production [6] (Figure 1) as well as results on the impact of the oxidation undergone by Aβ on the events related to Alzheimer's Disease.[7]

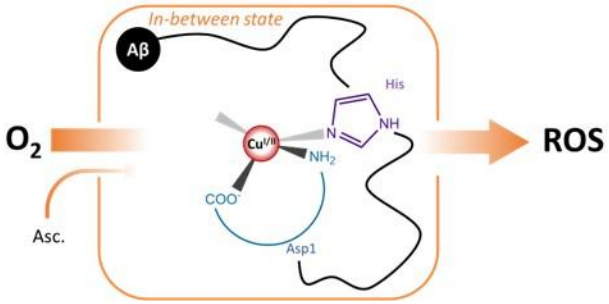


Figure 1: Schematic view of the proposed Cu-Aβ structure in the In-Between State during the metal-catalyzed ROS production.[6]

References

1) Hureau, C. and Faller, P., *Biochimie* **2009**, 91, 1212-1217.
2) Chassaing, S. et al., *Curr. Top. Med. Chem*. **2012**, 12, 2573-2595.
3) Balland, V. et al., *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 17113-17118.
4) Cassagnes, L.-E. et al., *Angew. Chem. Int. Ed*. **2013**, 52, 11110-11113.
5) Cheignon, C. et al., *Dalton Trans.* **2016**, *45*, 12627-12631.
6) Cheignon, C. et al., *submitted manuscript*.
7) Cheignon, C. et al., *Metallomics* **2016**, *8*, 1081-1089.

Acknowledgements: This work is supported by the ERC-2014-StG-638712 aLzINK grant and by the French agency for research ANR (grant ANR-13-BSV5-0016).

A bioinspired Nickel complex for the reduction of CO_2 into formate

Thibault Fogeron[a]; Tanya K. Todorova[a]; Maria Gomez-Mingot[a]; Lise-Marie Chamoreau[b]; Yun Li[a]; Marc Fontecave[a]

[a]*Laboratoire de Chimie des Processus Biologiques, UMR 8229 CNRS,UPMC Univ Paris 06, Collège de France, 11 Marcelin Berthelot, 75231 Paris Cedex 05, France ;* [b] *Sorbonne Universités, UPMC Université Paris 6, Institut Parisien de Chimie Moléculaire, UMR 8232 CNRS, 4 place Jussieu, 75252 Paris Cedex 5, France*

thibault.fogeron@college-de-france.fr

Abstract

Storage of diluted and intermittent sources of energy such as solar and wind energy is a great challenge of the 21st century. It can be accomplished by the conversion of electricity or light into chemical energy. For this reason, the reduction of CO_2 is particularly appealing since it also gives access to carbonated fuels. For these reasons, numerous labs have been focus in the development of catalysts over the last decades[1,2]. In order to find new catalysts with abundant metals we decide to take inspiration from Nature. More precisely, we have focused on molybdenum and tungsten dependent formate dehydrogenase which are known to reversibly catalyse the reduction of CO_2 into formate[3,4]. The active site has Mo/W coordinated to one or two molybdopterin (**MPT**) ligands and an O/S axial ligand (Figure 1). MPT is a highly unstable tricyclic organic molecule with a fused pyranopterin system and a dithiolene chelate moiety.

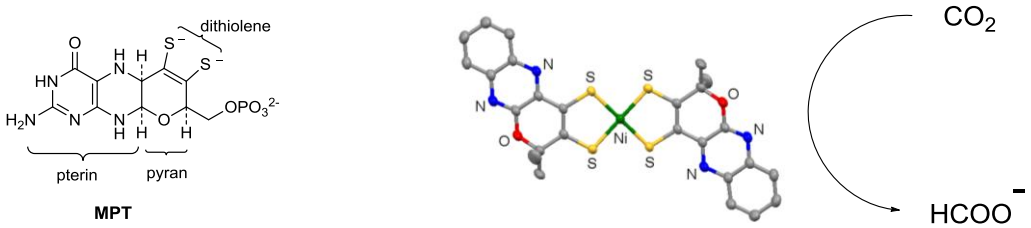


Figure 1: Structures of MPT (left) and ORTEP diagram of the $[Ni(qpdt)]^-$ catalyst (right).

We have previously reported the synthesis of a bio-inspired ligand (qpdt) mimicking the natural MPT ligand[5,6]. The corresponding bisdithiolene Molybdenum-oxo and cobalt complexes showed to be good catalysts for the reduction of protons into hydrogen[6,7]. We report here the synthesis of the $[Ni(qpdt_2)]^-$ complex. Interestingly, it has been found to be a catalyst for the electro-reduction of CO_2 and demonstrated a great stability. The main product of reduction was formate. Finally, the mechanism was investigated by DFT calculations.

References

[1] H. Takeda, C. Cometto, O. Ishitani, M. Robert, *ACS Catal.* **2017**, *7*, 70–88.
[2] J. Qiao, Y. Liu, F. Hong, J. Zhang, *Chem Soc Rev* **2014**, *43*, 631–675.
[3] T. Reda, C. M. Plugge, N. J. Abram, J. Hirst, *Proc. Natl. Acad. Sci.* **2008**, *105*, 10654–10658.
[4] A. Bassegoda, C. Madden, D. W. Wakerley, E. Reisner, J. Hirst, *J. Am. Chem. Soc.* **2014**, *136*, 15473–15476.
[5] J.-P. Porcher, T. Fogeron, M. Gomez-Mingot, L.-M. Chamoreau, Y. Li, M. Fontecave, *Chem. - Eur. J.* **2016**, *22*, 4447–4453.
[6] J.-P. Porcher, T. Fogeron, M. Gomez-Mingot, E. Derat, L.-M. Chamoreau, Y. Li, M. Fontecave, *Angew. Chem. Int. Ed.* **2015**, *54*, 14090–14093.
[7] T. Fogeron, J.-P. Porcher, M. Gomez-Mingot, T. K. Todorova, L.-M. Chamoreau, C. Mellot-Draznieks, Y. Li, M. Fontecave, *Dalton Trans.* **2016**, *45*, 14754–14763.

Synthesis and biological activity studies of theranostic half-sandwich Iridium-BODIPY dyads

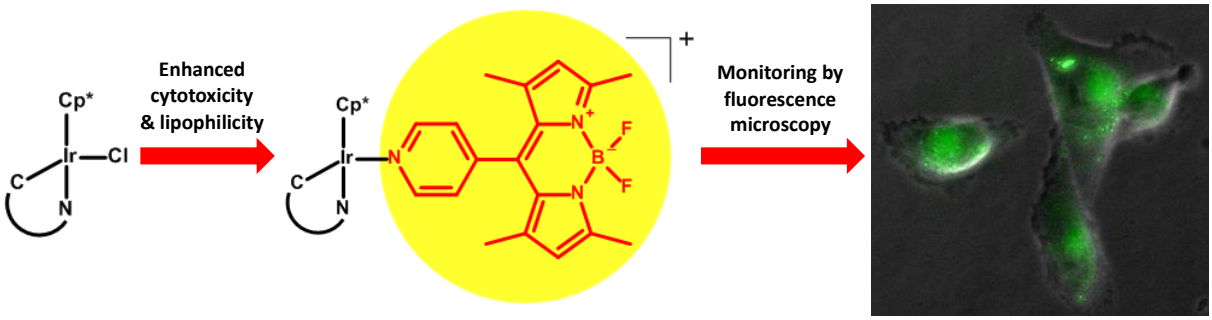
Kévin Passador,[a] Malcolm Jérémy Zimbron,[a] Claude-Marie Bachelet,[b] Candice Botuha,[a] Michèle Salmain,[a] Serge Thorimbert[a]

[a]*Sorbonne Universités, UPMC Univ Paris 06, UMR CNRS 8232, Institut Parisien de Chimie Moléculaire, 4 place Jussieu, 75005 Paris, France ;* [b]*Plateforme d'Imagerie Cellulaire, UPMC, Paris, France ;*
email : kevin.passador@upmc.fr

Abstract

For the last five years, cyclometalated half-sandwich Ir(III) complexes containing a C^N type ligand and a chloro ligand have particularly attracted interest since they were found to exhibit antiproliferative activity on various cancer cell cultures, including cisplatin resistant cell line.[1-4] Their cytotoxicity was further enhanced by replacing the chloro ligand by a pyridine to afford monocationic complexes. In order to get further insight into their mechanism of action and their pharmacokinetics, we designed new half-sandwich Ir(III) complexes bearing a fluorescent probe linked to the pyridine ligand. Our choice fell on BODIPYs (boron-dipyrromethene), which are among the most attractive fluorophores currently available, as they generally display high quantum yield, sharp absorption and emission bands, and easily tunable optical properties.[5]

To access these fluorescent theranostic metallodrug candidates, the half-sandwich Cp* Iridium(III) complex including 2-phenyl-pyridine chelating ligand was selected as scaffold. The optical imaging reporter was then introduced by replacing the pyridine ligand by meso-pyridyl BODIPYs. In this communication, we will present our results obtained on several fluorescent complexes with sub-micromolar cytotoxicity, and the successful monitoring of their internalization during fluorescence microscopy experiments.



References

(1) Jaouen, G.; Salmain, M., Ed. *Bioorganometallic Chemistry: Applications in Drug Discovery, Biocatalysis, and Imaging*; Wiley-VCH, Weinheim, 2015.

(2) Liu, Z.; Romero-Canelón, I.; Qamar, B.; Hearn, J. M.; Habtemariam, A.; Barry, N. P. E.; Pizarro, A. M.; Clarkson, G. J.; Sadler, P. J. The Potent Oxidant Anticancer Activity of Organoiridium Catalysts. *Angew. Chem. Int. Ed.* **2014**, *53*, 3941–3946.

(3) Liu, Z.; Habtemariam, A.; Pizarro, A. M.; Clarkson, G. J.; Sadler, P. J. Organometallic Iridium(III) Cyclopentadienyl Anticancer Complexes Containing C,N-Chelating Ligands. *Organometallics* **2011**, *30*, 4702–4710.

(4) Liu, Z.; Romero-Canelón, I.; Habtemariam, A.; Clarkson, G. J.; Sadler, P. J. Potent Half-Sandwich Iridium(III) Anticancer Complexes Containing CΛN-Chelated and Pyridine Ligands. *Organometallics* **2014**, *33*, 5324–5333.

(5) Loudet, A.; Burgess, K. BODIPY Dyes and Their Derivatives: Syntheses and Spectroscopic Properties. *Chem. Rev.* **2007**, *107*, 4891–4932.

Neodimium(III) Polyaminocarboxylate as Photostable Probe for Luminescence Imaging within the Biological Diagnostic Window

Ivana Martinić,[a] Svetlana V. Eliseeva,[a] Regis Delatouche,[b] Sylvain Routier,[b] Franck Suzenet,[b] Stéphane Petoud[a]

[a] *Centre de Biophysique Moléculaire, CNRS UPR 4301, 45071 Orléans, France*

[b] *Institut de Chimie Organique et Analytique, UMR 7311, 45067 Orléans, France*

email : svetlana.eliseeva@cnrs-orleans.fr

Abstract

Fluorescence optical imaging is a powerful tool for in vitro and in vivo biological imaging due to the high detection sensitivity and resolution at the cellular level. In addition, imaging within the biological diagnostic window is advantageous for enhanced signal-to-noise ratio and deeper penetration of light through tissues, a major advantage for non-invasive detection and diagnostic.[1] Lanthanides (Ln3+) possess unique optical properties that are complementary to those of organic fluorophores: sharp emission bands, spectral positions of which are not affected by pH and microenvironments, large difference between excitation and emission wavelengths and high resistance to photobleaching.

Herein, we report the synthesis, characterization and biological imaging with novel Ln^{3+}-based probe ($L1Nd^{3+}$), which possess excitation and emission wavelengths within biological diagnostic window. The synthesis of the ligand L1 has been performed by amide coupling between a triethylenetetramine-*N*,*N*,*N*',*N*'',*N*''',*N*'''-hexaacetic acid (TTHA) and an anthraquinone derivative. The complex formed with Nd^{3+} ($L1Nd^{3+}$) has been obtained *in situ* and the photophysical properties (quantum yields, luminescence lifetimes and photostability) have been studied. The internalization of $L1Nd^{3+}$ complex in lysosomes of living HeLa cells has been confirmed by visible/near-infrared epifluorescence and confocal microscopy. In addition, this probe demonstrated superior photostability over the commercial fluorescent probe LysoTracker Deep Red. Moreover, a deeper penetration of photons through tissues of different origin (up to 3 mm thickness) has been detected and an intense near-infrared signal arising from the $L1Nd^{3+}$ complex in the blood has been observed.

This research is supported through grants from the European Community's Seventh Framework Programmes (ITN Luminet, IEF Dendrimage), l'Agence National de la Recherche and La Ligue Contre le Cancer.

References

1) Martinic I., Eliseeva S.V., Petoud S., Near-Infrared Emitting Probes for Biological Imaging: Organic Fluorophores, Quantum Dots, Fluorescent Proteins, Lanthanide(III) Complexes and Nanomaterials. Journal of Luminescence. DOI : 10.1016/j.jlumin.2016.09.058.

New approaches on the treatment of Alzheimer's disease

Elena Atrián-Blasco, [a,b] Rufus Mitchell-Heggs, [a,b] Jan Hormann, [d] Sébastian Blanchard, [c] Nora Kulak,[d] Christelle Hureau, [a,b]

[a] CNRS, LCC (Laboratoire de Chimie de Coordination), 205 Route de Narbonne, F-31077, Toulouse, France ; [b]Université de Toulouse, UPS, INPT, LCC, F-31077, Toulouse, France ; [c] Institut Parisien de Chimie Moléculaire-UMR 8232-, Université Pierre et Marie Curie - Paris, 4 Place Jussieu, 75252 Paris, France ; [d] Freie Universität Berlin, Institut für Chemie und Biochemie, Fabeckstr. 34/36, 14195 Berlin, Germany

email : elena.atrian@lcc-toulouse.fr

Abstract

Metal-mediated Amyloid-β peptide aggregation[1] and ROS production[2] are well known to play an important role in the development of Alzheimer's disease.[3] For this reason, one of the proposed therapies is based on the chelation of implicated metal ions such as Cu, Zn and Fe.[4] Such chelators could sequestrate the metal ions from the amyloid aggregates and recover the normal metal homeostasis.

In this work we present the preliminary results obtained with different new approaches on metal chelation and inhibition of metal-mediated aggregation, using several families of ligands with differentiated actions.

In the recent years, the use of polyoxometales clusters (POMs) has been described successful in the inhibition of Amyloid-β aggregation.[5] We have studied the interaction of different Keggin-structure POMs with Cu and Zn, and how, through their interaction, they can modulate the metal-mediated aggregation of amyloid- β peptides.

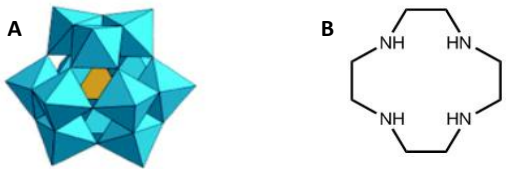


Figure 1. A) Structure of a Keggin-structure POM (from Chem. Soc. Rev., 2012, 41, 7537). B) cyclen.

Moreover, taking into account the effect that Zn could have on the action of Cu-chelators,[6] and the physiological relevance of Cu(I), we have studied different specific Cu(I) chelators based on the cyclen scaffold and their effect of inhibition of Cu(Aβ) ROS production and metal-mediated aggregation.

References

1) P. Faller, C. Hureau, O. Berthoumieu *et al.*, Inorg. Chem., 2013, 52, 12193-12206.
2) C. Hureau, P. Faller, Biochimie, 2009, 91, 1212-1217.
3) P. A. Adlard, A. I. Bush, J. Alz. Dis., 2006, 52, 12193-12206.
4) R. A. Cherny *et al.*, Neuron, 2001, 30, 665-676. S. Noël *et al.*, J. Inorg. Biochem., 2012, 117, 322-325. E. Atrián-Blasco *et al.*, Metallomics, 2015, 7, 1229-1232.
5) J. Geng *et al.*, Angew. Chem. Int. Ed., 2011, 50, 4184-4188. N. Gao *et al.*, Nature Comm., 2014, 5, 3422. N. Gao *et al.*, Nano Res., 2016, 9, 1079-1090.

Acknowledgement: This work is supported by the ERC-2014-StG-638712 aLzINK grant.

Necroptosis-induced by an alkynyl gold(I) complex

Inés Mármol[a], Javier Quero[a], Cristina Sánchez-de-Diego[a], Sonia Gascón[a], Mª Jesús Rodríguez-Yoldi[a] and Elena Cerrada[b]

[a] Departamento de Farmacología y Fisiología, Universidad de Zaragoza, CIBERobn, IA2, ISSAragón (España)

[,b] Departamento de Química Inorgánica, Universidad de Zaragoza, ICSHC-CSIC (España); email : ines.marmol9@gmail.com

Abstract

Colorectal adenocarcinoma is one of the main causes of cancer-related dead and the second most prevalent in Western countries. Treatment involves surgical resection followed by 5-fluoracil-based adjuvant therapy, but although it substantially improves survival, it is associated with side effects that affect overall health and the quality of life. Therefore, it is necessary to develop a novel, less toxic and more efficient therapy to treat this disease. Gold compounds have already been used in medicine as antibacterial drugs, but nowadays are in focus because several studies have demonstrated that organometallic compounds with a coordinated gold atom in the oxidation states +1 and +3 have strong cytotoxicity in different cancer cell lines. Most of these compounds produce cell death by apoptosis but due to the increase of tumors resistant to apoptosis, it is needed new drugs able to induce other forms of cell death. Necroptosis induction may have a great therapeutic potential on those apoptosis-resistant cancers, in spite of the inflammatory effects associated with it. We have synthetized and characterized an alkynyl gold(I) complex [Au(C≡C-2-NC_5H_4)(PTA)] whose anticancer effect was tested on colorectal adenocarcinoma Caco-2 cell line. With regard to its mechanism of action, this gold complex leads to an increase in ROS production, which triggers necroptosis. Necroptosis induction has been found dependent of TNF-α and TNFR1 binding, RIP1 activation and NF-κB signaling. Thus, this complex could be an interesting alternative to current chemotherapy drugs in cases of apoptosis resistance.

A novel dual-functional theranostic Ir(III) complex: biological and imaging properties.

Cristina Pérez-Arnaiz,[a,b] Natalia Busto,[a] Igor Echevarría,[a] Mª Isabel Acuña,[b] Gustavo Espino,[a] José María Leal,[a] Fernando Domínguez,[b] Begoña García.[a]

[a] Chemistry Department, University of Burgos; [b] Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela; email : cparnaiz@ubu.es.

Abstract

The development of multifunctional theranostic agents that combine therapy and diagnosis applications has attracted a great deal of attention, as they can avoid the undesirable differences in biodistribution and selectivity that exist between distinct imaging and therapeutic agents.[1,2] In this work, a novel cyclometallated Ir(III) complex that exhibits exceptional photochemical characteristics for bioimaging (high quantum yield, large Stokes shift and long emission lifetime) as well as potent anticancer properties is presented. Its mechanism of action involves mitochondrial dysfunction and intracellular ROS (reactive oxygen species) generation that leads to an apoptotic cell death process as evidenced by Time-Lapse and caspase-inhibition assays. In fact, subcellular colocalization studies demonstrates that this Ir(III) complex specifically accumulates in mitochondria (**Figure 1**).

In summary, a new tris-chelate Ir(III) complex with anticancer as well as bioimaging properties is presented, which puts forward the potential of this family of compounds to act as dual-functional theranostic agents.

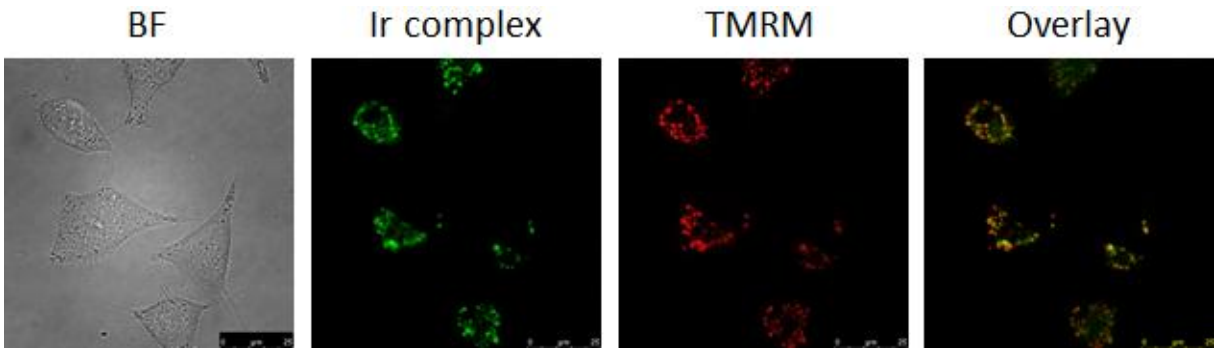


Figure 1. Confocal images of A549 cells treated with the Ir(III) complex (green) and the specific fluorescent probe for mitochondria "TMRM" (red).

Acknowledgements: this work was supported by Obra Social la Caixa (OSLC-2012-007), Ministerio de Economía y Competitividad (CTQ2014-58812-C2-2-R) and Junta de Castilla y León (BU042U16). C. Pérez-Arnaiz is grateful for the FPU13/00180 grant.

References

1. Kelkar, S. S. & Reineke, T. M. Theranostics: Combining imaging and therapy. *Bioconjug. Chem.* 2011, 22, 1879–1903.
2. Chen, M.-H. *et al.* Light-Up Mitophagy in Live Cells with Dual-Functional Theranostic Phosphorescent Iridium(III) Complexes. *ACS Appl. Mater. Interfaces*. 2017, Ahead of Print, doi:10.1021/acsami.7b01735.

ORGANOIRIDIUM HALF-SANDWICH COMPLEXES: STRUCTURE-ACTIVITY RELATIONSHIP STUDY

Ana R. Rubio[a], Natalia Busto[a], Marta Martínez[a], Gustavo Espino[a], José María Leal[a], Begoña García[a].

[a]*Chemistry Department, University of Burgos, Plaza Misael Bañuelos s/n, 09001Burgos, Spain. arrubio@ubu.es*

Abstract

The last efforts in chemotherapy have been dedicated to develop iridium metallodrugs[1] which exhibit positive antiproliferative activities against malignant cells and inhibit kinase enzymes. Their potency can be modulated varying the quelating ligands[2] or changing the substituents on Cp*.[3] Three analogous organoiridium half-sandwich complexes have been studied with ctDNA by a set of kinetic, circular dichroism spectroscopy, melting and viscosity experiments. In addition, the cytotoxic activity was evaluated towards A2780 (human ovarian carcinoma), A2780CIS (cisplatin resistant) and SW480 (human colon carcinoma) cell lines in order to elucidate the role of different atoms in the mode of action. These three complexes are schematized in figure 1.

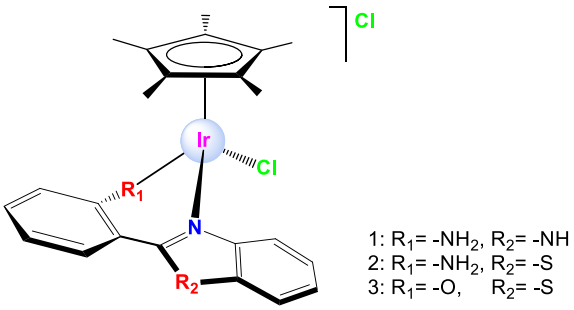


Figure 1. Chemical structure of the half sandwich iridium(III) complexes studied in this work.

Complex 3 is not cytotoxic towards SW480 cell line whereas it has the lowest IC_{50} value towards A2780 and A2780CIS cell lines. The covalent binding with N7 of guanine takes place in the three studied examples. Furthermore 2 and 3 are bifunctional complexes since both of them intercalate in the double helix of DNA. However only in presence of 2, thermal denaturation reveal an interesting stabilization. It is obvious that a simple atom leads the mode of binding.

Acknowledgements: This work was supported by Obra Social "la Caixa" (project OSLC-2012-007), MINECO Spain (CTQ2014-58812-C2-2-R, FEDER Funds) and Junta de Castilla y León (BU042U16). Ana R. Rubio is grateful for Junta de Castilla y León (ORDEN EDU/310/2015) and European Social Found.

References

1. Yellol, G. S. *et al.* On the antitumor properties of novel cyclometalated benzimidazole Ru(II), Ir(III) and Rh(III) complexes. *Chem. Commun. (Camb).* 2013 **49,** 11533–11535.
2. Liu, Z. *et al.* Contrasting reactivity and cancer cell cytotoxicity of isoelectronic organometallic iridium(III) complexes. *Inorg. Chem.* 2011 **50,** 5777–5783.
3. Liu, Z., Habtemariam, A., Pizarro, A. M., Clarkson, G. J. & Sadler, P. J. Organometallic Iridium(III) Cyclopentadienyl Anticancer Complexes Containing C,N-Chelating Ligands. *Organometallics.* 2011 **30,** 4702–4710.

Mitochondrion-targeted Platinum Complexes Inhibit Tumor Growth through Bioenergitic Metabolism

Zhenzhu Zhu,[a,b] Xiaoyong Wang,[a] Zijian Guo[b]

[a]*State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, P.R.China;*
[b]*State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, P.R.China; email : DG1324133@smail.nju.edu.cn;*

Abstract

Mitochondria play a central role in various cellular actions such as energy conversion, substance metabolism and apoptosis. In recent years, targeting mitochondria and affecting their function have become a new strategy for cancer therapy. Three mitochondrion-targeted monofunctional Pt^{II} complexes, $[Pt(o\text{-}PPh_3CH_2Py)(NH_3)_2Cl](NO_3)_2$ (OPT), $[Pt(m\text{-}PPh_3CH_2Py)(NH_3)_2Cl](NO_3)_2$ (MPT), and $[Pt(p\text{-}PPh_3CH_2Py)(NH_3)_2Cl](NO_3)_2$ (PPT) (PPh_3 = triphenylphosphonium, Py = pyridine), were investigated. OPT exhibited a high cytotoxicity against several human cancer cell lines, especially against non-small lung cancer cells (A549). In vivo antitumor efficacy was tested using A549-bearing nude mice. OPT exerted a strong inhibition towards the tumor growth. Cellular distribution assay showed that OPT accumulated more in mitochondria than in nuclei, while PPT accumulated more in nuclei than in mitochondria. The plasmid DNA unwinding activity of OPT was lower than that of PPT, while MPT showed little DNA-binding ability. Mitochondrial DNA lesion induced by OPT was similar to that induced by cisplatin. More importantly, OPT induced significant changes in mitochondrial functions, particularly in mitochondrial bioenergetics; furthermore, it also induced changes in the ultrastructural morphology and the membrane depolarization of mitochondria. In addition, the release of cytochrome c and nuclei DNA fragment induced by OPT were extremely higher than that responding to cisplatin. Therefore, the antitumor mechanism of these complexes differs from that of cisplatin.

References

1) X. Y. Wang, Z. J. Guo, Chem. Soc. Rev. 2013, 42, 202-224.

Structural Studies on the precious metal ions in the Atx1-like Copper Chaperons

Wei Wei,[a] Yang Sun,[b] Pengfei Li,[b] Yi Cao[b] and Jing Zhao[a] *

[a] *State Key Laboratory of Coordination Chemistry, Institute of Chemistry and BioMedical Sciences, School of Chemistry and Chemical Engineering, Collaborative Innovation Center of Chemistry for Life Sciences, Nanjing University, Nanjing 210093, China;* [b] *National Laboratory of Solid State Microstructure, Department of Physics, Nanjing University, Nanjing 210093, China; email : weiwei@nju.edu.cn*

Abstract

The coordination bond between gold and sulfur (Au-S) has been widely studied and utilized in many fields. However, detailed investigations on the basic nature of this bond are still lacking. A gold-specific binding protein, GolB, was recently identified, providing a unique opportunity for the study of the Au-S bond at the molecular level. We probed the mechanical strength of the gold-sulfur bond in GolB using single molecule force spectroscopy. We measured the rupture force of the Au-S bond to be 165 pN, much lower than Au-S bonds measured on different gold surfaces (~1000 pN). We further solved the structures of apo-GolB and Au(I)-GolB complex using X-ray crystallography. These structures showed that the average Au-S bond length in GolB is much longer than the reported average value of Au-S bonds. Our results highlight the dramatic influence of the unique biological environment on the stability and strength of metal coordination bonds in proteins.

The toxicity of silver to many living organisms has been generally attributed to its competitive binding to the metal coordination sites of metalloproteins. Here we report the crystal structure of silver with a copper trafficking protein Atox1. Surprisingly we find that silver could form a plane quadrangle and mediate the formation of Atox1 dimer. Using single molecule force spectroscopy we reveal that the silver-Atox1 complex is mechanically labile in solution and can easily fall apart by breaking the silver-silver bonds. The mechanical bond strength of the silver-silver bond is determined to be ~64 pN and this weak bond strength may explain the scarcity of silver nanoclusters. Our results not only provided the potential molecular basis for the detoxification of silver ions in vivo through copper chaperones, but also a biosynthetic route for metal nanoclusters.

References

1)： Wei, W.; Sun, Y.; Liu, X.; Sun, P.; Wang, F.; Gui, Q.; Meng, W.; Cao, Y.*; Zhao, J*. J. Am. Chem. Soc. 2015, 137, 15358−15361 (Highlighted by C&EN, Nat. Chem. Bio)

2)： Wei, W.; Li, P.; Wang, F.; Cao, Y.; Zhao, J*. Manuscript in preparation.

Siderophores in Cloud Waters and Potential Impact on Atmospheric Chemistry

Virginie Vinatier [a], Monica Passananti [a], Nolwenn Wirgot [a], Muriel Joly [a,b], Martine Sancelme [a], Laurent Deguillaume [b,c], Gilles Mailhot [a], Marcello Brigante [a] and Anne-Marie Delort [a]

[a] Université Clermont Auvergne, CNRS, SIGMA Clermont, Institut de Chimie (ICCF), F-63000 Clermont–Ferrand, France ; [b] L'Observatoire de Physique du Globe de Clermont-Ferrand (OPGC), Laboratoire de Météorologie Physique, Université Clermont Auvergne, Université Blaise Pascal, BP 10448, F-63000 Clermont-Ferrand, France [c)] CNRS, UMR 6016, L'Observatoire de Physique du Globe de Clermont-Ferrand (OPGC), Laboratoire de Météorologie Physique (LaMP/OPGC), BP80026, F-63177 Aubière, France ; email : virginie.vinatier@uca.fr

Abstract

One of the major sources of OH radical in atmospheric water has been attributed to iron species. However, iron chemistry in aqueous solution is a very complex process due to the existence and the stability of numerous iron species. Given the insolubility of ferric oxyhydroxides and oxides under natural waters conditions, the concentration of iron in equilibrium with these solids is very low and insufficient to drive substantial change in iron speciation or oxidant capacity of the medium. However, the concentration of dissolved iron can substantially increase by the presence of strong complexing agents as siderophores. The so formed iron complexes may absorb solar light and undergo a redox process leading to the reduction of Fe(III) and the formation of oxidant species (•OH). The presence of organic Fe-complexing ligands with high stability constants comparable to siderophores ones ($K>10^{20}$-10^{22}) has been shown in rainwaters[1] by striping cathodic voltametry. Their chemical nature has not been determined yet but the hypothesis that iron is complexed by siderophore in atmospheric waters could be emitted. Indeed metabolically active microorganisms are present in cloud water. To test our hypothesis a high-throughput method permitting the detection of siderophores and the characterization of the chemical functions involved in iron chelation has been set up. 450 strains isolated from cloud water at the puy de Dôme station (1465m, France) were identified and screened for siderophore production[2]. In our conditions 42% of strains were able to produce siderophores. The most frequently encountered genus *Pseudomonas* was also the most active. A mixture of pyoverdins produced by bacteria isolated from clouds was used to determine the photoreactivity of iron (III) pyoverdine complexes under simulated cloud conditions. The photolysis of these complexes led to the generation of Fe (II) and hydroxyle formation. Acetate formation was also observed suggesting a fragmentation following ligand-to-metal charge transfer[3]. Finally the presence of pyoverdine in cloud water could impact the composition and oxidative capacity of this environment.

References

(1) Cheize, M.; Sarthou, G.; Croot, P. L.; Bucciarelli, E.; Baudoux, A.-C.; Baker, A. R. Iron Organic Speciation Determination in Rainwater Using Cathodic Stripping Voltammetry. *Anal. Chim. Acta* **2012**, *736*, 45–54.

(2) Vinatier, V.; Wirgot, N.; Joly, M.; Sancelme, M.; Abrantes, M.; Deguillaume, L.; Delort, A.-M. Siderophores in Cloud Waters and Potential Impact on Atmospheric Chemistry: Production by Microorganisms Isolated at the Puy de Dôme Station. *Environ. Sci. Technol.* **2016**, *50* (17), 9315–9323.

(3) Passananti, M.; Vinatier, V.; Delort, A.-M.; Mailhot, G.; Brigante, M. Siderophores in Cloud Waters and Potential Impact on Atmospheric Chemistry: Photoreactivity of Iron Complexes under Sun-Simulated Conditions. *Environ. Sci. Technol.* **2016**, *50* (17), 9324–9332.

Transition metal catalysis in biological media and living cells

Miguel Martinez-Calvo, María Tomás-Gamasa, José R. Couceiro, José L. Mascareñas

*Centro Singular de Investigación en Química Biológica y Materiales Moleculares (**CIQUS**) and Departamento de Química Orgánica, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain;*
email : miguel.martinez@usc.es

Abstract

The living cell is a very complex, compartmentalized and dynamic entity, with a very high concentration of biomolecules, ions and other structures in complex equilibrium, and can therefore be considered as a very stringent reaction medium. Despite all these potential complications, recent data suggest that ruthenium derivatives can promote intracellular reactions through typical organometallic mechanisms.[1-3] Contributions in the area dealing with the use of palladium complexes suggest that obtaining good results requires the use of heterogeneous nanostructured palladium species.[4–6] All these data confirm that achieving organometallic catalytic reactions of exogenous substrates in complex biological media and within living cells is certainly tricky, and has not yet unambiguously demonstrated.

While the field is in its infancy and further progress requires the development of new biocompatible transformations, there are many other questions that remain to be addressed. Is it possible to concentrate the catalyst within a specific organelle/environment while keeping its activity, and without generating toxicity? Would it be possible to visualize the catalyst within the cell and the organelles? Is it possible to use the confined catalyst to generate a differentiated biological effect? Could this localization provide a functional advantage?

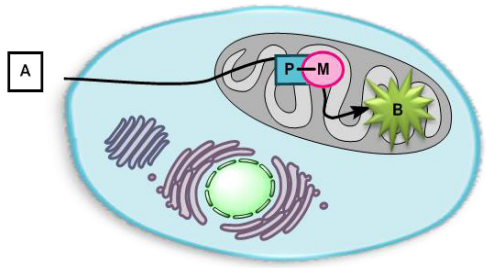


Figure 1. Preferential accumulation of the metal catalyst in the mitochondria owing to the targeting group P. Abbreviations: M = metal complex, P = mitochondria targeting group.

Herein we provide some answers to these questions and among other things we will describe designed ruthenium conjugates that accumulate in the mitochondria of living cells and promote a localized uncaging of alloc/allyl protected exogenous substrates (Fig. 1).

Acknowledgements

We are thankful for the support given by the Spanish grants SAF2013-41943-R and IJCI-2014-19326, the Xunta de Galicia (GRC2013-041, 2015-CP082 and 2016-PG015), the ERDF, and the European Research Council (Advanced Grant No. 340055).

References

1) Streu, C. & Meggers, E. *Angew. Chem. Int. Ed.* **2006,** 45, 5645–5648.
2) Volker, T., Dempwolff, F., Graumann, P. L. & Meggers, E. *Angew. Chem. Int. Ed.* **2014***, 53,* 10536–10540.
3) Tomás-Gamasa, M., Martínez-Calvo, M., Couceiro, J. R., Mascareñas, J. L. *Nat. Chem.* **2016**, 7, 12538.
4) Yusop, R. M. *et al*. *Nature Chem.* **2011**, *3,* 239–243.
5) Weiss, J. T. *et al. J. Med. Chem.* 2014, *57,* 5395–5404.
6) Li, J. *et al. Nature Chem.* 2014, ***6,*** 352–361

Towards the discovery of new antibacterial agents

Jaione Saez Cabodevilla[ab], Martin Clemancey[a], Jean-Marc Latour[a], Patricia Amara[b], Juan Fontecilla-Camps[b], Olivier Hamelin[a], Yung-Sing Wong[c], Sandrine Ollagnier de Choudens[a]

[a]*Equipe BioCat, Laboratoire de chimie et biologie des métaux, CEA, CNRS, UGA, 17 Av. des Martyrs, 38054 Grenoble, cedex 9*

[b]*Groupe Métalloprotéines, IBS, CEA, CNRS, UGA, 71 Av des Martyrs, 38044 Grenoble, Cedex 9*

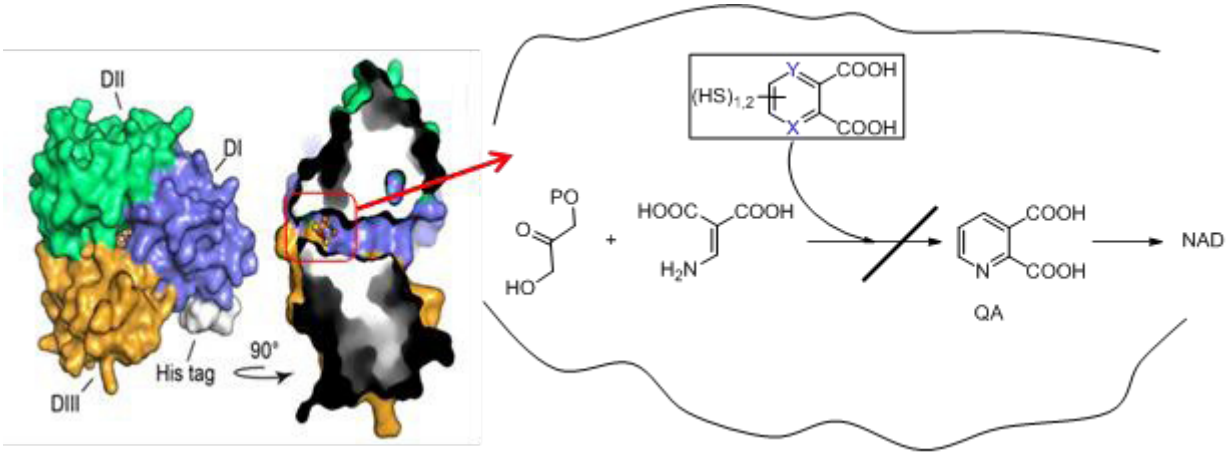
[c]*Département de Pharmacochimie Moléculaire, Batiment André RASSAT, 470 rue de la chimie, 38400 St Martin d'Heres*

jaione.cabodevilla@cea.fr

As it is well known nowadays, more and more resistance to antibiotics is being developed by different pathogens, becoming a world-wide health risk: Resistant microorganisms are able to withstand drugs, these become ineffective, and disease persist. This is why the research of new drugs is of big importance on the current days.

In our laboratory, we are interested in the development of new antibacterial drugs against the "quinolinate synthase" enzyme, which is essential in some pathogen organisms such as *Mycobacterium leprae*, cause of leprosy disease; and *Helicobacter pylori*, cause of gastric ulcers and cancers.

This enzyme, also called NadA, is a $[4Fe\text{-}4S]^{2+}$ enzyme. The cluster is essential for its activity and it is coordinated by 3 cysteine ligands and a water molecule [1]. Previously in our laboratory, the first *in vitro* and *in cellulo* inhibitor of NadA was described: the dithiohydroxyphtalic acid or DTHPA (X=Y=CH; $(SH)_2$. IC_{50}=10µM; 1:1 with regard to the protein). It was demonstrated that it inhibits NadA activity by interaction through its thiolate groups with the iron site of the cluster coordinated by the water molecule in the resting state [2]. However, a lack of selectivity was observed at high concentrations both *in vitro* and *in vivo*. Based on a structure-activity study and some docking studies [1], with the aim of finding new, stronger and more specific inhibitors, we have designed, synthesized, studied and tested a family of molecules. These results will be presented and discussed in the poster.



[1] Cherrier M.V., Chan A., Darnault, C., Reichmann, D., Amara, P., Ollagnier de Choudens, S., Fontecilla-Camps, J.C. The Crystal Structure of Fe4S4 Quinolinate Synthase Unravels an Enzymatic Dehydration Mechanism That Uses Tyrosine and a Hydrolase-Type Triad. J. Am. Chem. Soc. 2014, 136, 5253−5256

[2] Chan, A., Clémancey, M., Mouesca J.M., Amara P., Hamelin O., Latour, J.-M., Ollagnier de Choudens, S. Studies of Inhibitor Binding to the [4Fe-4S] Cluster of Quinolinate Synthase. Angew. Chem. Int. Ed. 2012, 51, 7711 –7714

High throughput screening on P_{1B}-type ATPase: toward new inhibitors of CadA

Xavier Maréchal[a], Caroline Barette[b], Roger Miras[a], Olivier Neyrolles [c], Patrice Catty[a]

[a] Laboratoire de Chimie et Biologie des Métaux (LCBM) UMR5249 UGA/CNRS/CEA, BIG/CEA-Grenoble, 17 rue des Martyrs, 38054 Grenoble cedex 09.
[b] Laboratoire BGE U1038 INSERM-CEA-UGA, BIG/CEA-Grenoble, 17 rue des Martyrs, 38054 Grenoble cedex 09.
c IPBS, UMR5089 CNRS/UPS Equipe "Interactions des Mycobactéries avec les Cellules Hôtes", 205 route de Narbonne, 31077 Toulouse cedex 04
e-mail: xavier.marechal@cea.fr

Abstract

Cellular ion homeostasis is essential for cells. Key components of this process are P-Type-ATPases, membrane proteins that actively transport many cations by using the energy of ATP hydrolysis. P_{1B}-type ATPases are a large family that gathers Zn^{2+}, $Cu^{+/2+}$, Cd^{2+}, Pb^{2+}-ATPases. CadA from *Listeria monocytogenes* belongs to this family and was found responsible for the resistance to cadmium however its physiological role very likely relies on zinc homeostasis [1].

In *L. monocytogenes* and in other intracellular pathogens like *Mycobacterium tuberculosis*, P_{1B}-type ATPases are also involved in the resistance of metal based poisoning triggered by macrophages in response to infection [2]. P_{1B}-type ATPases could then be relevant as new therapeutic targets, given that inhibition of these transporters could somehow decrease bacterial virulence and then help in the treatment of various diseases

Inhibitors could also constitute new pharmacological tools useful in the understanding of P_{1B}-type ATPases. For now little is known about P_{1B}-type ATPase structure. Only two structures are available, one of the Cu^{+}-ATPase CopA from *Legionnella pneumophila*, the other of the Zn^{2+}-ATPase ZntA from *Shigella sonnei*. This two structures have been obtained without the N-terminal metal binding domain, a key domain in ATPase function, and in an unmetalled conformation. The stabilization of different conformations of P_{1B}-type ATPases by inhibitors (or ligands in general) could be helpful in the acquisition of new crystallographic structures and thus in a better understanding of the mechanism of metal transfer by these transporters.

With these two main objectives, we decided to find new inhibitors of P_{1B}-type ATPases and for that to develop the first High Throughput Screening (HTS) assay on this kind of transporters. We chose CadA as model, whose enzymatic characteristic we know in details [3]. Measurement of the ATP hydrolysis using the Fisk-Subarrow method is the readout of the HTS assay. The latter was optimized to 384 wells plate format and orthovanadate, an analogue of inorganic phosphate that non-specifically blocks all the P-type ATPases, used as reference inhibitors. Eleven hit compounds (IC_{50} value below 50 µM) were identified from the first screen performed on the Prestwick chemical library® (1280 molecules). Screening of other chemical libraries are in progress. Due to the similarity of their enzymatic cycle, this assay could easily be adapted to other members of the P_{1B}-type ATPase family.

References

[1] Lebrun M; Audurier A; Cossart P. Plasmid-borne cadmium resistance genes in Listeria monocytogenes are present on Tn5422, a novel transposon closely related to Tn917. *J. Bacteriol*. **1994**, 176, 3049-61.
[2] Botella H; Peyron P; Levillain F; Poincloux R; Poquet Y; Brandli I; Wang C; Tailleux L; Tilleul S; Charrière GM; Waddell SJ; Foti M; Lugo-Villarino G; Gao Q; Maridonneau-Parini I; Butcher PD; Castagnoli PR; Gicquel B; de Chastellier C; Neyrolles O. Mycobacterial p(1)-type ATPases mediate resistance to zinc poisoning in human macrophages. *Cell. Host. Microbe*. **2011**, 10, 248-59.
[3] Wu C C, Gardarin A, Catty P, Guillain F, Mintz E. CadA, the Cd2+-ATPase from Listeria monocytogenes, can use Cd^{2+} as co-substrate. *Biochimie*. **2006**, 88, 1687-92.

Live-lung Cancer Cell IR Imaging of a Cytotoxic Ruthenium Nitrosyl Complex by Spectromicroscopy Analysis

Fernando Postalli Rodrigues, [a,] Leandro N. C. Máximo,[b,] Fernanda C. Sales,[c,] Juliana C. Biazzotto, [a,] Frank N. Crespilho[c,] Roberto Santana da Silva,[a,]

a Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. Café s/n, 14040-903 Ribeirão Preto, SP, Brazil.

b Departamento de Química, Instituto Federal de Educação, Ciência e Tecnologia Goiano, Campus Urutaí, Rodovia, Prof. Geraldo Silva Nascimento, Km2,5, 75790-000 - Urutaí, GO - Brasil.

c Departamento de Físico-Química, Instituto de Química de São Carlos, Universidade de São Paulo. Av. Trab. São-carlense, 400. Centro, 13560970 - São Carlos, SP - Brasil

e-mail : ferpostalli@hotmail.com

Abstract

Some biological studies of metallocomplexes include particular events such as (i) drug uptake by the cells, (ii) interaction with biomolecules, (iii) co-localization, (iv) stability of the complexes in solution, and more. These events could be monitored using the FTIR – Fourier Transform Infrared spectromicroscopy (microFT-IR), presented here as a new tool for ruthenium metal complex studies. Ruthenium nitrosyl complexes are promising NO donor agents with numerous advantages for the biologic applications of NO. In this context, we have characterized the NO release from the ruthenium nitrosyl complex *trans-*$[Ru(NH_3)_4(isn)(NO)]^{3+}$ (**I**), presenting the chemical and cytotoxicity properties against lung carcinoma cell line, A549. Its biological effect on the A549 cell line was studied in the absence of visible light irradiation, releasing NO via reducing process. The results indicated that oxidation of exogenous NADH promotes NO release from (**I**), using an electrode for detection. Interestingly, the NO release was also observed by monitoring the NO stretching (1939 cm^{-1}, ν_{NO}) via microFT-IR, after 30 minutes of cell treatment, in the moment that was detected the internalization of (I). When tested for their cytotoxic potency, the species showed IC50 values in the low µM rage. MicroFT-IR analysis allowed detecting the (**I**) concentration in specific sites of the cell environment which corroborate with the determination of the cytoplasmatic co-localization. Then, live-lung cell IR imaging was generated for this class of ruthenium nitrosyl, describing the real-time determination of interactions and dynamics of metallocomplexes with cells. The interactions with cellular biomolecules are being under investigation using this advanced label-free analysis.

Posters

Session A

Thursday June 8th

HD	**Metals in health and disease**
DG	**Metal-based drugs**
EN	**Metals in environment and toxicology**
IM	**Metals in imaging and sensing**
EZ	**Metalloenzymes, inspiration, mimics function and inhibition**
BM	**Metals and biomolecules**

The role of Amyloid beta protein, metal ions and oxidative stress in the pathophysiology of Alzheimer's disease: recent findings

Giuseppe Grasso,[a] Hiroaki Komatsu,[b] Paul H. Axelsen[b]

[a] *Dipartimento di Scienze Chimiche, Università di Catania, Italy;* [b] *Departments of Biochemistry and Biophysics, and Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA email : grassog@unict.it*

Abstract

Aβ peptides are associated in various ways with metal ions and with mediating oxidative stress in Alzheimer's disease (AD). That oxidative stress, acting on ω-6 and ω-3 polyunsaturated fatty acyl chains, produces 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-hexanal (HHE) respectively, which can covalently modify the Aβ peptides that helped producing it. To examine possible feedback pathways involving Aβ peptides, metal ions and HNE, the interactions were examined by mass spectrometry and fluorescence spectroscopy. Results indicate that metal ions, particularly copper(II), interfere with the modification of His side chains by HNE, but that once modified, metal ions can still bind to Aβ with high affinity. Moreover, a first attempt to monitor the relative amounts of unmodified Aβ and HNE/HHE modified Aβ in vivo is also reported.

These results provide insight into a network of biochemical reactions that may be operating as a consequence of oxidative stress in AD, or as part of the pathogenic process.

IMPACT OF METAL-CATECHOL COMPLEXES FORMATION ON THE REGIOSELECTIVITY OF THE PICTET-SPENGLER REACTION

Nicolas HAVET[a,b],Laury LIVEMONT[b],Emmanuel BAUDRIN[a],

[a]*LG2A, UMR 7378 CNRS, Université de Picardie Jules Verne, UFR de Pharmacie, 1 rue des Louvels F-8000 Amiens ;*

[b]*Laboratoire UNITHER – Service Développement et Validation, 151 rue André Durouchez F-80084 ; email : nicolas.havet@unither-pharma.com*

Dopamine belongs to the catecholamines family, a widespread class of neurotransmitters which are active in the central nervous system. Parkinson's disease is characterized by a deficit of dopamine and abnormal accumulation of diverse metals, such as iron, copper and aluminium, in the *substantia nigra* is part of numerous neurodegenerative diseases. This does not come out of the blue since catecholamines decomposition is known to be catalyzed by metal ions leading to formaldehyde[1] which can in a second step lead to two regioisomers of tetrahydroisoquinolines[2] (THIQ) through the Pictet-Spengler Reaction (PSR). The induced THIQs are found in significant amount in *substantia nigra* of parkinsonian and alcoholic people.

The aim of our work is to study *in vitro*a subsequent effect of metal ions on this degradation process. In a previous work[3], it was shown that the kinetic of the PSR reaction was affected by the presence of transition metals, namely Cu^{2+} and Fe^{3+}. We report here the impact of the presence of some metal ions on the regioselectivity of the Pictet-Spengler Reaction and show that the effect is highly correlated to the complexation strength of these metal ions with dopamine. Hence, Fe^{3+}, Al^{3+} and Cu^{2+}could have a significant impact on the toxicity level of neurotoxic THIQs depending on the relative amount of each regioisomer.

Figure 1. Formation of two regioisomers of tetrahydroisoquinolines through a Pictet-Spengler reaction

References

[1] A. Napolitano, A. Pezzella, and G. Prota, *Chem. Res. Toxicol.*, **1999**, *12*, 1090
[2] H. A. Bates., *J. Org. Chem.*, **1981**, *46*, 4931
[3] P. Manini, M. d'Ischia, R. Lanzetta, M. Parrilli, G. Prota, *Bioorgan. Med. Chem.,***1999**, *7*, 2525

Iron-Sulfur Clusters Biogenesis and diseases: the role of mammalian ISCA proteins

S. Ollagnier de Choudens, LK. Beilschmidt, M.A. Hograindleur, M. Clémancey, G.

Blondin, I. Sanakis, A. Martelli and H. Puccio

Abstract

Iron-sulfur clusters (Fe-S) are ancient and essential cofactors that participate in a number of cellular processes ranging from mitochondrial respiration to DNA metabolism. Within the past decade, their biogenesis has being studied by genetic and biochemical approaches in bacteria, yeast, plants and mammalian demonstrating that it is a complex process involving multiple components highly conserved from bacteria to humans. The process of Fe-S protein maturation is a fundamental and essential biosynthetic pathway. In human, mutations in several components of this biosynthetic machinery are associated with severe disorders such as neurodegeration, myopathy and anemia pointing to the importance of this pathway for normal cellular function. Therefore, it is crucial to investigate such a process at a molecular level in order to better understand these diseases. In eukaryotes, Fe-S clusters are synthesized by the mitochondrial ISC machinery and the cytosolic CIA system. In the mammalian ISC machinery, the initial stage of nascent Fe-S cluster biosynthesis occurs by a multimeric complex allowing the assembly of a transiently-bound Fe-S cluster on a scaffold protein from inorganic sulfide and iron. Once assembled, the labile cluster is transferred directly or indirectly from the scaffold to target mitochondrial proteins. Mammalian A-type proteins, ISCA1 and ISCA2 are proteins involved in this later step and it was shown that mutation in ISCA2 in human lead to severe genetic disorders in young children. We have characterized mouse ISCA1 and ISCA2 using biochemical, spectroscopic and in vivo approaches. Collectively, our data point to different requirements of ISCA1 and ISCA2 *in vivo* in contrast to previous published results.

Copper-induced proteasome inhibition: the role of metals*a laconciergerie* of the core particle

Anna Maria Santoro[a], Irene Monaco[a,b], Francesco Attanasio[a] , Valeria Lanza[a],Giuseppe Pappalardo[a], Marianna Flora Tomasello[a], Alessandra Cunsolo[c], Enrico Rizzarelli[a,b], Ada De Luigi[d], Mario Salmona[d] and Danilo Milardi[a*]

[a]Istituto di Biostrutture e Bioimmagini - CNR Sede di Catania, Via P. Gaifami, 9- 95126 Catania, Italy, [b]FondazioneRiMed, Via Bandiera 11, 90133, Palermo, Italy,[c]Dipartimento di ScienzeChimiche, Università di Catania, Viale Andrea Doria 6, 95125 Catania, Italy[d]IRCCS-Istituto di RicercheFarmacologiche "Mario Negri", Via Giuseppe La Masa 19, 20156, Milano, Italy.email : valeria.lanza@cnr.it

Abstract

Copper is essential for living cells and itsdyshomeostasisis involved in many diseases, such as neurodegenerative disorders and cancer. In particular, growing evidences suggest a causative relationship linking between derangement of the ubiquitin proteasomesystem(UPS)and copper mishandling. Based on this, a number of Copper(II) chelating ligands (including 8-hydroxyquinoline, clioquinol, and dithiocarbamate) have been tested as proteasome inhibitors (PIs) and proposed as anticancer agents.[1]Although it is known that their pro-apoptotic activity is potentiated by Cu(II) ions, the exact mechanisms by which they inhibit proteasome activity is not yet elucidated.[2]

In the present study we show that Cu(II) ions simultaneously inhibit the three peptidase activities of isolated 20S proteasomes with potencies (IC_{50}) ranging from 1.0 to 1.4 μM. Spectroscopic studies and native gel electrophoresis assays suggest that Cu(II) ions, in the experimental conditions adopted for cell-free assays, neither catalyze red-ox reactions nor disrupt the assembly of the core particle but, rather, promote conformational changes that may be associated with impaired gating dynamics. A mutant proteasome (α3ΔN) which is in a permanent open gate conformation resulted insensitive to Cu(II) ions, thus confirming their role in proteasome gate closing. Notably, proteasome activity of live HeLa cells grown in a Cu(II)-supplemented medium was significantly inhibited with a maximum effect observed at about 40 μM. However, this inhibitory effect resulted attenuated in the presence of an antioxidant.[3]Finally, the our results have demonstrated that Cu(II)-inhibited 20S activities may be associated to conformational changes that favor the closed state of the core particle, on the other hand the complex effect induced by Cu(II) ions in intact cancer cells is the result of several concurring events including ROS-mediated proteasome flooding, and disassembly of the 26S proteasome into its 20S and 19S components. Knowledge of all these phenomena is expected to better guide the design of novel and more effective Cu(II)-based PIs.

References

1)Marzano, C., Pellei, M., Tisato, F. &Santini, C. Copper complexes as anticancer agents. Anti-Cancer Agents. Med. Chem. 2009,9, 185–211

2) Daniel, K. G., Gupta, P., Harbach, R. H., Guida, W. C. & Dou, Q. P. Organic copper complexes as a new class of proteasome inhibitors and apoptosis inducers in human cancer cells. Biochem. Pharmacol. 2004,67, 1139–1151.

3) Santoro,A.M. Monaco I.,Attanasio F., Lanza V.,Pappalardo G.,Tomasello M.F.,Cunsolo A.,Rizzarelli E., De Luigi A.,Salmona M., Milardi D. Cu(II) ions affect the gating dynamics of the 20S proteasome: a molecular and *in cell* study.*Sci. Rep.*2016,6,33444 DOI: 10.1038/srep33444

Synthesis, Characterization and Determination of Biological Properties of Benzotriazole/Acesulfamate Mixed Ligand Coordination Compounds of Cu(II) and Zn(II) Ions

Ömer Yurdakul[a], Dursun Ali Köse[a], Emre Avcı[b], Onur Şahin[c]

[a]*Hitit University Department of Chemistry, 19030, Çorum/Turkey*

[b]*Hitit University Department Molecular Biology and Genetics, 19030, Çorum/Turkey*

[c]*Sinop University, Scientific and Technological Research Application Center, 57010, Sinop/Turkey*

omeryurdakul@hitit.edu.tr

Abstract

In this study, coordination compounds of biofunctional ligands benzotriazole[1] and acesulfamate[2] with Cu(II) and Zn(II) metal cations were synthesized. The structure of these compounds is characterized by Elemental Analysis, Single Crystal X-Ray Diffraction, FT-IR and UV-Vis Spectroscopy, Thermal Analysis Techniques DTG-DTA methods. As a result of these analyzes Zn(II) complex has been determined to be tetrahedral geometry (Figure 1).

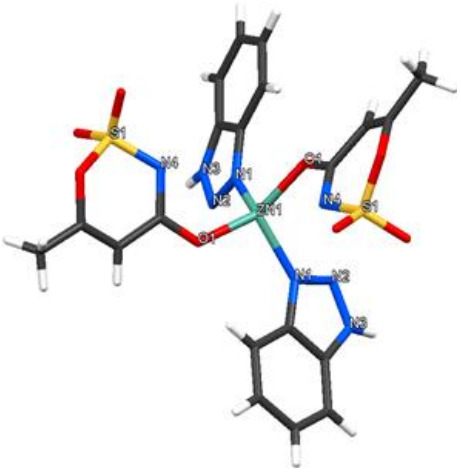


Figure 1. Structure of Zinc Complex (bis(acesulfamato-O)bis(benzotriazole)zinc(II))

Subsequently, the biological activities of the synthesized complexes were investigated by disk diffusion method as antibacterial, antimicrobial and antifungal.

Keywords: Biological Properties, Acesulfamate, Benzotriazole

References

1) Raju R. Kale, Virendra Prasad, Prabhu P. Mohapatra, Vinod K. Tiwari, Recent developments in benzotriazole methodology for construction of pharmacologically important heterocyclic skeletons, 2010, 141, 1159-1182.

2) Cavicchioli M, Massabni AC, Heinrich TA, Costa-Neto CM, Abrao EP, Fonseca BA, Castellano EE, Corbi PP, Lustri WR, Leite CQ, Pt(II) and Ag(I) complexes with acesulfame: crystal structure and a study of their antitumoral, antimicrobial and antiviral activities, 2010, 104(5), 533-540.

Modeling Cu-Abeta oligomers to understand dioxygen activation in Alzheimer's disease

Giovanni La Penna, [a,b] Pham Dinh Quoc Huy,[c,d] Mai Suan Li[c,d]

[a] National research council of Italy, ICCOM, Firenze; [b] National institute of nuclear physics, Roma-Tor Vergata; [c] Polish academy of sciences, IF, Warsaw ; [d] Institute for Computational Science and Technology, Ho Chi Minh City, Vietnam; email : glapenna@iccom.cnr.it

Abstract

Recent research [1,2] shows that soluble complexes of amyloid beta (AB) peptides and copper are efficient catalysts in dioxygen activation and, therefore, are potentially dangerous species triggering an irreversible oxidative pathway in Alzheimer's disease. Starting from dimeric models already investigated within a joint collaboration [3], we built statistical models of oligomeric Cu-AB complexes up to [Cu-AB(1-42)] octamers. The binding of Cu is assumed in all cases to Asp 1 (N,O), His 6 (Nd1) and His13 (Ne2) of each protein chain.

The simulations of these models show that in all cases there is a significant pool of configurations displaying higher solubility in water compared to Cu-free species, and with Cu largely exposed to the water solvent. The burying of Cu within the protein assembly appears disfavoured in transient AB oligomers with Cu:AB 1:1. The relationship between the structural statistics and the activity of Cu sites for dioxygen activation is disclosed by the models.

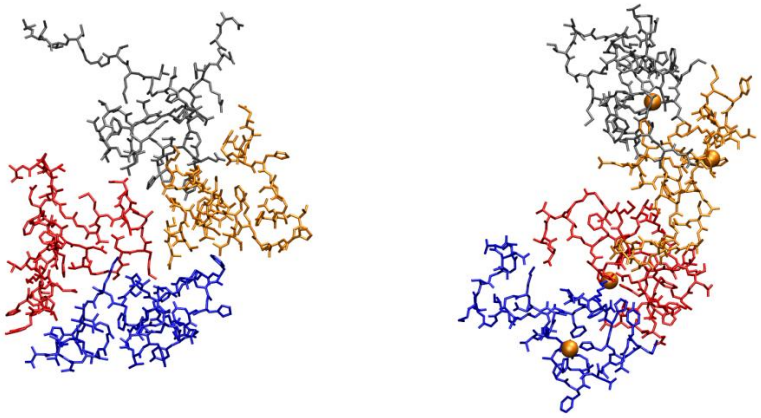


Fig. 1 Tetramers of Cu-AB(1-42) with high solvent-accessible surface with no Cu (left) and with Cu:AB 1:1 (right). Monomers are blue (A), red (B), orange (C) and gray (D); Cu atoms are in orange; atomic and bond radii are arbitrary; H atoms are not displayed.

References

1) A. Mirats et al., Dioxygen activation in the Cu-amyloid beta complex , Phys. Chem. Chem. Phys., 2015, 17, 27270

2) K. Reybier et al., Free superoxide is an intermediate in the production of H2O2 by copper(I)-Abeta peptide and O2, Angew. Chem., 2016, 55, 1085

3) P.D.Q. Huy et al., Impact of Cu(II) binding on structures and dynamics of Abeta42 monomer and dimer: Molecular dynamics study, ACS Chem. Neurosci., 2016, 10, 1348

A new tris-hydroxypyridinone chelating agent with potential biomedical interest: Synthesis, chemical and biochemical studies

Rosita Cappai,[a] Joanna I. Lachowicz,[a] Valeria M. Nurchi, [a] M. Amélia Santos,[b] Karam Chand,[b] Lurdes Gano,[c] M. Antonietta Zoroddu,[d] Massimiliano Peana[d]; rositacappai@gmail.com

[a]Dipartimento di Scienze Chimiche e Geologiche, Università di Cagliari, Cittadella Universitaria, 09042 Monserrato-Cagliari, Italy; [b]Centro de Quimica Estrutural, Instituto Superior Técnico, Universidade Técnica de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal; [c]Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, 2695-066 Bobadela LRS, Portugal; [d]Dipartimento di Chimica e Farmacia, Università di Sassari, Via Vienna 2, 07100 Sassari, Italy.

Abstract

In the industrial age, exposure to metal ions is ever increasing, and so their toxic effects to humans. The behavior of a metal in the physiological conditions depends on the different forms in which it is located in a particular environment. Metal ions can exert conflicting roles in body physiology: some are essential, some are toxic and some essential can transform in toxic depending on concentration.

For instance, iron is necessary for the normal metabolic functions, but can be toxic at high concentration, interfering with functions of various organs like the central nervous system, liver, kidneys, etc. The use of selective metal chelators as therapeutic agents for the prevention, diagnosis and treatment of cancer, diabetes, thalassemia, Alzheimer's, Parkinson's and Wilson's diseases, has received increasing attention [1-3].

The family of hydroxypyridinones, and that of close analogous hydroxypyrones, are particularly versatile classes of ligands. Easily functionalizable, these *O*,*O*-donor chelators allow the formation of a range of divalent and trivalent stable metal-complexes and can include tissue molecular targeting features [4-9].

A new tripodal 3-hydroxy-4-pyridinone derivative has been synthesized and fully characterized using different spectroscopic techniques ^{1}H NMR, ^{13}C NMR and ESI-MS. Its protonation and metal complex formation equilibria with iron, aluminium and gallium have been studied through potentiometry, UV-visible and ESI-MS spectroscopy. Moreover, biodistribution studies with ^{67}Ga in mice indicate that the ligand has a high *in vivo* chelating ability promoting the rapid elimination of the radiometal from the animal body. The results are discussed in comparison with reported data for homologous compounds.

References

1) G. Crisponi, V. M. Nurchi, M. Crespo-Alonso and L. Toso, Curr. Med. Chem., 2012, *19*, 2794-1003.
2) S. J. S. Flora and Vidhu Pachauri, Int. J. Environ. *Res. Public Health*, 2010, *7*, 2745-2788.
3) S. J. S. Flora, M. Mittal and A. Metha, *I. J. Med. Res*., 2008, *128*, 501-523.
4) V. M. Nurchi, G. Crisponi, M. Arca, M. Crespo-Alonso, J. I. Lachowicz, D. Mansoori, L. Toso, G. Pichiri, M. A. Santos, S. M. Marques, J. Niclos Gutiérrez, J. M. Gonzàlez-Pérez, A. Dominguez-Martin, D. Choquesillo-Lazarte, Z. Szewczuk, M. A. Zoroddu and M. Peana, *J. Inorg. Biochem*., 2014, *141*, 132-143
5) Y.-Jung Zhou, Xiao-Le Kong, Jun-Pei Li, Yong-Min Ma, Robert C. Hider and Tao Zhou, *Med. Chem. Commun.*, 2015, *6*, 1620-1625.
6) K. H. Thompson, C. A. Barta and C. Orvig, *Chem. Soc. Rev*., 2006, 35, 545-556.
7) S. Chaves, S. M. Marques, A. M. F. Matos, A. Nunes, L. Gano, T. Tuccinardi, A. Martinelli and M. A. Santos, *Chem. Eur. J.*, 2010, *16*, 10535-10545.
8) M A. Santos, S. Chaves, *Future Med. Chem*, 2015, *7*, 381-408.
9) J.I. Lachowicz, V.M. Nurchi, G. Crisponi, M.G. Jaraquemada-Pelaez, M. Arca, A. Pintus, M.A. Santos, C. Quintanova, L. Gano, Z. Szewczuk, M.A. Zoroddu, M. Peana, A. Dominguez-Martin, D. Choquesillo-Lazarte, *Dalton Trans* 2016, *45*, 6517-6528.

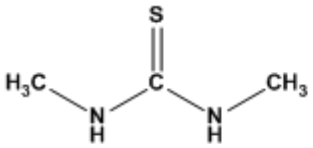
Synthesis, Characterization and Biological Activity of Antimony(III) Halide Complexes with Thiourea Derivatives

I.I. Ozturk [a], C.N. Banti [b], N. Kourkoumelis [c], A.J. Tasiopoulos [d], S.K. Hadjikakou [b]

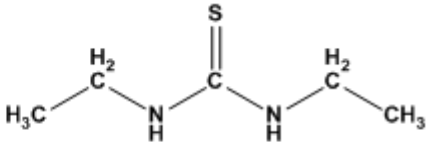
[a]Department of Chemistry, Namık Kemal University, 59030 Tekirdag, Turkey [b]Section of Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece [c]Medical Physics Laboratory, Medical School, University of Ioannina, Greece [d]Department of Chemistry, University of Cyprus, 1678 Nicosia, Cyprus
e.mail: iiozturk@nku.edu.tr

Abstract

Thiourea derivatives have been used in medicine as antibacterial, antiviral and antifungal agents.[1] The chemical and medicinal interest of thiourea derivatives rise from their ambidentate behavior since they can coordinate to metal ions through either the sulfur or nitrogen atoms, while they possess binding sites relevant to those of living organisms.[2] The coordination chemistry of thiourea derivatives with p-block elements is less studied than that of transition metals and so this is a matter of research interest.[3] Among the elements of 15th group, antimony and arsenic are less studied than bismuth. Recently, antimony(III) thione or thiolate complexes have been shown antitumor properties.



N,N'-dimethylthiourea



N,N'-diethylthiourea

Scheme 1. Formulae of the ligands used.

In the progress of our work in the design and development of new cytotoxic compounds based on the structure activity relationship studies, we report here the synthesis and spectroscopic characterization of new antimony(III) halide complexes (SbX_3, X = Cl and Br) with thioureas (Scheme 1), N,N-dimethylthiourea (DMTU) and N,N-diethylthiourea (DETU) of formulae [fac-$SbCl_3$(DMTU)$_3$] (**1**), [mer-$SbBr_3$(DMTU)$_3$] (**2**) and ([$SbCl_3$(DETU)$_2$] (**3**). The ligands and their antimony(III) halide complexes were evaluated for their in vitro cytotoxic activity against human breast adenocarcinoma (MCF-7) and human cervical adenocarcinoma (HeLa) cells. The toxicity of the complexes was studied against human fetal lung fibroblast cells (MRC-5). Cell cycle arrest was employed in order to verify the mechanism of action of compounds **1–3**. The factors such as (i) structural (geometry, molecular weight, volume), (ii) electronic (intermolecular interactions), (ii) lipophilicity (LogP) and (iii) antioxidant activity, which govern the biological activity of these compounds, is discussed in relation with those of other related compounds reported previously by our group.

Acknowledgements: This research was support by Namık Kemal University, Scientific Research Projects Unit (Project No: NKUBAP.01.GA.16.014).

References

1) S. Bourne, K.R. Koch, J. Chem. Soc., Dalton Trans. 1993, 13, 2071.

2) E. Rodríguez-Fernández, J.L. Manzano, J.J. Benito, R. Hermosa, E. Monte, J.J. Criado, J. Inorg. Biochem. 2005, 99 (8), 1558.

3) (a) A. Han, I.I. Ozturk, C.N. Banti, N. Kourkoumelis, M. Manoli, A.J. Tasiopoulos, A. M. Owczarzak, M. Kubicki, S.K. Hadjikakou, Polyhedron, 2014, 79, 151, (b) S.K. Hadjikakou, I.I. Ozturk, C.N. Banti, N. Kourkoumelis, N. Hadjiliadis, J. Inorg. Chem. 2015, 153, 293.

THE HUMAN ENZYME TOPOISOMERASE IB IS INHIBITTED BY Ru(II) DIPHENYLPHOSPHINE COMPOUNDS

Rafael Gomes da Silveira[1,4], Monize M. da Silva[1], Victor M. Deflon[2], Alessandro Desideri[3] and Alzir A. Batista[1]

[1]Department of Chemistry, Federal University of São Carlos, 13565-905 São Carlos, SP, Brazil; [2] São Carlos Institute of Chemistry, University of São Paulo, 13560-570 São Carlos, SP, Brazil; [3] Department of Biology, University of Rome Tor Vergata, 00173 Rome, Italy; [4]Department of chemistry, IF Goiano – campus Ceres 76300-000, Ceres, GO, Brazil.

Abstract

Cancer is the second leading cause of death worldwide. So far, about 50-70% of all tumors are treated with platinum based compounds. Due to severe side effects caused by platinum drugs and the resistance (intrinsic or acquired), complexes with other metal ions than platinum, have been investigated for their anticancer properties. Ruthenium appears as a strong candidate for drug development showing low toxicity, selectivity for tumors, inhibition of metastasis progression and anti-angiogenic properties. Many studies have shown that topoisomerases are important therapeutic targets in cancer chemotherapy. Therefore, the aim of this work was the synthesis of two Ru(II) complexes, the evaluation of their citotoxicity against normal and lung cancer cells and their capacity to inhibit the human topoisomerase IB activity. The ruthenium compounds with formula [Ru(mpca)(bipy)(P-P)]PF6 (**1**) and [Ru(mpca)2(dppb)] (**2**) where mpca=6-mercaptopyridine-3-carboxylic, bipy = 2,2'-bipyridine and dppb = 1,4-bis (diphenylphosphino)butane were obtained. The complexes were characterized by elemental analysis, spectroscopic techniques, including X-ray crystallography, conductimetry, cyclic voltammetry and 31P{H} NMR experiments. Additionally, preliminary tests were carried out, in order to evaluate the cytotoxicity activity of the complexes to performed the MTT-assay for cell viability on cancer cell lines of the new complexes, and the free ligands, against A549 (human lung cancer) and the MRC-5 (human lung) cell lines. The inhibition of ruthenium compounds on topoisomerase enzyme activity was evaluated by dose-dependent analysis, performed by simultaneously adding enzyme (TopIB) and the supercoiled plasmid in the presence of the ruthenium compounds, using DMSO as control. The complexes showed cytotoxicity (IC50A549 11.74 ± 0.52 (**1**) and 25.06 ± 0.29 (**2**) µmol L–1) and selectivity indexes (SI = IC50MRC-5/IC50A549) within 1.03-1.87, when compared to cisplatin (IC50A549 = 14.48 ± 1.45µmol L–1 and SI= 2.02) the results are as good as. DNA supercoiled relaxation was inhibited for all evaluated compounds showing the inhibitory effect of the compounds on topoisomerase IB activity. Compound **1** totally inhibits the activity of the TopIB at the concentration of 100 µmol L–1 whereas complex **2** partially inhibits the activity of the enzyme. DNA interaction studies were carried out by means of viscosity measurements, square wave voltammetry and gel electrophoresis, and it was suggested that the complexes do not interact strongly with the DNA, but their interaction with this molecule is predominantly electrostatic or through binding hydrogen bonding between the complexes and the DNA. The ruthenium complexes are cytotoxic against the A549 cells and a possible action mechanism consists on TopIB inhibition, encouraging further studies on their potential use on cancer therapy.

Key words: Ruthenium, cytotoxicity, topoisomerase inhibitors.

Acknowledgements: This research is supported by grants from CNPq, CAPES, IF Goiano and FAPESP.

Azaindoles as a Suitable Scaffold for Development of Anticancer Transition Metal Complexes

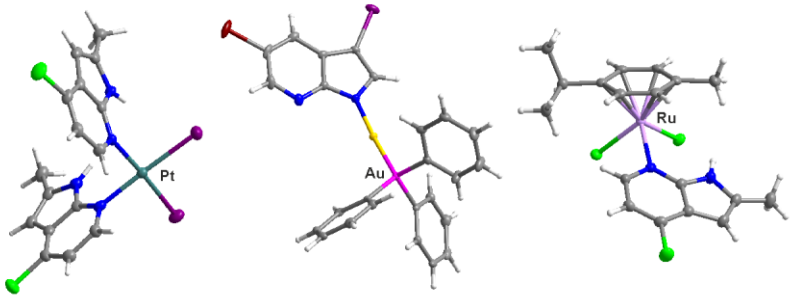
Pavel Štarha,[a] Zdeněk Dvořák,[b] Zdeněk Trávníček[a]

[a] *Department of Inorganic Chemistry,* [b] *Department of Cell Biology and Genetics, Regional Centre of Advanced Technologies and Materials, Faculty of Science, Palacký University in Olomouc, 17. listopadu 12, 771 46 Olomouc, Czech Republic; email: zdenek.travnicek@upol.cz*

Abstract

A group of azaindoles, formally representing the dideazapurines, consists of four isomers, namely 1*H*-pyrrolo[3,2-*b*]pyridine (4-azaindole), 1*H*-pyrrolo[3,2-*c*]pyridine (5-azaindole), 1*H*-pyrrolo[2,3-*c*]pyridine (6-azaindole) and 1*H*-pyrrolo[2,3-*b*]pyridine (7-azaindole; 7aza). Among them, 7-azaindole and its derivatives (*n*7aza) have been recently used as ligands of highly *in vitro* cytotoxic platinum(II) complexes of the general formulas *cis*-[PtX_2(*n*7aza)$_2$] and [Pt(Y)(*n*7aza)$_2$], where X symbolizes Cl^-, I^- or decanoato, and Y stands for oxalato, cyclobutane-1,1-dicarboxylato or malonato ligands. For example, the iodido complexes *cis*-[PtI_2(*n*7aza)$_2$] exhibited significant *in vitro* cytotoxicity against a panel of human cancer cell lines. Their mechanism of action seems to be different from the conventional *cisplatin*, as resulted from the studies of cell cycle modulation or DNA interactions [1]. In addition, these complexes showed encouraging anticancer potency against the L1210 mouse leukaemia cells even at the *in vivo* level.

With respect to overall positive cytotoxicity of platinum complexes containing 7-azaindoles, we decided to utilize these ligands as a scaffold for the preparation of other transition metal complexes showing promising anticancer activity. Thus, we also prepared, thoroughly characterized and studied gold(I) and ruthenium(II) complexes. The [Au(*n*7aza$^-$)(PPh_3)] complexes showed significant potency against the A2780 human ovarian carcinoma cells and promising selectivity towards the mentioned cancer cells over the MRC-5 non-cancerous ones [2]. We also prepared a series of organometallic half-sandwich dichlorido complexes of the type [Ru(η^6-*p*cym)(*n*7aza)Cl_2] (*p*cym = *p*-cymene) [3]. Nowadays, we strive to prepare iridium(III) complexes of the composition [Ir(η^5-Cp*)(*n*7aza)Cl_2] (Cp* = 1,2,3,4,5-pentamethylcyclopentadienyl). An overview of the biological properties of the complexes will be discussed with the framework of the presentation.



References

1) Štarha, P.; Vančo, J.; Trávníček, Z.; Hošek, J.; Klusáková, J.; Dvořák, Z. PLoS ONE 11 (2016) e0165062.
2) Štarha, P.; Trávníček, Z.; Drahoš, B.; Dvořák, Z. Int. J. Mol. Sci. 17 (2016) 2084.
3) Štarha, P; Hanousková, L; Trávníček, Z. PLoS ONE 10 (2015) e0143871.

Bismuth Hydroxamic Acid Complexes Targeting Multidrug Bacterial Resistance

Darren M. Griffith, [a] Donal M. Keogan, [a] Deirdre Fitzgerald-Hughes [c]

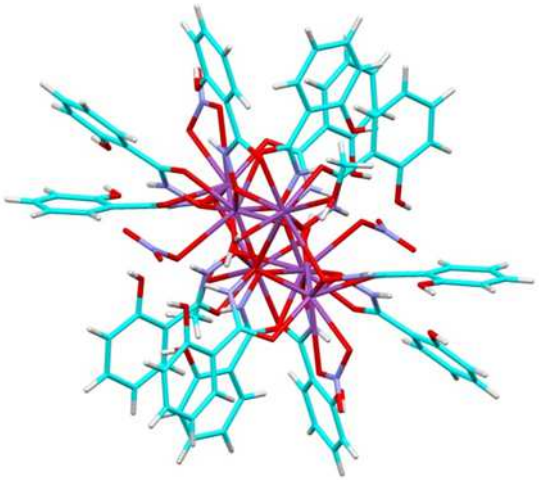
[a] Centre for Synthesis & Chemical Biology, Department of Pharmaceutical and Medicinal Chemistry, RCSI, Dublin 2, Ireland ; [b] RCSI Microbiology, Royal College of Surgeons in Ireland, RCSI Education & Research Centre, Beaumont Hospital, Beaumont, Dublin 9, Ireland; email : dgriffith@rcsi.ie

Abstract

Hydroxamic acids, of general formula RCONHOH, represent a very important family of bioligands.[1] They are effective metal chelators and as ligands exhibit versatile and fascinating coordination chemistry. In turn hydroxamic acids have been employed to develop (i) a diverse range of interesting complexes including metallacrowns, coordination polymers and cluster complexes and (ii) important inhibitors of metalloenzymes such as urease and peptide deformylase.[1]

Infection involving multidrug bacterial resistance represents an emergent global disease and significantly one of the greatest threats to human health. Given that the prospect of untreatable bacterial infections is now becoming a reality, there is an urgent need to develop new classes of effective antibacterial agents.[2]

Progress, including synthesis and biological activity, in relation to the development of novel multifunctional bismuth hydroxamic acid complexes, that target multidrug bacterial resistance will be reported.[3,4]



References

1) D. Griffith, M. Devocelle and C. J. Marmion, in Amino Acids, Peptides and Proteins in Organic Chemistry, ed. A. Hughes, Wiley-VCH, Weinheim, 2009.
2) D. Keogan and D. Griffith, Molecules, 2014, 19, 15258-15297.
3) D. M. Keogan, B. Twamley, D. Fitzgerald-Hughes and D. M. Griffith, Dalton Trans., 2016, 2016, 45, 11008-11014
4) D. M. Keogan, L. E. Fagan, T. M. Passos, H. Müller-Bunz, B. Quilty, D. M. Griffith, Inorg. Chim. Acta, 2017, DOI: 10.1016/j.ica.2016.09.021.

Evaluation of metal polypyridyl complexes as anticancer agents

João Costa Pessoa,[a] Isabel Correia,[a] Nádia Ribeiro,[a] Cristina Matos,[a] Patrique Nunes,[a] Fernanda Marques[b]

[a]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Portugal; [b]Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Portugal; email : joao.pessoa@ist.utl.pt

Abstract

A great variety of metal-complexes, namely 2,2′-bipyridine and 1,10-phenanthroline (phen) containing complexes, have been tested as cytotoxic agents and showed antitumor activity in several *in vitro* and a few *in vivo* experiments.[1-3] Many of these compounds, namely Cu-, V-, Zn-, Fe- and Ru-complexes containing phen and derivatives have been reported to bind DNA by intercalative and non-intercalative interactions; several of these compounds have been also characterized as agents for its cleavage and oxidative modification.[1-5] The mechanism of cytotoxic action for these complexes may go beyond the DNA cross-linking and adduct formation; it may involve disruption in the mitochondrial and of cellular redox balance by producing reactive oxygen species and GSH depletion, as well as inhibition of topoisomerases, and proteasome inhibition or oxidation.[2,3,5]

Several studies were reported on the cytotoxicity, bactericidal, antiparasitic, DNA and HSA binding properties and mechanism of action of mixed ligand Cu-, V- and Zn-complexes containing phen and derivatives. In many cases relatively low concentrations of the compounds were used, e.g. 1-10 μM, and low IC_{50} or MIC values were reported. However, the stability of the compounds at these low concentrations in aqueous media, and which are the species present in the experimental conditions used, is not accessed.

In this work we prepare several XM(phen) and $XM(phen)_2$ complexes (M = Cu(II), V(IV) or Zn(II) and X = anion to balance charge), and report preliminary results on cytotoxicity, as well as speciation analysis, to clarify which might be the relevant complexes that might be present in the aqueous media in contact with the cells during the experiments to evaluate the cytotoxicity. These results are compared with those previously reported with the mixed ligand Cu-, V- or Zn-complexes containing phen as co-ligand.

We also evaluate the experimental conditions of spectroscopic techniques (e.g. fluorescence, circular dichroism), used to access the binding constants of the complexes to DNA or HSA.

References

1) I. Correia, S. Roy, C. P. Matos, S. Borovic, N. Butenko, I. Cavaco, F. Marques, J. Lorenzo, A. Rodriguez, V. Moreno, J. Costa Pessoa, J. Inorg. Biochem., 2015, 147, 134.
2) C. Santini, M. Pellei, V. Gandin, M. Porchia, F. Tisato, C. Marzano, Chem. Rev., 2014, 114, 815.
3) I. E. León, J. F. Cadavid-Vargas, A. L. Di Virgilio, S. B. Etcheverry, Curr. Med. Chem., 2016, 23, 1.
4) A. K. Patra, S. Roy, A. R. Chakravarty, Inorg. Chim. Acta, 2009, 362, 1591.
5) C. Acilan, Z. Adiguzel, B. Cevatemre, D. Karakas, E. Ulukaya, N. Ribeiro, I. Correia, J. Costa Pessoa, Biochem. Biophys. Acta - Gen Subj., 2017, 1861, 218.

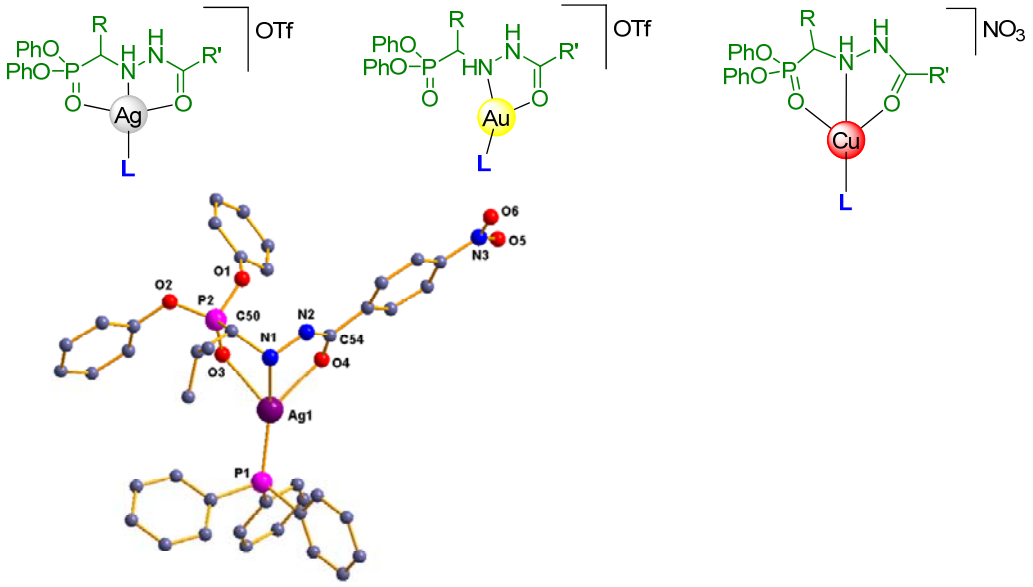
First synthesis of group 11 metal complexes with α-hydrazidophosphonate ligands and biological study

Raquel P. Herrera,[a] Daniel Salvador-Gil,[b] Lourdes Ortego,[b] and M. Concepción Gimeno*,[b]

[a] Laboratorio de Organocatálisis Asimétrica. Departamento de Química Orgánica. Instituto de Síntesis Química y Catálisis Homogénea (ISQCH), CSIC-Universidad de Zaragoza. E-50009 Zaragoza, Spain; [b] Departamento de Química Inorgánica, Instituto de Síntesis Química y Catálisis Homogénea (ISQCH), CSIC-Universidad de Zaragoza, E-50009 Zaragoza, Spain; email : raquelph@unizar.es

Abstract

Phosphonyl derivatives have aroused a great interest because of the important biological properties that these compounds exhibit.[1] In the context of our research program focused on the search of new biologically active metal complexes,[2] the coordination chemistry of α-hydrazidophosphonates towards group 11 metals has been studied by the first time. Thus, Au(I), Ag(I) and Cu(I) complexes with α-hydrazidophosphonates ligands[3] have firstly been prepared and characterized. The *in vitro* cytotoxic activity of the resulting metal complexes was further tested against two tumor human cell lines (HeLa and A-549). IC_{50} values were compared with those obtained for the α-hydrazidophosphonate ligands alone and *cisplatin*. Interestingly, several complexes exhibited excellent cytotoxic activities (IC_{50} < 5 μM) compared with the ligands alone and even better than that shown by *cisplatin*.[4]



References

1) M. Balali-Mood, M. Abdollahi, in *Basic and Clinical Toxicology of Organophosphorus Compounds*. Springer-Verlag London, **2014**

2) See for instances: a) V. Fernández-Moreira, I. Marzo, M. C. Gimeno, *Chem. Sci.* **2014**, *5*, 4434-4446. b) S. Montanel-Perez, R. P. Herrera, A. Laguna, M. D. Villacampa, M. C. Gimeno, *Dalton Trans.* **2015**, *44*, 9052-9062.

2) R. P. Herrera, D. Roca-López, G. Navarro-Moros, *Eur. J. Org. Chem.* **2010**, 1450-1454.

3) D. Salvador, L. Ortego, R. P. Herrera, M. C. Gimeno *Submitted manuscript.*

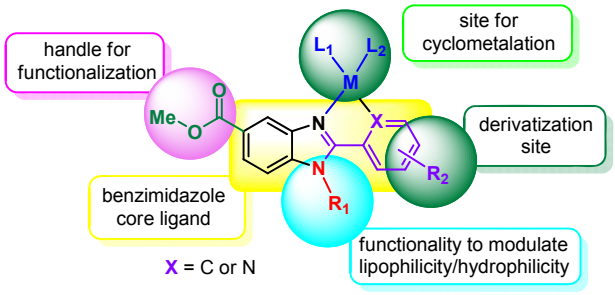
Synthesis and Development of Novel Metallodrugs Based on Benzimidazole with Therapeutic Activity

José Ruiz,[a] Jyoti Yellol,[a] Sergio Pérez,[a] Juraj Zajac,[b] Gloria Vigueras,[a] Gorakh Yellol,[a] Vojtech Novohradsky,[b] Viktor Brabec,[b] Antonio Donaire,[a] Kevin J Barnham,[c] Ana Zamora,[a] Christoph Janiak,[d] Albert Gandioso,[e] Vicente Marchán[e]

[a]Departamento de Química Inorgánica, Universidad de Murcia, Murcia, Spain; [b] Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic; [c] The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Australia; [d] Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany; [e] Departament de Química Inorgànica i Orgànica, Universidad de Barcelona, Barcelona, Spain;
email: jruiz@um.es

Abstract

The design concept of the presently synthesized target has originated from the recognition of the biological role of the benzimidazole which exhibits a wide range of pharmacological properties including anticancer, anti-alzheimer and HIV-1 integrase inhibition. Hence the benzimidazole moiety linked with a phenyl or pyridyl ring is selected as the main core of the design (Figure 1). This type of ligand has allowed us preparation of novel ruthenium and iridium organometallic complexes that exhibit outstanding potency in anticancer,[1,2] antiangiogenic[1] and anti-Alzheimer[3] studies. We successfully synthesized also the iridium compounds (C^N or N^N) functionalized with linear OH-$(PEG)_n$-OH, and OH-$(PEG)_n$-OMe, NH_2-$(PEG)_n$-NH_2 (n = 3) as linkers to improve the lipophilicity and cytotoxicity. In addition, an intensive exploration of functionalization of these structures is currently underway. Furthermore heteroleptic iridium(III) complexes are strong luminescent emitters in cells, being therefore good candidates as theranostic agents.



References

1) Yellol J., Pérez S., Buceta A., Yellol G., Donaire A., Szumlas P., Bednarski J., Makhloufi G., Janiak C., Espinosa A., Ruiz J. *J. Med. Chem.* **2015**, 58, 7310.
2) Yellol J., Pérez, S. A., Yellol, G., Zajac, J., Donaire, A., Vigueras, G., Novohradsky, V., Janiak, C., Brabec, V., Ruiz, J. *Chem. Commun.* **2016**, 52, 14165.
3) Yellol G., Yellol J., Kenche V. B., Liu X. M., Barnham K. J., Donaire A., Janiak C., Ruiz J. *Inorg. Chem.* **2015**, 54, 470.

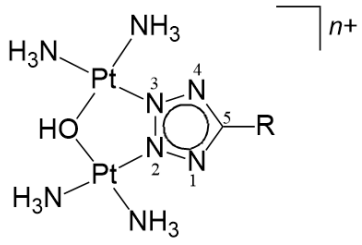
Structure-activity relationships, DNA compaction efficiencies and intracellular accumulations on anticancer-active tetrazolato-bridged dinuclear platinum(II) complexes

Seiji Komeda,[a] Masako Uemura,[a] Keiichi Hiramoto,[a] Yuko Yoshikawa,[b] Kenichi Yoshikawa,[b] Hiroki Yoneyama,[c] Shinya Harusawa[c]

[a] Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science, Suzuka, Mie 513-8670, Japan; [b] Faculty of Life and Medical Sciences, Doshisha University, Kyotanabe, Kyoto 610-0394, Japan ; Faculty of Pharmaceutical Sciences, [c] Osaka University of Pharmaceutical Sciences, Takatsuki, Osaka 569-1094, Japan; email : komedas@suzuka-u.ac.jp

Abstract

Structure-activity relationships, DNA compaction efficiencies and intracellular accumulations on recently synthesized tetrazolato-bridged dinuclear Pt(II) complexes [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-5-R-tetrazolato-*N2*,*N3*)]$^{2+}$ (tetrazolato-bridged complexes, Figure 1) with diverse substituents at tetrazolate C5 will be presented to update our drug development research. The series of tetrazolato-bridged complexes is known to be among next-generation anticancer drug candidates, which are effective on cancers with intrinsic or acquired resistance to the currently available Pt-based drugs such as cisplatin. Tetrazolato-bridged complexes are cationic Pt(II) complexes, and the framework consists of two sets of *cis*-diammineplatinum(II) coordination plane bridged by tetrazolato and hydroxo group, which acts as a leaving group for bifunctional DNA binding at purine bases. Tetrazolato-bridged complexes exhibited markedly high DNA compaction potency through non-covalent interactions (Pt···DNA), followed by slow covalent interactions (Pt—DNA), indicating that the mechanism of action involves a multimodal DNA binding. In addition, highly efficient intracellular uptake into cancer cells was observed.[1, 2] The mechanistic uniqueness of tetrazolato-bridged complexes therefore resulted to bring cytotoxicity profiles totally different from Pt-based drugs[3] and markedly high *in vivo* antitumor efficacies against pancreatic[4] and colon cancer (Figure 2, SK38).



Tetrazolato-bridged complexes

Figure 1. Chemical Structure of tetrazolato-bridged dinuclear platinum(II) complexes.

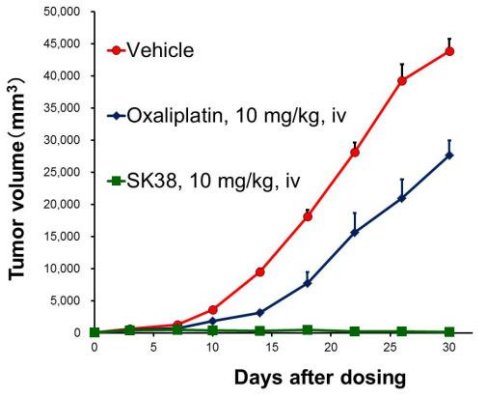


Figure 2. Mouse xenograft study for treatment of colon-26 murine colon cancer (n = 5).

References

1) Uemura, M. et al., Metallomics 2015, 7, 1488-96.
2) Komeda, S. et al., Inorganic Chemistry 2017, 56, 802-811.
3) Komeda, S. et al., Metallomics 2013, 5, 461-8.
4) Komeda, S. et al., ChemMedChem 2011, 6, 987-90.

Development of Trackable Platinum-Based as Anticancer Agents

Eolann Kitteringham[a], Dan Wu[a], Shane Cheung[a], Brendan Twamley[b], Donal F. O'Shea[a], Darren M. Griffith[a]

[a]Dept of Pharmaceutical and Medicinal Chemistry, 123 St. Stephen's Green, RCSI, Dublin 2, Ireland; [b]School of Chemistry, Chemistry Building, Trinity College Dublin, Dublin 2, Ireland. email: eolannkitteringham@rcsi.ie

Abstract

Metal based drugs provide an excellent platform for the development of novel anticancer therapeutic and diagnostic agents, and the prediction and control of the pharmacodynamics and pharmacokinetics of such compounds. Individual metals can offer characteristic or accessible geometries, coordination numbers and redox states. Furthermore manipulation of such variables, by selection of appropriate ligands, can lead to the fine tuning of electronic, chemical and photophysical properties of metal complexes (1-2). Given that many clinical chemotherapeutic metallodrugs, such as cisplatin, are associated with adverse side effects and acquired resistance, non-conventional or next generation complexes are in high demand (1). We are interested in the development of platinum (Pt) complexes as (i) fluorescent probes capable of monitoring cellular uptake and trafficking of Pt-drugs within cells and (ii) theranostic agents, capable of dual therapeutic and diagnostic activity. We have employed copper-free alkyne-azide cycloaddition (AAC) click chemistry to conjugate a derivative of the Pt-based anticancer drug, carboplatin, with a near-infrared (NIR) fluorophore (3). The synthesis, characterisation, *in vitro* activity and cellular imaging of the Pt-NIR fluorophore conjugate will be described.

References

(1)Bertrand B. et al., Dalton Trans., 2016; 45: 13005-13011.
(2) Gaynor D., Griffith D.M., Dalton Trans., 2012; 41(43): 13239-13257.
(3) D. Wu, RSC Adv., 2016, 6, 87373.

Funded by the RCSI Apjohn PhD Scholarship programme.

Monohydroxamate complexes of half-sandwich platinum metal ions – A solution and solid state study

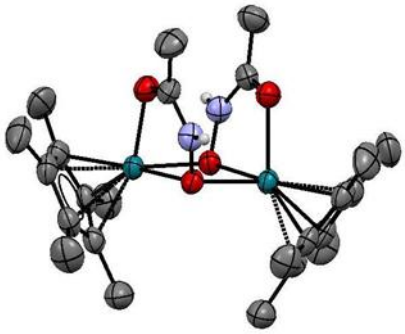
Péter László Parajdi-Losonczi, [a] Norbert Lihi, [a] Attila Csaba Bényei, [b] Diána Mikó, [a] Anita Horváth, [a] Linda Bíró, [a] Etelka Farkas, [a] Péter Buglyó [a]

[a] *Department of Inorganic and Analytical Chemistry, University of Debrecen, Hungary;* [b] *Department of Physical Chemistry, University of Debrecen, Hungary; email : parajdip@science.unideb.hu*

Abstract

Half-sandwich platinum metal complexes represent one of the most extensively studied class of anticancer metallodrugs in recent times. It is also known that some hydroxamic acids possess antiproliferative activity. In the past few years we have combined these two biologically active classes of compounds into single molecules, in order to synthesize metal complexes with increased antitumor effects.[1,2]

In this work we used four different metal ions which can be classified into two types: the para-cymene ruthenium- and osmium aqua ions bearing the η^6-bonding mode, and the Cp* rhodium- and iridium ions of the η^5-bonding mode. The studied ligands represent both the primary- and the secondary monohydroxamates, allowing us to explore all details affecting these metal ion - ligand interactions. To determine the composition and stability of the species existing in solution in these systems, solution equilibrium studies were carried out by means of pH-potentiometry, ESI-MS and ^{1}H NMR.[1] For these experiments we have selected the acetohydroxamic acid and its secondary analogue, N-methyl-acetohydroxamic acid. In order to synthesize these hydroxamate-complexes extensive solid state studies with various ligands were also carried out (see the scheme). In addition, we tested some of these compounds for their anticancer activity *in vitro*, however, they didn't show cytotoxic effect in the studied concentration range.[2]



This presentation will summarize the results of this work, which will show, that interesting differences can occur in the complex formation, depending on the type of the ligand and also on the type of the metal ion.

References

1) P. Buglyó, E. Farkas; Dalton Trans., 2009, 8063
2) A.J. Godó, A.Cs. Bényei, B. Duff, D.A. Egan, P. Buglyó; RSC Adv., 2012, 2, 1486

Acknowledgements

This work was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008. The work was also supported by the Hungarian Scientific Research Fund (OTKA K112317).

Polyoxometalate-chitosan nanocomposites for innovative anticancer approaches

Matteo Croce [a], Simona Conti[b], Caroline Maake[b], Greta R. Patzke[a]

[a] *University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zurich, Switzerland;*

[b] *University of Zurich, Institute of Anatomy, Winterthurerstrasse 190, 8057 Zurich, Switzerland;*

matteo.croce@chem.uzh.ch

Abstract

Polyoxometalates (POMs), negatively charged metal-oxide clusters, are active against different biological targets including cancer cells and viruses.[1] However, their clinical use is not yet feasible, because of their often undefined stability at physiological conditions and their high unspecific cytotoxicity. To investigate how the encapsulation in a biocompatible polymer influences the POMs' stability and cytotoxicity, the two bioactive POMs $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$[2] and $K_6[P_2W_{18}O_{62}]$[3] were synthesized and used to produce nanoparticles with chitosan and its derivate carboxymethyl chitosan. The nanocomposites (range 100-200 nm) were characterized by dynamic light scattering (DLS), FT-IR, and electron microscopy. The stability of the POMs and of the nanocomposites was investigated at different pH values by ^{31}P-NMR, DLS and UV-Vis spectroscopy, showing an improved stability of $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$ when encapsulated.

The viability of HeLa cells as monolayers and 3-dimensional spheroids was tested after 24 h of incubation with the nanoparticles as well as with the pristine components, revealing two opposite behaviours. The encapsulation, depending on the POM, either increased (in the case of $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$) or reduced (for $K_6[P_2W_{18}O_{62}]$) the toxicity of the inorganic drug. To better understand the underlying mechanisms, whole transcriptome RNA sequencing was performed. Preliminary analysis revealed an overexpression of the genes of the immune response.

We work towards a triggered release of the encapsulated POMs, using the different stability of the composites at physiological pH and at slightly acidic pH to target cancer cells.

The biological activity of $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$ and $K_6[P_2W_{18}O_{62}]$, respectively, was thoroughly investigated and their association with chitosan is proposed as new possibility to improve their stability and selectivity toward cancer cells.

References

1) T. Yamase, Anti-tumor, -viral, and -bacterial activities of polyoxometalates for realizing an inorganic drug, 2005, 15, 4773.
2) B. L. Moskovitz, Clinical Trial of Tolerance of HPA-23 in Patients with Acquired Immune Deficiency Syndrome, 1988, 32, 1300–1303.
3) A. Flutsch, T. Schroeder, M. G. Grutter, G. R. Patzke, HIV-1 protease inhibition potential of functionalized polyoxometalates, 2011, 21, 1162–1166.

Reversible Activation Dynamics of Tethered Ruthenium(II) Arene Complexes

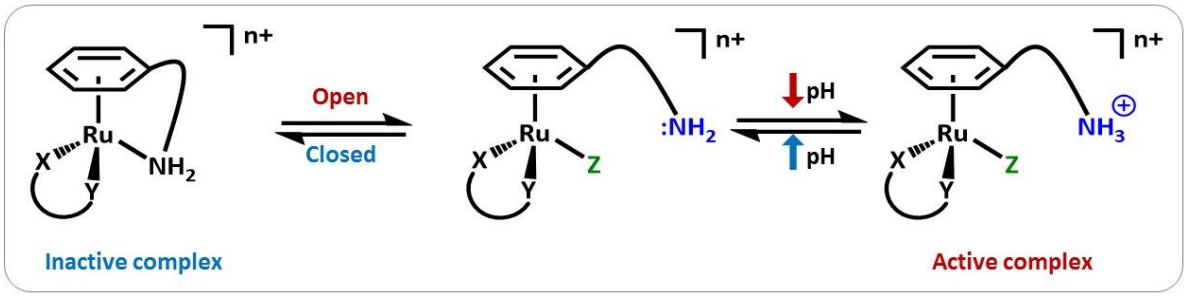
Francisco Martínez-Peña, Ana M. Pizarro

Nanobiotecnología (IMDEA-Nanociencia), Unidad Asociada al Centro Nacional de Biotecnología (CSIC);

email : ana.pizarro@imdea.org

Abstract

Tethered ruthenium(II) arene complexes of general formula $[Ru(\eta^6{:}\kappa^1\text{-}C_6H_5(C_6H_4)NH_2)(XY)]^{n+}$ (closed tether-ring) and $[Ru(\eta^6\text{-}C_6H_5(C_6H_4)NH_2)(XY)Cl]^{(n-1)+}$ (open tether) bearing different chelating XY ligands (XY = aliphatic diamine, phenylenediamine, oxalato, bisphosphine) have been synthesized and characterized either as solid state or in solution. The activation of these complexes (closed-to-open tether-ring conversion) occurs in methanol and dimethylsulfoxide at different rates, and to different reaction extents at equilibrium. Most importantly, Ru complex activation (cleavage of the $Ru–N_{tether}$ bond) occurs in acidic aqueous solution. The activation dynamics can be modulated by rational variation of the XY chelating ligand. The electron-donating capability and steric hindrance of XY have a direct impact on the Ru–N bond reactivity, with XY = NN'-dimethyl, NN'-diethyl, and NNN'N'-tetramethylethylenediamine affording complexes more prone to activation. Such activation in acidic media is fully reversible, and proton concentration governs the deactivation rate, i.e. tether ring closure occurs slower the lower the pH. Interaction of closed-tether complex $[Ru(\eta^6{:}\kappa^1\text{-}C_6H_5(C_6H_4)NH_2)(dimethylethylenediamine)]^{2+}$, and the corresponding open-tether $[Ru(\eta^6\text{-}C_6H_5(C_6H_4)NH_2)(dimethylethylenediamine)Cl]^{+}$ with 5'-GMP indicates selectivity of the active form (open tether complex) towards nucleobase interaction. This work presents ruthenium tether complexes as exceptional pH-dependent switches with possible applications in cancer research.



Immuno-chemotherapy by Platinum-based Inhibitors of Indoleamine-2,3-dioxygenase

David Stíbal,[a] Samuel G. Awuah,[a,b] Stephen J. Lippard[a,c]

[a] *Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

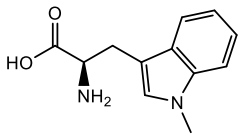
[b] *Department of Chemistry, University of Kentucky, 505 Rose Street, Lexington, KY 40506, USA*

[c] *The Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

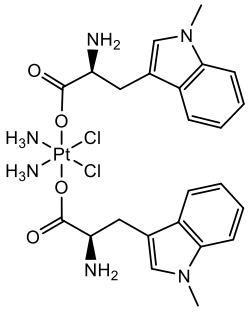
email : stibald@mit.edu

Abstract

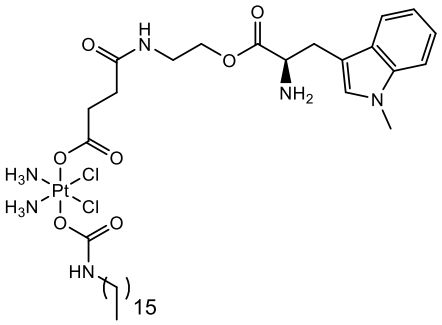
We report the synthesis and biological properties of two Pt(IV) complexes conjugated to an indoleamine-2,3-dioxygenase (IDO) inhibitor 1-methyl-D-tryptophan (1-MT). In vitro, the compounds are reduced to cisplatin and 1-MT, inducing DNA damage and inhibiting IDO, respectively.



(D)-1-MT



1



2

Compounds **1** and **2** were synthesized by oxidation of cisplatin to form *cis,cis,trans*-$[Pt(NH_3)Cl_2(OH)_2]$, followed by attachment of 1-MT either directly or through a short chemical linker. Both complexes were characterized by HRMS, ^{1}H, ^{13}C and ^{195}Pt NMR spectroscopy, and by elemental analysis. Treatment of SKOV3 cells by **2** results in the overexpression of phosphorylated proteins ATR, ATM, Chk1, Chk2, BRCA1, H2AX and p53, indicative of DNA damage. The cellular distribution as well as RNAi signature studies confirm the DNA-binding character of **2** and demonstrate that it maintains the typical mode of action of platinum complexes. The ability of **2** to inhibit the function of IDO was assessed by measuring the concentrations of the substrate L-tryptophan (Trp) and its metabolite L-kynurenine (Kyn) in cell medium. A significant increase in the Trp/Kyn ratio was observed between untreated SKOV3 cells and cells treated with complex **2**. To study the effects of **2** on T-cell proliferation in vitro, a mixed leukocyte reaction was performed. Co-cultures of peripheral blood mononuclear cells (PBMCs) and SKOV3 cells treated with **2** showed a clear increase in the proliferation of $CD3^{high}$ T-cells, consistent with the inhibition of IDO and a decrease in the Kyn levels.

The Mixed Ligand Complexes of CoII, NiII, CuII and ZnII with Coumarilic Acid/1,10-Phenanthroline. Synthesis, Crystal Characterization and Biological Applications

Dursun Ali Köse

Hitit University, Faculty of Science and Arts, Department of Chemistry ; email : dalikose@hitit.edu.tr

Abstract

The coumarilate (coum-) and 1,10-phenanthroline (phen) mixed ligand complexes of Co^{II} (**I**), Ni^{II} (**II**) Cu^{II} (**III**) and Zn^{II} (**IV**) were synthesized and structural characterizations were performed by using elemental analysis, magnetic susceptibility, solid state UV–Vis, FTIR spectra, thermoanalytic TG-DTG/DTA, and single crystal X-ray diffraction methods. The **I** and **II** complexes are salt type compounds and they have two moles phen ligands bound as bidentate, two moles aqua ligands in coordination sphere and two moles anionic coum- ligand outside of the coordination unit as counter ion of the molecular structure. At the same time, the **I** and **II** complexes have five moles of aqua ligands as hydrate water outside of the molecules. It was obtained that **III** and **IV** complex structures contain 1 mole of phen ligand, two moles coordinated ($coum^-$) ligand, and one mole aqua ligand; and the molecules (**III** and **IV**) have five-fold structure and obey square pyramidal geometry [1]. Thermal decomposition of each complex started with dehydration (first removal is the dehydration of complex **I** and **II** as removal of hydrate-aqua molecules) and then the decomposition of organic parts was observed. The thermal dehydration of the complexes takes place in one (**III** and **IV**) or two (**I** and **II**) steps. The decomposition mechanism, thermal decomposition steps and thermal stability of the investigated complexes provides useful data for the interpretation of their structures [2,3]. The final decomposition products were found to be metal oxides. Some biological applications (total antioxidant activity (TAC) and anti-fungal / anti-bacterial) were performed using structurally characterized compounds [4].

References

1) Ö. Dağlı, D. A. Köse, O. Şahin, Z. S. Şahin, The Synthesis and Structural Characterization of Transition Metal Coordination Complexes of Coumarilic Acid, 2017, Journal of Thermal Analysis and Calorimetry, DOI 10.1007/s10973-016-6053-y.
2) D. A. Köse, B. Öztürk, O. Şahin, O. Büyükgüngör, Mixed Ligand Complexes of Coumarilic Acid / Nicotinamide with Transition Metal Complexes. Synthesis and Structural Investigation, Journal of Thermal Analysis and Calorimetry, 2014, 115(2):1515–1524.
3) A. Karaliota, O. Kretsi, C. Tzougraki, Synthesis and characterization of a binuclear coumarin-3-carboxylate copper(II) complex. Journal of Inorganic Biochemistry, 2001, 84:33–37.
4) M. Hattori, S. Hada, A. Watahiki, H. Ihara, Y. Z. Shu, N. Kakiuchi, T. Mizuno, T. Namba, Studies on dental caries prevention by traditional medicines. X. Antibacterial action of phenolic components from mace against Streptococcus mutans, Chemical and Pharmaceutical Bulletin 1986, 34, 3885-3893.

Biological testings of mechanochemicaly obtained aroyl hydrazones as perspective ligands for metal complexes

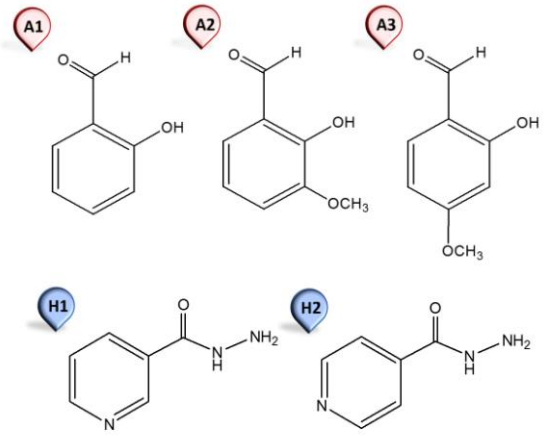
Jana Pisk,[a] M. Rubčić,[a] D. Cvijanović,[b] V. Damjanović,[b] J. Lovrić,[b] M. Cindrić,[a] V. Vrdoljak[a]

[a] *Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia*

[b] *University of Zagreb, School of Medicine, Šalata 3, 10000 Zagreb, Croatia ; email : jana.pisk@chem.pmf.hr*

Abstract

In recent years, mechanochemistry is attracting interest and sympathy of many scientist [1]. In general, it is promoted by hand grinding or ball milling [2]. Ball milling is an efficient method, commonly used both in organic and inorganic chemistry. Hydrazones are class of as chelating agents, used in coordination chemistry and their metal complexes are of a great importance. The significance of studying hydrazones arises from the fact that they have increased biological activity and they form particular materials with unique properties [3,4]. In particular are known as antioxidants, vasidilatators, agents again tuberculosis and tumors, ect. [5,6] Moreover, molybdenum complexes with hydrazone ligands have been reported to possess interesting antibacterial activities.



Scheme 1. Hydrazone starting compounds.

The presented research considers green protocol and highly efficient synthesis of series of aromatic hydrazones. [7]. Ball milling was deliberated as solventless method promoting eco-friendly reaction conditions. The ligands were prepared by the condensation of salicylaldehyde derivative (A1-3) and appropriate hydrazide (isoniaside or nicotinic acid hydrazide, H1-3), as shown in the Scheme 1. Solid state preparative procedures were also followed by conventional synthetic procedure, in solution, and different forms of ligands were isolated (polymorphs and solvates), whose molecular and crystal structure was determined by the single crystal diffraction method. Molybdenum(VI) mononuclear and dinuclear complex with the obtained ligands were synthesized and characterized. The prepared compounds were biologically tested. With the targeted synthetic policy and recognized correlation of structure and activity, biologically active compounds can be evolved and expanded.

References

1. G.-W. Wang, *Chem. Soc. Rev*. 2013, 42, 7669.
2. P. F. M. Oliveira, , A. Chamayou, C. André-Barrès, B. Guidetti, M. Baltas, *RSC Advances*, 2014, 00, 1.
3. G. Verma, A. Marella, M. Shaquiquzzaman, M. Akhtar, M. R. Ali, M. M. Alam. *J Pharm Bioall Sci* 2014, 6, 69.
4. S. Rollas, Ş. Güniz Küçükgüzel, *Molecules* 2007, 12, 1910.
5. N. Belkheri, D. Bougerne, F. Bedos-Beval, H. Duran, C. Bernis, R. Salvayre, A. Négre-Salvayre, M. Baltas, *Eur. J. Med. Chem*., 2010, 45, 3019.
6. D. R. Dabideen, K. F. Cheng, B. Aljabari, E. J. Miller, V. A. Pavlov, Y. Al-Abed, *J. Med. Chem*., 2007, 50, 1993.
7. This work has been fully supported-supported in part by Croatia Science Foundation under the project IP-2016-06-4221.

Novel squaramide-based photoactivatable Pt(II) anticancer compounds

Òscar Palacios,[a] Kevin Morales,[a] Katia G. Samper,[a] Quim Peña,[a] Mercè Capdevila,[a] Marta Figueredo,[a] Julia Lorenzo,[a] Pau Bayón[a]

[a] Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès (Barcelona) ;[b] Institut de Biotecnologia i Biomedicina, Dept. Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès (Barcelona) ; email : oscar.palacios@uab.cat

Abstract

Platinum complexes, such as cisplatin and carboplatin, are widely used in chemotherapy due to its anticancer activity. It is generally accepted that DNA is the main target for platinum complexes, inducing structural modifications on the helix, thus promoting apoptosis. However, side effects as well as loss of effectiveness of the drug, which are produced due to the interactions of the platinum-complexes with proteins, lead to drug resistance in some tumors. For that reason, the development of novel platinum-based-drugs with lower protein interactions and higher DNA affinity is still required. Photoactivatable complexes can be good candidates in order to avoid these problems, allowing modulating drug-reactivity, *i.e.*, the biological activity. In the last years, many efforts have been devoted to the field of photoactivated chemotherapy (PACT), *i.e.*, the use of an external radiation, focused on a tumour region, in order to initiate a photochemical process. This type of therapy is useful to have temporal and spatial control over drug activation, which supposes a less aggressive and more specific cancer treatment.

In this work, different platinum(II) complexes containing a functionalized squaramide unit as metal-ligands have been prepared (Figure 1). Their activity, before and after irradiation, have been checked by different techniques and under several experimental conditions, always in aqueous media. The high stability (low reactivity and low citotoxicity) of the synthesized complexes in front of the high reactivity of the species formed after irradiation, comparable with those of other Pt-anticancer drugs, allows to propose these Pt(II)-squaramide complexes as good photoactivatable candidates for the treatment of cancer.

Figure 1. Activation of the photosensitive platinum moiety by the use of light. The deactivation depends on the solvent used.

References

1) M. T. Nguyen and G. Raspoet *Can. J. Chem*., 1999, **77**, 817.
2) X. Hao, R. Liu, J. Lusztyk, J. Ma, M. A. McAllister, Y. Rubin, K. Sung, T. T. Tidwell and B. D. Wagner, *J. Am. Chem. Soc.* 1997, **119**, 12125.

A "sweet approach" to the targeted anticancer chemotherapy: gold-based glycoconjugates

A. Pettenuzzo,[a] J. Wölker,[b] A. Terenzi,[c] I. Ott,[b] L. Ronconi[a]

[a]School of Chemistry, National University of Ireland Galway, Galway (Ireland); [b]Institute of Medicinal and Pharmaceutical Chemistry, Technische Universität Braunschweig, Braunschweig (Germany); [c]Institute of Inorganic Chemistry, University of Vienna, Vienna (Austria); email: a.pettenuzzo1@nuigalway.ie

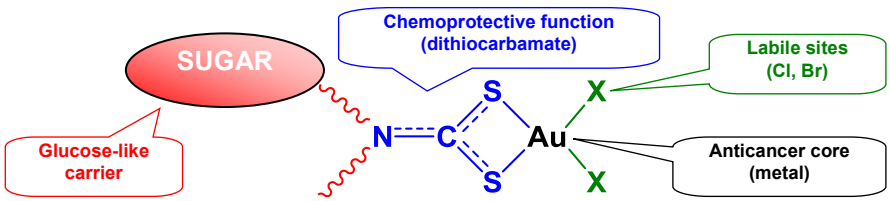
Abstract

Rapidly dividing tumor cells requires higher amounts of nutrients and energy for their fast proliferation, and glucose is no exception. Accordingly, such increased demand of glucose by cancer cells makes it very attractive to selectively target tumor sites. In particular, tailored glucose-like substrates can be conjugated to chemotherapeutics (including metal-containing anticancer agents) to attain the site-specific delivery of drugs into the affected tissues.[1]

Accordingly, we have been focusing on the design of gold(III)-dithiocarbamato glycoconjugates which can combine the antitumor properties and the favorable toxicological profile of the gold(III)-dithiocarbamato scaffold,[2] along with an improved selectivity and cellular uptake provided by the glucose-containing ligands coordinated to the metal center, through the exploitation of the glucose-mediated cellular internalization provided by glucose transporters (GLUTs).

In this communication, we report on a new generation of gold-dithiocarbamato-glucose conjugates generated *via* an innovative synthetic approach. All compounds have been fully characterized by spectroscopic techniques, and some solution studies have been carried out in PBS by means of UV-Vis spectrophotometry.

The cytotoxic activity of the glycoconjugates has been evaluated *in vitro*. Moreover, docking studies for some selected compounds with GLUT1 suggest that there are no sterical constraints within the binding pocket of the transporter.



Acknowledgements

Financial support by NUI Galway (*College of Science Scholarship* to AP; *Millennium Fund Minor Project 2013* to LR), Irish Research Council (*Postgraduate Scholarship* to AP), and COST Action CM1105 (*STSM Grant*) is gratefully acknowledged.

References

1) A. Pettenuzzo, R. Pigot, L. Ronconi *Metallodrugs* **2015**, *1*, 36
2) E.M. Nagy, L. Ronconi, C. Nardon, D. Fregona, *Mini-Rev. Med. Chem.* **2012**, *12*, 1216

Ru(II)-naphthoquinone complexes as new potential anticancer agents

Katia M. Oliveira[a,b], Erica J. Peterson[b], Nicholas P. Farrell[b], Alzir A. Batista[a]

[a] Universidade Federal de São Carlos – Departamento de Química, [b] Virginia Commonwealth University – Department of Chemistry and Massey Cancer Center

email : kmoliveiraq@gmail.com

Abstract

Ruthenium has become a popular metal used in the pursuit of anticancer agents because of its special characteristics such as hexavalent coordination, easy modulation by different ligands and the accessible oxidation states (II and III) in physiological solutions[1]. The coordination of a biologically active ligand to a metal is an interesting method to obtain new compounds, which could improve the activity of the free ligand. This approach can lead to increased cellular uptake and synergistic effects, providing different modes of action of the complex with respect the free ligand[2]. In this direction naphthoquinones are an interesting class of natural products with a broad variety of biological activities. Thus, lapachol is a naphthoquinone characterized by diverse properties such as antimicrobial, antiparasitic, antiviral and anticancer[3]. Therefore, in this work we have synthesized, characterized and evaluated the biological properties of new ruthenium complexes with the general formula $[Ru(L)(dppb)(bipy)]PF_6$ and $[Ru(L)(dppm)_2]PF_6$, where L = lapachol, dppb = 1,4-bis(diphenylphosphine)butane, dppm = 1,1-bis(diphenylphosphine)methane and bipy = 2,2'-bipyridine. The IC_{50} of these complexes showed they are nearly eighteen time more active than cisplatin against the MDA-MB-231 (breast cancer) and when tested against the noncancerous MCF-10A (breast) cell line, showed a high selectivity for cancer cells. Moreover, these complexes inhibit cell migration, arrest the cell cycle, and induce p53-independent cell death by caspase-3 mediated apoptosis. Oxidative stress is a plausible mechanism of cell death for these complexes, evidenced by increased reactive oxygen species, mitochondrial depolarization, and robust activation of protective cell signaling through HSP27 and SAPK/JNK pathways.

References

1) C. Mari, V. Pierroz, S. Ferrari, G. Gasser, Combination of Ru(II) complexes and light: new frontiers in cancer therapy. Chem. Sci., 2015, 6, 2660.
2) A. K. Renfrew. Transition metal complexes with bioactive ligands: mechanisms for selective ligand release and applications for drug delivery. Metallomics, 2014, 6, 1324.
3) K. W. Wellington. Understanding cancer and the anticancer activities of naphthoquinones – a review. RSC Adv., 2015, 5, 20309.

Around osteopontin/uranium interaction using hyphenated CE-ICP/MS

T.N.S. Huynh[b], S. Le Hir[a,b], C. Vidaud[b], A. Hagège[a,b]

[a] Univ Lyon, CNRS, Université Claude Bernard Lyon 1, Ens de Lyon, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, 69100 Villeurbanne, France ; [b] CEA, DRF, Biosciences and biotechnologies Institute (BIAM), 30207 Bagnols-sur-Cèze, France; email : agnes.hagege@isa-lyon.fr

Abstract

L'os fait partie des organes majeurs dans lesquels s'accumule l'uranium et il est vraisemblable que les interactions entre l'uranium et la matrice organique de l'os soient d'une importance considérable dans cette accumulation.[1]

La forte affinité pour l'uranium de l'ostéopontine, protéine impliquée dans le turn-over osseux, a même été démontrée, suggérant le rôle de cette protéine dans l'accumulation de l'uranium.[2] Selon les travaux de Safi *et al.,* alliant spectroscopies, thermodynamique et calcul théorique,[3] une structure du site de fixation de l'uranium dans une séquence peptidique de l'OPN a été proposée.

L'obtention de données thermodynamiques fiables décrivant les interactions protéine-uranium fait actuellement l'objet de nombreuses publications. Les méthodes spectroscopiques (UV, fluorescence, dichroïsme circulaire) ont démontré tout leur intérêt dans ce domaine. Cependant, ces méthodes ne présentent pas toujours les sensibilités requises et les volumes d'échantillons nécessaires (de l'ordre de plusieurs dizaines de µl) limitent les études à des protéines disponibles en quantités importantes. Dans ce contexte, nous avons mis au point un système basé sur le couplage entre l'électrophorèse capillaire (CE) et la spectrométrie de masse à ionisation par plasma (ICP/MS). Son intérêt réside dans la possibilité d'étudier les interactions protéine/métal à partir de petits volumes d'échantillons.

Dans ce cadre, le défi est de maintenir l'intégrité des espèces jusqu'au point de détection. Le choix des conditions de mise en contact et de séparation en particulier s'avère primordial si l'on veut refléter la répartition réelle de l'uranium.

Nous démontrons la possibilité de déterminer la première constante de complexation de l'uranium à partir de 4 nmol de protéine. Nous montrons qu'il est également possible de mettre en évidence très rapidement la présence de plusieurs sites d'interaction et leur coopérativité (< 2h) et la formation d'espèces ternaires (~ 10 min) en présence d'une protéine partenaire de l'OPN (lactoferrine).

References

[1] D. Bourgeois, B. Burt-Pichat, X. Le Goff, J. Garrevoet, P. Tack, G. Falkenberg, L. Van Hoorebeke, L. Vincze, M. A. Denecke, D. Meyer, C. Vidaud and G. Boivin, *Anal. Bioanal. Chem*., 2015,**407**, 6619–6625.

[2] S. Safi, G. Creff, A. Jeanson, L. Qi, C. Basset, J. Roques, P. L. Solari, E. Simoni, C. Vidaud and C. Den Auwer, *Chemistry*, 2013, **19**, 11261–11269.

[3] L. Qi, C. Basset, O. Averseng, E. Quéméneur, A. Hagège and C. Vidaud, *Metallomics,* **2014, 6, 166–176.**

Uptake, bioaccumulation and health risk assessment of Cd in cacao beans in areas impacted by oil activities in Ecuador

Fiorella Barraza[a], E. Schreck[a], G. Uzu[b], T. Lévêque[c], F. Lopez[a], J. Ruales[c] and L. Maurice[a]
[a]*Géosciences Environnement Toulouse (GET), Observatoire Midi Pyrénées, Université de Toulouse, CNRS, IRD, Toulouse, France ;* [b]*IGE, Université Grenoble Alpes, CNRS, IRD, Grenoble, France ;* [c] *Escuela Politécnica Nacional, Department of food Science and Biotechnology, Quito, Ecuador*
email : *fiorella.barraza@get.obs-mip.fr* / fiorella.barraza-castelo@univ-tlse3.fr

Abstract

In Ecuador, oil and gas exploitation in the northeastern Amazon and refinery along the Pacific coast has already caused serious environmental and social impacts. (San Sebastián and Hurtig, 2005). Both regions have vast extensions of cacao crops, an exportation product recognized worldwide for its special flavor, the fine aroma. Due to their toxicity, metals and hydrocarbons released during oil production not only impact ecosystems, but also represent potential health risk to the populations after inhalation or by ingestion of contaminated crops.

Recent studies have shown the presence of trace metals, such as Cd, in cacao beans and byproducts (Bertoldi et al., 2016; Chavez et al., 2015; Gramlich et al., 2016). Nevertheless, the ways of Cd transfer (by foliar or root uptake) to cacao crops, its main sources and the fraction that potentially affects human health after ingestion are still poorly described.

Thus, this study aims to assess trace metals uptake by cacao beans in areas impacted by oil activities and the potential health risks involved. Cacao (leaves and pods) and soil samples (at different depths) were collected in different locations in the Amazon basin (oil production) and in Esmeraldas (refinery). Soil physico-chemical properties (pH, CEC, organic carbon) were determined. Cacao beans were separated from the entire pod in order to focus on the consumed tissue. All samples were freeze-dried and grounded before acid digestion and metal content analysis by ICP-MS. Human bioaccesibility after ingestion was investigated by the BARGE *ex vivo* test in cacao beans.

Results show that Cd is bioaccumulated in cacao beans (1.5 mg kg^{-1}) reaching values higher than the critical level (0.8 mg kg^{-1}) established by Ecuador and the European Union for dark chocolate. In leaves and pod husks, Cd concentrations can reach values of 2.08 and 1.02 mg kg^{-1} respectively. Even its low content in soils (<0.5 mg kg^{-1}), Cd remains phytoavailable. Between 80 and 100% of the total Cd content is bioaccessible after ingestion, suggesting potential risks for human health and raising concerns of safety in the consumption of cacao-based chocolate. However, Cd origins in soils (natural sources, fertilizers, irrigations or oil contamination) remain unclear.

Further studies are in progress to assess more precisely local population's exposure to metals-PAHs cocktails. The global study of socio-environmental impacts of oil activities in Ecuador takes place in the frame of the French ANR-MONOIL Project.

References

Bertoldi, D., Barbero, A., Camin, F., Caligiani, A., Larcher, R., 2016. Multielemental fingerprinting and geographic traceability of Theobroma cacao beans and cocoa products. Food Control 65, 46–53. doi:10.1016/j.foodcont.2016.01.013

Chavez, E., He, Z.L., Stoffella, P.J., Mylavarapu, R.S., Li, Y.C., Moyano, B., Baligar, V.C., 2015. Concentration of cadmium in cacao beans and its relationship with soil cadmium in southern Ecuador. Sci. Total Environ. 533, 205–214. doi:10.1016/j.scitotenv.2015.06.106

Gramlich, A., Tandy, S., Andres, C., Chincheros Paniagua, J., Armengot, L., Schneider, M., Schulin, R., 2016. Cadmium uptake by cocoa trees in agroforestry and monoculture systems under conventional and organic management. Sci. Total Environ. doi:10.1016/j.scitotenv.2016.12.014

San Sebastián, M., Hurtig, A.K., 2005. Oil development and health in the Amazon basin of Ecuador: the popular epidemiology process. Soc. Sci. Med. 60, 799–807. doi:10.1016/j.socscimed.2004.06.016

Direct DNA interaction and genotoxic impact of three metals: cadmium, nickel and aluminum

Catherine Belliardo, Carole Di Giorgio, Florence Chaspoul, Philippe Gallice, David Bergé-Lefranc

IMBE UMR 7263 IRD 237 - *Aix Marseille Université, Campus Timone, Faculté de Pharmacie 27 Boulevard Jean Moulin, 13385 Marseille CEDEX 5 ; email : catherine.belliardo@gmail.com*

Abstract

This study investigates simultaneously direct DNA interaction and genotoxic impact of three typical metals: aluminum, cadmium and nickel, whom the high concentration in soils and the developed industrial use result in a daily human exposure not insignificant. Their harmful effects on human are not to demonstrate anymore[1,2,3] even if their mechanisms of action remain mostly to elucidate. The three of them are suspected to be involved in carcinogenesis which implies genomic lesions. We propose to study their genotoxic impact first in vivo on primary normal human dermal fibroblast (NHDF) cells with comet assay to measure DNA breaks occurrence and then, to characterize the interaction metal/DNA by isothermal titration calorimetry (ITC).

Under physiological conditions (pH7 and 37°C), alkaline comet assay was performed, on NHDF exposed 2h at different metal concentrations. And ITC was performed for each metal with 2 $mmol.L^{-1}$ of phosphate groups (DNA) and with an ionic strength from 5 to 300 $mmol.L^{-1}$ of NaCl. The binding between metal cations and phosphate groups of DNA were studied using McGhee von Hippel model[4].

Comet assay shows that Cd and Ni are genotoxic, they are responsible of DNA breaks starting from 1.10^{-4} $mol.L^{-1}$ and 5.10^{-2} $mol.L^{-1}$, respectively. Al has no effect on DNA at pH7. Cd and Ni present an electrostatic interaction with DNA phosphate groups. Interaction intensity decreases with metal concentration. At high Cd concentration, a DNA condensation is observed. Al has no interaction with DNA phosphate groups at pH 7 and 37°C, but at pH 4 the electrostatic interaction is strong and the same DNA condensation phenomenon is observed. Metal genotoxic effect seems linked to the electrostatic interaction on DNA phosphate groups. Genotoxic power evolved in parallel to DNA phosphate interaction strength as Cd > Ni > Al. If these study shows that metals do not directly break DNA, this binding could be a preferential site for damage due to reactive oxygen species.

References

1) L. Jarup, M. Berglund, C. G. Elinder, Health effects of cadmium exposure a review of the literature and a risk estimate, 1998,24, 240
2) D. Schaumloffel, Nickel species: analysis and toxic effects, 2012, 26, 1–6
3) G. Crisponi, V. M. Nurchi, G. Faa, M. Remelli, Human Diseases Related to Aluminum Overload, 2011, 14, 31–340
4) A. Velazquez-Campoy, Ligand binding to one-dimensional lattice-like macromolecules: analysis of the McGhee-von Hippel theory implemented in isothermal titration calorimetry, vol. 348, pp. 94-104, 2006.

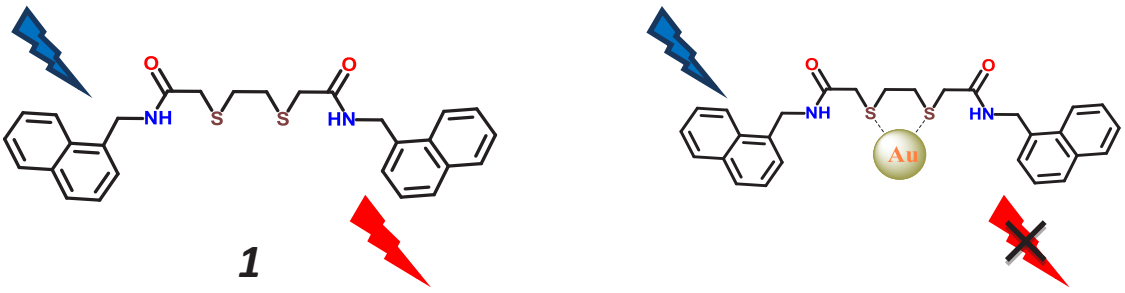
Turn-Off fluorescent sensors toward Au(III) detection

Hisila Santacruz,[a] Sarahy López,[a] Lorena Machi,[a] Rocío Sugich,[a] Enrique F. Velazquez,[a] Luis Miguel López,[b] Adrián Ochoa Terán[c]

[a] *Universidad de Sonora, Blvd Luis Encinas y Calle Rosales. Hermosillo, Sonora, México, CP 83000 ;* [b)] *Instituto Tecnológico de Hermosillo,* Ave. Tecnológico y Periférico Poniente S/N C.P. 83170 Colonia Sahuaro, Hemosillo Sonora, México; [b)] *Instituto Tecnológico de Tijuana, AP 1166, Tijuana BC, México. CP 22500 email : hisila@polimeros.uson.mx*

Abstract

Recently, the molecular design and synthesis of new fluorescent chemosensors has currently attracted significant interest because of their potential application in medicinal and environmental research.[1] The development of selective and sensitive chemosensors for the detection of transition- and heavy-metal cations with a high degree of specificity even at low concentrations nowadays has received considerable attention,[2] as these ions play important roles in living systems and have an extremely ecotoxicological impact on the environment and human.[3] In this work, we report the synthesis, characterization and evaluation of two fluorescence probes (***1*** and ***2***) which contains in their structure thioether and amide as donor groups, and naphthalene as indicator unit. Furthermore, was evaluated the capacity of the ligand to recognize certain metal ions (Au^{3+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Ag^{+} y Hg^{+}) by fluorescence spectroscopy technique. The fluorescents probes showed good selectivity and sensitivity to Au^{3+}, caused complete fluorescence quenching. The detection limit of the probes was $2.94X10^{-8}$ M for ***1*** and $4.88X10^{-8}$ M for ***2***; the excellent linear relationship suggest that the probe ***1*** are potentially useful for quantitative detection of Au^{3+} in water.



References

1) Avijit Kumar Das, Shyamaprosad Goswami, Sensors and Actuators B: Chemical, "-Hydroxy-1-naphthaldehyde: A versatile building block for the development of sensors in supramolecular chemistry and molecular recognition, 2017, 245, 1062.
2) Subhankar Singha, Dokyyoung Kim, Hyewon Seo, Won Cho and Kyo Han Ahn. Chem. Soc. Rev. Fluorescebce sensing systems for gold and silver species, 2015, 44, 4367.
3) Yolanda Aguilar-Hinojosa, Diana Meza-Figueroa, Arturo I. Villalba-Atondo, Martín Encinas-Romero, Jesús L. Valenzuela-García, Agustin Gómez-Alvarez, Water Air Soil Pollut. Mobility and Bioavailability of Metals in Stream Sediments Impacted by Mining Activities: the Jaralito and the Mexicana in Sonora, Mexico. 2016, 227, 345.

Indirect Bioimaging Studies of Metallocenyl-Nucleobases in Living Human Cancer Cells

Konrad Kowalski,[a] Joanna Skiba,[a] Claudia Schmidt,[b] Petra Lippmann,[b] Philipp Ensslen,[c] Hans-Achim Wagenknecht,[c] Rafał Czerwieniec,[d] Fabian Brandl,[d] Ingo Ott,[b] Tytus Bernaś,[e] Dominik Szczukocki,[f] Barbara Krawczyk[f]

[a] Department of Organic Chemistry, Faculty of Chemistry, University of Łódź, Tamka 12, 91403 Łódź, Poland ; [b]Institute of Medicinal and Pharmaceutical Chemistry, Technische Universität Braunschweig, Beethoven 55, 38106 Braunschweig, Germany; [c]Institute of Organic Chemistry, Karlsruhe Institute of Technology, Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany; [d]Institute für Physikalische und Theoretische Chemie, Universität Regensburg, Universitätsstraße 31, 93040 Regensburg, Germany; [e]Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, 02093 Warsaw, Poland; [f]Department of Inorganic and Analytical Chemistry, Faculty of Chemistry, University of Łódź, Tamka 12, 91403 Łódź, Poland email : kondor15@wp.pl

Abstract

Ferrocene (**1**) and ruthenocene (**2**) are aromatic, non-luminescent, organometallic compounds with a three-dimensional sandwich-like molecular structure. These metallocenes have shown great promise in medicinal chemistry. To date, bioimaging techniques that would allow for visualization of **1**, **2**, and their derivatives in living cells, have not been reported. In my talk, the first such technique for this visualization will be proposed. It is based on the structural similarity of metallocenes **1** and **2** to their metal-free [2.2]paracyclophane (**3**) congener. Accordingly, the syntheses of three structurally similar aromatic compounds **4**-**6** (Fig. 1) bearing a common uracil-ethynylpyrenyl substituent was achieved.[1]

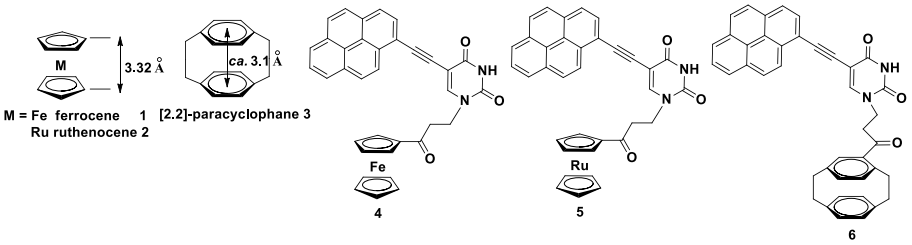


Fig. 1 Structures of compounds **1**-**6**

The pyrenyl group acts as a luminescent reporter that enables bioimaging of the [2.2]paracyclophane nucleobase (**6**) in living HeLa cells with confocal microscopy. Compound **6** localized in cell membranes and the cytoplasm. The distribution of **6** in the cytoplasm was not uniform, and this may originate from mitochondrial staining. Derivatives **4** and **5** are non-luminescent due to the presence of a metallocenyl quencher group. Confocal microscopy studies were further correlated with high-resolution continuum source atomic absorption spectroscopy (HR-CS AAS) measurements of Ru-containing derivative **5**. HR-CS AAS measurements confirmed the cellular uptake of **5** into cancer cells. Owning to their similar structures, same log$P_{o/w}$ values, and the HR-CS AAS measurement results, it can be hypothesized that non-luminescent uracil derivatives **4** and **5** show the same cellular biodistribution pattern as luminescent compound **6**. Therefore, **6** can be regarded as a metal-free luminescent probe for the indirect bioimaging of metallocene derivatives **4** and **5**.

References

1) Skiba J.; Schmidt C.; Lippmann P.; Ensslen P.; Wagenknecht H.-A.; Czerwieniec R.; Brandl F.; Ott I.; Bernaś T.; Krawczyk B.; Szczukocki D.; Kowalski K. *Eur. J. Inorg. Chem.* 2017, 297-305.

pH-responsive amine PARACEST contrast agents

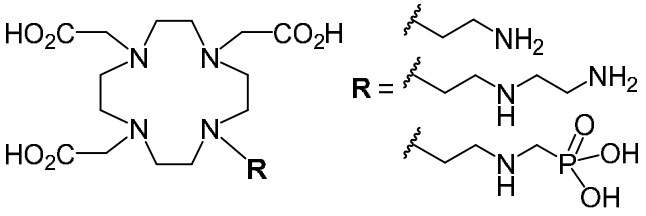
Tereza Krchová, [a] Jan Kotek, [a] Petr Hermann [a]

[a] *Universita Karlova (Charles University), Faculty of Science, Department of Inorganic Chemistry, Hlavova 2030, 12843 Prague 2, Czech Republic*

Abstract

Over the past two decades, the ability to provide information about tissue pH has become increasingly important as, e.g., the tumour extracellular microenvironment is often more acidic than healthy tissue. Such information can be also obtained by magnetic resonance imaging (MRI). Several MRI strategies (and appropriate contrast agents, CAs) to measure tissue pH have been investigated. The CAs previously studied are mostly relaxation-based MRI CAs (Gd(III) complexes) with longitudinal relaxivity (r_1) dependent on pH (e.g. through a change in the number of inner-sphere water molecules). Some time ago, another class of MRI CAs based on the chemical exchange saturation transfer (CEST) has been introduced. Rate of the proton exchange between the CA and bulk water protons generally depends on pH. If the method utilizes as CAs complexes of paramagnetic metal ions (often lanthanide(III) ions as Pr, Nd, Eu, Yb), it is called PARACEST.

In the contribution, PARACEST concept based on slow coordination/decoordination of amine group (which is pH-dependent) coupled with a fast proton exchange on the (temporarily) free amine group will be introduced. The ligands (Figure) are formal DOTA analogues and basicity of their amine pendant arm is close to the physiological pH. The ligand complexes (with trivalent Eu or Yb) are octa- or nonacoordinated as their amine-containing pendant arm is, depending on the solution pH, fully/partially metal-bound. It gaves proton exchanging pools which are detectable at different pH's and, thus, it leads to a ratiometric (i.e. concentration independent) pH probe. In addition, the lanthanide(III) complexes of the phosphonate-containing ligand can be used as ^{31}P-MRS pH probe (MRS = magnetic resonance spectroscopy) as the complexes exhibit unique large differences (tens–hundred ppm, caused by different position of phosphonate group in relation to the magnetic axis of the complexes) between ^{31}P NMR chemical shifts of the protonated and deprotonated forms. For practical utilization, the ^{31}P-MRS shift difference is tuneable by the lanthanide(III) ions. The Gd(III) complexes of the ligands can be also used as MRI pH probes as their relaxivity is changed with solution pH due to modification of their hydration state.



References

1. T. Krchová, J. Kotek, D. Jirák, J. Havlíčková, I. Císařová, P. Hermann, Lanthanide(III) complexes of aminoethyl-DO3A as PARACEST contrast agents based on decoordination of the weakly bound amino group. *Dalton Trans.* **2013**, *42*, 15735–15747.
2. T. Krchová, A. Gálisová, D. Jirák, P. Hermann, J. Kotek, Ln(III)-complexes of a DOTA analogue with an ethylenediamine pendant arm as pH-responsive PARACEST contrast agents. *Dalton Trans.* **2016**, *45*, 3486–3496.
3. T. Krchová, V. Herynek, A. Gálisová, J. Blahut, P. Hermann, J. Kotek, Eu(III) complex with DO3A-amino-phosphonate ligand as a concentration-independent pH-responsive contrast agent for magnetic resonance spectroscopy (MRS). *Inorg. Chem.* **2017**, accepted.

Nanocomposite of Functionalized Iron Oxide and Gadolinium Borate for Biomedical Applications

Okan Icten[a], Narayan S. Hosmane[b], Sherine F. Elsawa[c], Jason A. Misurelli[c], Dursun Ali Kose[d], Birgul Zumreoglu-Karan[e]

[a]Nanotechnology and Nanomedicine Division, Hacettepe University, Turkey; [b]Department of Chemistry and Biochemistry, Northern Illinois University, USA; [c]Department of Biological Sciences, Northern Illinois University, USA; [d]Department of Chemistry, Hitit University, Turkey; [e]Department of Chemistry, Hacettepe University, Turkey.
okanicten@hacettepe.edu.tr

Abstract

Advances in nanotechnology led to the production of magnetic materials for medical applications. Magnetic properties of iron oxides associated with the electrons in the material have been utilized for drug targeting, magnetic separation and magnetic hyperthermia while Gd-based paramagnetic materials or superparamagnetic iron oxide particles, associated with the protons can be utilized in powerful imaging techniques, such as magnetic resonance imaging (MRI).[1] However, recent studies have been focused on the development of multifunctional hybrid systems to address the needs of simultaneous imaging and more efficient therapy.

Here, we present the synthesis and characterization of iron oxide-gadolinium borate composites with potential applications in Boron Neutron Capture Therapy (BNCT), Gadolinium Neutron Capture Therapy (GdNCT) and combined application for MRI as contrast agent and as a drug delivery agent for GdBNCT in cancer treatment[2]. Accordingly, the Fe_3O_4-$GdBO_3$ nanocomposites were prepared from biocompatible starting materials (magnetite, borax and a simple Gd salt). The XRD and FTIR confirmed vaterite-type $GdBO_3$ formation over Fe_3O_4 support. Surface modification with fluorescein isocyanate doped silica (FITC-SiO_2), followed by conjugation with folic acid (FA) was then performed. The resulting nanocomposites of ca. 50 nm size were found to possess soft ferromagnetic properties (σ_s= 20.3 emu/g) with the following chemical compositions:

1. ^{157}Gd:^{10}B=0.7 that is appropriate for magnetically targeted NCT, and
2. Gd:Fe=0.5 that is suitable for MRI applications.

Fluorescence microscopy, flow cytometry and cell viability tests with XTT assay showed that the particles of nanocomposite penetrated MIA PaCA-2 cells without any significant negative effect on the cell growth.

References

1) Ramanujan, R. V., Magnetic particles for biomedical applications, 2009, In R. Narayan (Ed.), Biomedical materials, Springer, 477-491.

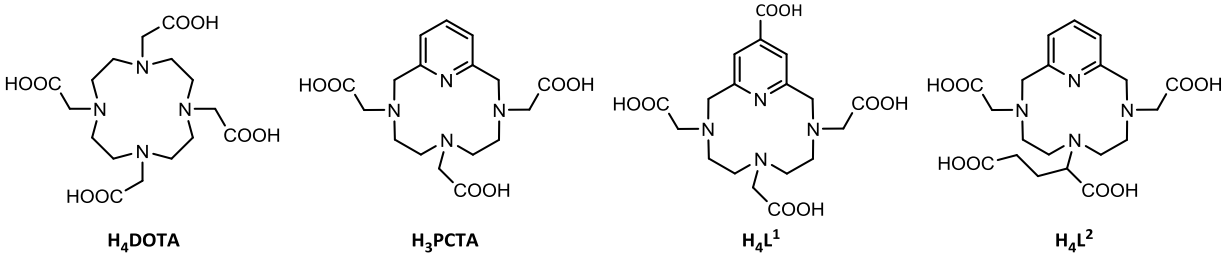
2) Hosmane, N.S., Maguire, J.A., Zhu, Y., Takagaki, M., Boron and Gadolinium Neutron Capture Therapy for Cancer Treatment, 2012, World Scientific.

Ln^{III} complexes derived from functionalized PCTA structure: synthesis, properties and applications

Morgane Enel,[a] Nadine Leygue,[a] Eric Benoist,[a] Luce Vander Elst,[b] Sophie Laurent,[b] Chantal Galaup,[a] Claude Picard[a]

[a]Synthesis and Physicochemistry of Molecules of Biological Interest Laboratory ,UMR CNRS 5068, Paul Sabatier University, 118, route de Narbonne, 31062 Toulouse, France ; [b]NMR and Molecular Imaging Laboratory, University of Mons, Avenue Maistriau, 19, B-7000 Mons, Belgium ; enel@chimie.ups-tlse.fr

In order to improve properties of DOTA-like complexes, heptatentate ligands containing pyridine ring as a part of the macrocycle have been synthesized for more than thirty years. Lanthanide(III) complexes of such ligands show high kinetic inertness due to their rigid structure comparing to those of their acyclic counterparts. The most investigated member of this family is heptadentate H_3PCTA (PCTA = pyridine-containing cyclen triacetic acid), mostly because of its potentiality to act as MRI contrast agent (Gd^{III} complex). More recently, some of its derivatives were successfully evaluated for optical imaging (Tb^{III} complex) or nuclear imaging (Cu^{64}, Ga^{68} complex for TEP and In^{111} complex for SPECT).[1-3]



H_4DOTA H_3PCTA H_4L^1 H_4L^2

Due to these promising results, the synthesis of functionalized H_3PCTA derivatives could be interesting for bioconjugating applications. Currently, only two bifunctional derivatives of PCTA have been linked to biomolecules: the first one bearing an isothiocyanate function on the carbon backbone, and the second one carrying an aliphatic carboxylic acid function on the *meta*-position of the pyridine ring.[4][5]

We report here the synthesis of two new bifunctional chelating agents H_4L^1 and H_4L^2 and the relaxometric and luminescent evaluation of some of their lanthanide(III) complexes. First trials of coupling reactions will also be presented.

References

1) W.D. Kim *et al.*, *Inorg. Chem.*, **1995**, 34, 2233
2) D. J. Bornhop *et al.*, *Appl. Spectrosc.*, **2003**, 57, 1216
3) E. W. Price *et al.*, *Chem. Soc. Rev.*, **2014**, 43, 260
4) C. L. Ferreira *et al.*, *Eur. J. Nucl. Med. Mol.*, **2010,** 37, 2117
5) G. Bort *et al.*, *Eur. J. Med. Chem.*, **2014**, 87, 843

Reactivity and Properties of Pt(II) Complexes with Triazole-based C^N^N Ligands

Marian Hebenbrock,[a] Linda Stegemann,[b] Nikos L. Doltsinis,[c]

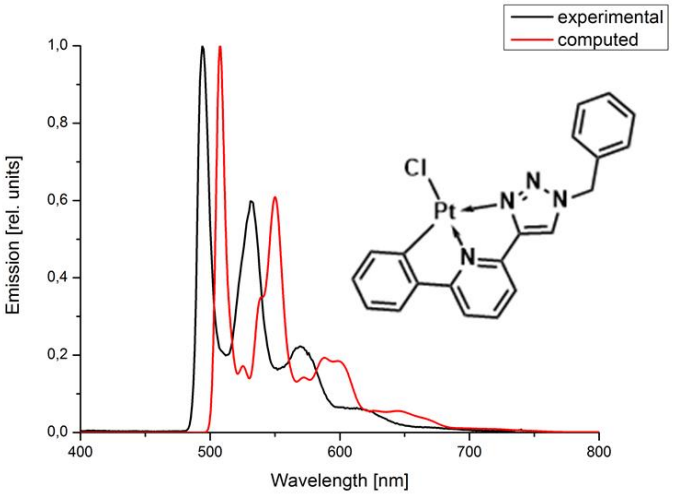
Jens Müller[a]* und Cristian A. Strassert[b]*

a) Westfälische Wilhelms-Universität Münster, Germany, Fachbereich Chemie und Pharmazie, Institut für Anorganische und Analytische Chemie, Corrensstraße 28/30, 48149 Münster, Germany; b) Physikalisches Institut, Heisenbergstraße 11, 48149 Münster, Germany; c) Institut für Festkörpertheorie, Wilhelm-Klemm-Straße 10, 48149 Münster, Germany. marian@muellerlab.org

Abstract

The square planar geometry of d^8 metal complexes opens the possibility of secondary orbital interactions by d_z^2 orbitals.[1] This leads to unique photophysical properties based on metal-pertubed ligand-centred triplet states (^{3}MP-LC) as monomer up to metal-metal-to-ligand charge-transfer states (^{3}MMLCT) or excimers as aggregates.[1,2] Many examples of tridentate ligands in platinum(II) complexes are known, in which the luminescent properties depend on the grade of carbometallation.[3]

We report here the synthesis of a tridentate chelate ligand and its platinum(II) complexes including their photophysical properties.[4] Starting from 2,6-dibromopyridine and phenylboronic acid the ligand was available in a four step synthesis with an overall yield of 23%. The complexation with potassium tetrachloroplatinate yielded a yellow chlorido complex in which the ancillary ligand could be displaced by pyridine or in a Sonogashira-type reaction with phenylacetylene. All complexes showed phosphorescence with quantum yields up to 35% in solution. Moreover in case of the pyridine complex the formation of excimers was observed. For a deeper insight into the photophysical processes quantum chemical studies based on DFT calculations have been used. Here the simulation of the experimental emission spectra based on vibrationally resolved analyses including different solvent models was successful. Future application of this ligand motif and its platinum complexes in bioimaging is projected.



References

[1] M. Mauro, A. Aliprandi, D. Septiadi, N. S. Kehr, L. De Cola, *Chem. Soc. Rev.* **2014**, *43*, 4144; [2] J. A. G. Williams, *Top. Curr. Chem.* **2007**, *281*, 205; [3] S. Huo, J. Carroll, D. A. K. Vezzu, *Asian J. Org. Chem.* **2015**, *4*, 1210; [4] M. Hebenbrock, L. Stegemann, J. Kösters, N. L. Doltsinis, J. Müller, C. A. Strassert, *Dalton Trans.* **2017**, *46*, (doi:10.1039/c7dt00393e) in press.

Synthesis, electrochemistry and photophysical studies of rhenium(I) tricarbonyl complexes bearing arenethiolate ligands

Menglan He, [a,b,c] H. Y. Vincent Ching,[a,b] Clotilde Policar[a,b], Hélène C. Bertrand[a,b]

[a] *Departement de Chimie, Ecole Normale Superieure, PSL Research University, UPMC Univ Paris 06, CNRS, Laboratoire des Biomolecules (LBM), 24 rue Lhomond, 75005 Paris, France*

[b] *Sorbonne Universites, UPMC Univ Paris 06, Ecole Normale Superieure, CNRS, Laboratoire des Biomolecules (LBM), Paris, France*

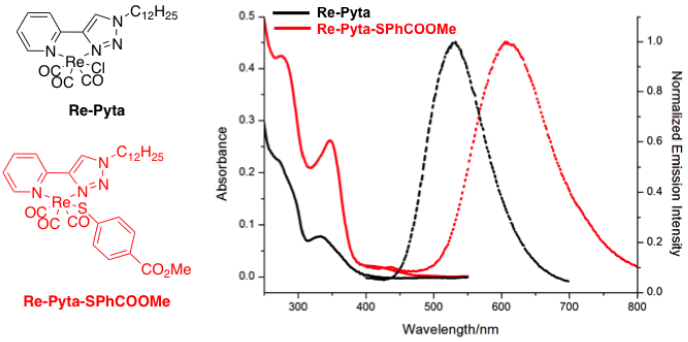
[c] *Shanghai Key Laboratory of Green Chemistry and Chemical Processes, School of Chemistry and Molecular Engineering, East China Normal University, 3663 N. Zhongshan Road, Shanghai, China*

email : menglan.he@ens.fr

Abstract

Luminescent Re(I) tricarbonyl complexes have attracted particular interest for chemists as cellular imaging agents in recent years[1] owing to their photophysical properties and cellular uptake properties, biological stability and low toxicity. Additionally, in terms of luminescent sensors for bioimaging and sensing, there is an increasing demand for compounds that both emit in red or near-infrared area and absorb at longer wavelengths. Incorporation of thiolate ligands in the coordination sphere of luminescent organometallic complexes has been suggested to induce a bathochromic-shift of their emission spectrum.[2]

Therefore, a series of novel Re(I) tricarbonyl complexes based on a Pyta or Tapy ligand[3] and bearing different arenethiolate ligands has been designed and synthesized. We will describe the synthesis of this new class of complexes, their photophysical properties (UV-Vis absorption and fluorescence spectroscopy) and their electrochemical behaviour. DFT calculations have been performed in an attempt to rationalize our observations. This study not only enriches the library of luminescent rhenium(I) tricarbonyl complexes but also offers some inspiration for the design of new rhenium(I) complexes.



References

1) a. K. K.-W. Lo, *Acc. Chem. Res.* **2015**, *48*, 2985 – 2995; b. M. P. Coogan, R. P. Doyle, J. F. Valliant, J. W. Babich, J. Zubieta, *J. Label Compd. Radiopharm* **2014**, *57*, 255 – 261; c. S. Clède, C. Policar *Chem Eur. J.* **2015**, *21*, 942 – 958.

2) W. A. Tarran, G. R. Freeman, L. Murphy, A. M. Benham, R. Kataky, J. A. G. Williams *Inorg. Chem.* **2014**, *53*, 5738−5749.

3) H. C. Bertrand, S. Clède, R. Guillot, F. Lambert, C. Policar, *Inorg. Chem.*, **2014**, *53* (12), 6204 – 6223.

4) M. He, H. Y. V. Ching, C. Policar, H. C. Bertrand, manuscript in preparation

Pyridinophane: a versatile platform for the synthesis of water soluble and stable lanthanide complexes

Aritz Perez e Iñiguez De Heredia [a,b], Nadine Leygue [a,b], Stéphane Balayssac [a,b], Chantal Galaup [a,b], Eric Benoist [a,b], Laurent Lamarque [c], Claude Picard [a,b]

[a] CNRS; Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR-5068; 118 Route de Narbonne, F-31062 Toulouse cedex 9, France;[b] Université de Toulouse; UPS; Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB; 118 route de Narbonne, F-31062 Toulouse cedex 9, France;[c] CisBio Bioassays, Parc Marcel Boiteux, BP 84175, 30200 Codolet, France. galaup@chimie.ups-tlse.fr.

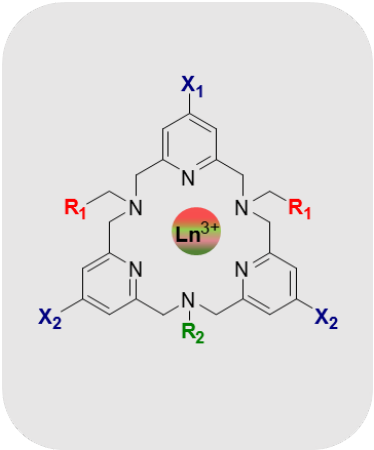
Abstract

In the field of medical diagnostics, lanthanide complexes occupy a prime position as T_1-type contrast agents for Magnetic Resonance Imaging (Gd^{3+} complexes) and also represent a promising category of luminescent probes for imaging (Eu^{3+}, Tb^{3+}, Yb^{3+}, Nd^{3+} complexes). Each of these techniques requires the association of a lanthanide ion with an organic chelate whose structure must favor the observation of the desired magnetic or optical properties while ensuring a thermodynamic stability and a kinetic inertia of the complex in biological environment.

In this context, we explore the relevance of a nonadentate system derived from a Pyridinophane platform TPP for the development of lanthanide complexes as luminescent bioprobes.

The TPP platform contains three pyridine rings and three secondary amino groups in a highly C3-symmetrical 18-membered macrocyclic structure.

We will show in this work how this structure can be easily functionalized with different pendant arms (R_1, R_2) on the amino group or variable substituents (X_1, X_2) on the pyridine ring. The characterization and the photophysical properties of the lanthanide complexes will be also discussed.



Potential MRI contrast agents for neuroimaging

Maamar Jlali, Jean-François Morfin, Célia Bonnet, Eva Tóth

Centre de biophysique Moléculaire, UPR CNRS4301 ,45071 Orléans, France
jean-francois.morfin@cnrs-orleans.fr

Neurotransmitters play a crucial role in neural activity. All imaging modalities included, methods to detect neurotransmitters, directly or indirectly, are still very limited and neurotransmitter-sensitive probes are just beginning to be developed. Concerning MRI, only a single probe able to sense dopamine has been reported to show the potential to track neurotransmitter concentration changes *in vivo*.[1]

Recently a series of smart MRI contrast agents has been conceived, capable of giving an MRI response to concentration changes of zwitterionic amino acid neurotransmitters.[2] Given the low sensitivity of MRI, such *direct* detection of neurotransmitters with smart MRI probes can be only applied to certain amino acids (glutamate, GABA, glycine) which are present at millimolar concentration, however, it is not adapted to other neurotransmitters such as dopamine, present at much lower concentrations.

Here we propose a fundamentally different strategy to assess dopamine via the detection of enzymatic activities involved in its degradation. The detection of enzymatic activities is particularly interesting in MRI, as a small quantity of the enzyme can convert, in catalytic cycles, a large quantity of the MRI probe, thus considerably reducing the detection limit. Smart MRI probes have been designed to function as substrates of the enzymes that degrade dopamine, in particular monoamine oxidases (MAO). These probes are macrocyclic lanthanide-based complexes that provide a PARACEST response to the enzymatic activity of monoamine oxidase, which can be translated to a contrast change in MR images.

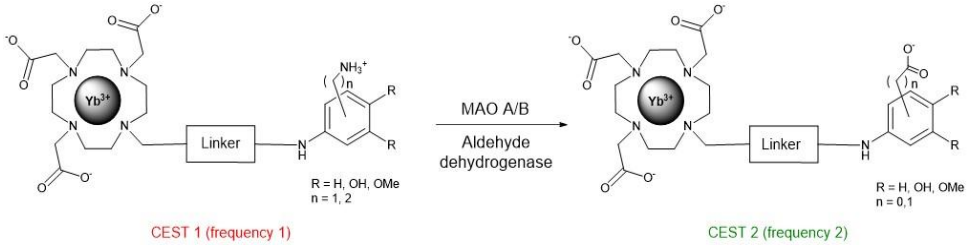


Figure 1. The enzymatic reaction on the neurotransmitter moiety is expected to generate a change in the CEST properties, detectable on the MR images

The structure of the contrast agents have been modulated trough several linkers and substrates. The CEST properties and the enzymatic recognition by MAO have been studied. This allowed us to understand which combinations were good compromise between the CEST effects and fast recognition and, to select the best candidates for enzymatic assay in CEST experiments.

1. Shapiro M. G., Westmeyer G. G., Romero P. A., Szablowski J. O., Kuster B., Shah A., Otey C. R., Langer R., Arnold F. H., Jasanoff A., *Nat. Biotechnol.* **2010**, *28*, 264-270.
2. Oukhatar F., Même S., Même W., Szemereta F., Logothetis N.K., Angelovski G., Tóth E., *ACS Chem. Neurosci.* **2015**, *6, 219.*

Study of the activity and speciation, intracellular distribution and quantification of a manganese superoxide dismutase mimic on a cellular model of inflammatory bowel diseases

E. Mathieu,[a] A.-S. Bernard,[a] N. Delsuc,[a] E. Quévrain,[a] G. Gazzah,[a] B. Lai,[c] F. Chain,[d] P. Langella,[d] M. Bachelet,[b] J. Masliah,[b] P. Seksik,[b] C. Policar [a]

[a] Département de Chimie, Ecole Normale Superieure, PSL Research University, UPMC Univ Paris 06, CNRS, Laboratoire des Biomolecules (LBM), 24 rue Lhomond, 75005 Paris, France; [b] Sorbonne Universites, UPMC Univ Paris 06 Département de Chimie, Ecole Normale Superieure, PSL Research University CNRS, INSERM, APHP, INRA, Laboratoire des Biomolecules (LBM), 27 rue de Chaligny, 75012 Paris, France; [c] X-ray Science Division, Argonne National Laboratory (ANL), Argonne, Illinois 60439, United States; [d] Commensal and Probiotics-Host Interactions Laboratory, Micalis Institute, INRA, AgroParisTech, UniversitéParis-Saclay, F-78350 Jouy-en-Josas, France
email: emilie.mathieu@ens.fr

Abstract

Inorganic complexes can be described in terms of binding affinity, geometry, outer-sphere ligand(s) interaction or redox potential. When designing a metallodrug, these different characteristics are used to build inorganic complexes with specific roles such as imaging agent, catalysts, prodrugs,…[1] In addition to the investigation of the bio-activity of a metallodrug (anticancer, antiinflammatory, antimicrobial,…), one should also explore the questions of its cellular uptake, location inside cells, and stability in intricate biological environments.[2-5]

Our group has designed bio-inspired Mn^{II}-complexes superoxide dismutase mimics,[6-8] and demonstrated the ability of one of these complexes (Mn1) to efficiently reduce superoxide concentration in activated macrophages.[9] Following on, we have further investigated the effect of Mn1 on a cellular model of Inflammatory Bowel Diseases (IBD), in which oxidative stress and inflammation arise. Indeed, it is reported that superoxide dismutases are either over-produced in an inactive form or under-expressed in patients with IBD.[10] The speciation of Mn1, its quantification, and its distribution have been investigated in detail in epithelial cells activated with lipopolysaccharide, and correlated with its activity. We have demonstrated that Mn1 exerts an intracellular anti-inflammatory activity in this model, and efficiently complements MnSOD in oxidative stress condition. Additionally, we have investigated Mn1 activity in a DNBS-induced murine model of colitis and showed that it improves the health status of mice.[5]

References

1) K. D. Mjos, C. Orvig, Chem. Rev., 2014, 114, 4540-4563
2) B. A. Gilston, T. O'Halloran, Mechanisms controlling the metal economy. In Metals in cells; V. Culotta, R. A. Scott, Eds.; Wiley, London, 2013.
3) C. S. Dam, S. A. Perez Henarejos, T. Tsolakou, C. A. Segato, B. Gammelgaard, G. S. Yellol, J. Ruiz, I. H. Lambert, S. Stürup, Metallomics 2015, 7, 885–895
4) G. Gasser, Inorganic Chemical Biology: Principles, Techniques and Applications; Wiley: London, 2014
5) E. Mathieu, A.-S. Bernard, N. Delsulc, E. Quevrain, G. Gazzah, B. Lai, F. Chain, P. Langella, M. Bachelet, J. Masliah, P. Seksik, C. Policar, Inorg.Chem., 2017, doi10.1021/acs.inorgchem.6b02695
6) F. Cisnetti, A.-S. Lefevre, R. Guillot, F. Lambert, G. Blain, E. Anxolabeń er e-Mallart, C. Policar, Eur. J. Inorg. Chem., 2007, 4472–4480

Artificial metalloenzyme design: finding the protein-siderophore anchor

Ellis J. Wilde[1,2], Adam Hughes[1], Elena V. Blagova[1], Olga V. Moroz[1], Ross P. Thomas[2], Johan P. Turkenburg[1], Daniel J. Raines[2], Keith S. Wilson[1] and Anne-Kathrin Duhme-Klair[2]

[1] *Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, UK*

[2]*Department of Chemistry, University of York, Heslington, York YO10 5DD, UK; email:ew555@york.ac.uk*

Abstract

Iron is essential for growth of almost all bacteria. Many bacteria utilise iron-chelating siderophores and a dedicated set of transport proteins for iron uptake.[1] It was previously thought that siderophores must be hexadentate, however CeuE, the periplasmic ferric siderophore binding protein in *C. jejuni,* studied via X-ray crystallography, revealed the interactions with tetradentate siderophores.[2] As CeuE binds ferric complexes of tetradentate ligands, such as 4-LICAM^{4-} with nanomolar affinities,[2, 3] these systems make good candidates as anchoring groups in artificial metalloenzymes (Figure A).[4]

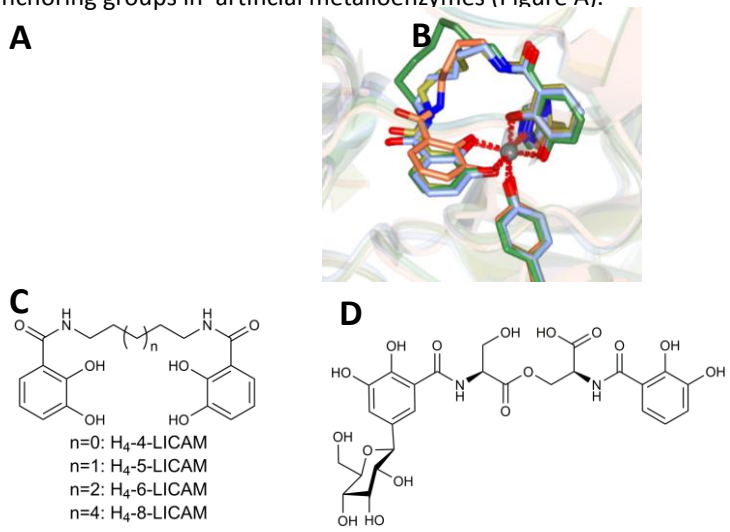


Figure A: Schematic diagram of potential artificial metalloenzyme.
Figure B: Ferric 4-,5-,6- and 8-LICAM in the binding pocket of CeuE, where Tyr and His residues complete the octahedral coordination.
Figure C: The H_4-n-LICAM siderophore mimic series.
Figure D: Salmochelin S1, a stealth siderophore.

Using co-crystal structures, circular dichroism and intrinsic fluorescence quenching experiments, a series of Fe(III) bound siderophores and siderophore mimics was screened against the periplasmic binding proteins CeuE (*C. jejuni*), FepB (*E. coli*) and VctP (*V. cholerae*). Fe(III)-n-LICAM^{-} tetradentate siderophore mimics (Figure C) were found to bind to CeuE (Figure B). Linker lengths between the iron-binding catecholamide units were increased from four carbon atoms (4-LICAM^{4-}) to five, six and eight (5-, 6-, 8-LICAM^{4-}). CeuE recognises and binds all four mimics and selects Λ-configured complexes. Mutagenesis studies show that Y288 is fundamental to Fe(III)-siderophore binding, and H227 provides favorable Fe(III) coordination. Interestingly, CeuE preferentially binds Λ-configured tetradentate ligands, whereas FepB favours hexadentate ligands. VctP, with conserved His and Tyr, is able to bind both tetradentate and hexadentate ligands, but also binds stealth siderophore mimics, whilst CeuE and FepB do not. It is proposed that the preference for Λ-configuration may confer enantioselective properties to an attached inorganic catalyst in an artificial metalloenzyme (Figure A).

1. D. J. Raines, T. J. Sanderson, E. J. Wilde and A. K. Duhme-Klair, in *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*, Elsevier, 2015, DOI: http://dx.doi.org/10.1016/B978-0-12-409547-2.11040-6.
2. D. J. Raines, O. V. Moroz, E. V. Blagova, J. P. Turkenburg, K. S. Wilson and A.-K. Duhme-Klair, *Proc. Nat. Acad. Sci.*, 2016, **113**, 5850–5855.
3. D. J. Raines, O. V. Moroz, K. S. Wilson and A. K. Duhme-Klair, *Angew. Chem. Int. Ed.*, 2013, **52**, 4595-4598.
4. M. Hoarau, C. Hureau, E. Gras and P. Faller, *Coordination Chemistry Reviews*, 2016, **308**, 445-459.

Bio-inspired molybdenum catalysts for implantable glucose fuel cells

Philipp Kurz and Jann Sonnenfeld

Institut für Anorganische und Analytische Chemie und Freiburger Materialforschungszentrum (FMF), Albert-Ludwigs-Universität Freiburg, Albertstrasse 21, 79104 Freiburg, Germany
philipp.kurz@ac.uni-freiburg.de

In implantable fuel cells (IFCs), endogenous substances (like glucose) and oxygen react and the resulting electric current can be used to supply power to medical implants like pacemakers or sensors inside a patient's body. As both reactants for the IFC are replenished from the body fluids, such cells can operate independently over extended time periods, making implantable fuel cells an interesting alternative to batteries.[1] On the other hand, the development of electrocatalysts for both the anode and the cathode reactions is challenging, as a good long-term performance in serum is required. For example, platinum particles show small overpotentials for glucose oxidation in the laboratory but are often poisoned in serum within hours of IFC operation.

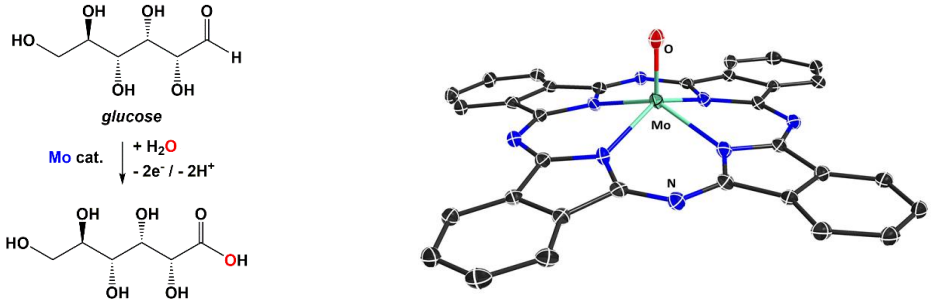


Figure. *left:* oxidation of glucose to gluconic acid; *right:* structure of the unsubstituted Mo^{IV}-complex [MoOPc] from the analysis of single-crystal X-ray diffraction data.

Molybdenum enzymes like sulphite or aldehyde oxidase catalyse reactions in which an oxygen atom is transferred to substrates at mild oxidation potentials. These processes are closely related to the first step of glucose oxidation, the transformation of glucose to gluconic acid (Fig., left), which requires ~0 V *vs.* NHE at physiological conditions. Inspired by these metalloproteins and also an earlier (but today rather forgotten) report,[2] we prepared oxido-phthalocyanato-complexes of molybdenum ([MoOPc], see Fig., right) to use these compounds as glucose-oxidation catalysts. Phthalocyanine metal complexes are attractive not only due to their accessibility, but also because of their very good thermal and chemical stabilities.

By variation of the substituents on the phthalocyanine macrocycle, different [MoOPc]-type complexes could be synthesised, characterized and investigated for their spectroscopic and electrochemical properties in solution. By immobilization of water-insoluble compounds on conductive supports, we were also able to prepare [MoOPc]-impregnated electrodes which could be successfully used in electrolysis cells for glucose oxidation. Molybdenum phthalocyanines thus emerge as promising new electrocatalysts for the anodes of implantable fuel cells.

References

[1] S. Kerzenmacher, J. Ducrée, R. Zengerle, F. von Stetten, *J. Power Sources* **2008**, *182*, 1–17.
[2] G. G. Arzoumanidis, J. J. O'Connell, *J. Phys. Chem.* **1969**, *73*, 3508–3510.

Tuning the redox potential of the first electron transfer relay to modulate the catalytic activity of the periplasmic nitrate reductase from *Rhodobacter sphaeroides*

Kamal ZEAMARI,[a] Guillaume Gerbaud,[a] Sandrine GROSSE,[b] Pascal ARNOUX,[b] Monique SABATY,[b] David PIGNOL,[b] Bruno GUIGLIARELLI,[a] Bénédicte BURLAT[a]

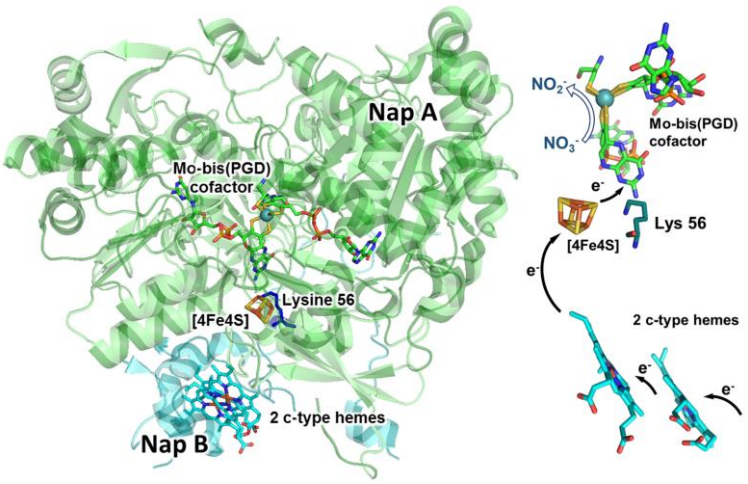
[a] *Laboratoire de Bioénergétique et Ingénierie des Protéines – UMR 7281 CNRS, Aix-Marseille Université, 31, chemin Joseph Aiguier, 13009 Marseille, France ;* [b]*Laboratoire de Bioénergétique Cellulaire – SBVME UMR 6191 CNRS CEA, Aix-Marseille Université, CEA Cadarache, 13108 Saint-Paul-lez-Durance France ;*

kzeamari @imm.cnrs.fr

Abstract

Molybdoenzymes are ubiquitous in living organisms and catalyze, for most of them, oxidation-reduction reactions using a large range of substrates. These enzymes are involved in the biogeochemical cycles of essential elements (C, S, N) and their studies may find applications in the field of energy, environment or health (1).

Periplasmic nitrate reductase (NapAB) from *Rhodobacter sphaeroides* catalyzes the 2-electron reduction of nitrate into nitrite (2). Its active site is a Mo bis-(pyranopterin guanine dinucleotide) found in all molybdoenzymes from the bacterial DMSO reductase family. A [4Fe4S] cluster and two *c*-type hemes form the intramolecular electron transfer chain (3). Lysine 56 (K56) is a highly conserved amino acid within this enzyme family which is located on the intramolecular electron transfer pathway. This residue connects, through hydrogen-bond network, the [4Fe-4S] center to the edge of one pyranopterin ligand of the Mo and is expected to be involved in the electron transfer.



In this work, we investigated the role of the K56 amino acid by combining site-directed mutagenesis, activity assays measurements, redox titrations and EPR and HYSCORE spectroscopies. Our data show that removal of the positively charged residue at position 56 strongly decreases the redox potential of the [4Fe-4S] cluster at pH8 by 300 to 400 mV in the K56H and K56M mutants respectively, thus strongly affecting the kinetics of intramolecular electron transfer. Interestingly, this charge effect can be reversed by protonation of the imidazole ring of the histidine at pH 6 in the K56H mutant. Overall, our study demonstrates clearly the importance of Lys residue in fine tuning the [4Fe-4S] reduction potential in NapAB *Rs* and related Mo-enzymes.

References

1) Grimaldi, S.; Schoepp-Cothenet, B.; Ceccaldi, P.; Guigliarelli, B.; Magalon, A., *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2013,** *1827* (8–9), 1048-1085.

2) Arnoux, P.; Sabaty, M.; Alric, J.; Frangioni, B.; Guigliarelli, B.; Adriano, J. M.; Pignol, D., *Nature Structural Biology* **2003,** *10* (11), 928-934.

3) Jacques, J. G. J.; Fourmond, V.; Arnoux, P.; Sabaty, M.; Etienne, E.; Grosse, S.; Biaso, F.; Bertrand, P.; Pignol, D.; Leger, C.; Guigliarelli, B.; Burlat, B., *Biochimica Et Biophysica Acta-Bioenergetics* **2014,** *1837* (2), 277-286.

Crystal structures of a native Hydroquinone 1,2-dioxygenase and of substrate and inhibitor complexes.

Andrea Scozzafava

Department of Chemistry, University of Florence

Abstract

The crystal structure of hydroquinone 1,2-dioxygenase from Sphingomonas sp. strain TTNP3, has been solved (1) by Molecular Replacement, using the coordinates of PnpCD from Pseudomonas sp. strain WBC-3 (2). The enzyme ,a Fe(II) ring cleaving dioxygenase which oxidizes a wide range of hydroquinones to the corresponding 4-hydroxymuconic semialdehydes, is a heterotetramer, constituted of two subunits α and two β of 19 and 38kDa, respectively. Both the two subunits fold as a cupin, but that of the small α subunit lacks a competent metal binding pocket. Two tetramers are present in the asymmetric unit. Each of the four β subunits in the asymmetric unit bind one Fe(II) ion. The iron ion in each β subunit is coordinated to three protein residues, His258, Glu264, and His305 and a water molecule. The crystal structures of the complexes with the substrate methylhydroquinone, obtained under anaerobic conditions, and with the inhibitors 4-hydroxybenzoate and 4-nitrophenol were also solved. Significant differences where found in the structures of these derivatives with respect to PnpCD, the other hydroquinone 1,2-dioxygenase of known structure, and in particular a different coordination at the metal center.

References

(1) Ferraroni M., DaVela G.,Kolvenbach BA, Corvini PF, Scozzafava A., The crystal structures of native Hydroquinone 1,2-dioxygenase from Sphingomonas sp. TTNP3 and of substrate and inhibitor complexes.Biochim. Biophys. Acta, in press

(2) . Liu, S., Su, T., Zhang, C., Zhang, W-M., Zhu, D., Su, J., Wei, T., Wang, K., Huang, Y., Guo, L., Xu, S., Zhou, N-Y. & Gu, L.. J. Biol. Chem., Crystal Structure of PnpCD, a Two-subunit Hydroquinone 1,2-Dioxygenase, Reveals a Novel Structural Class of Fe^{2+}-dependent Dioxygenases. (2015) 290, 24547-24560.

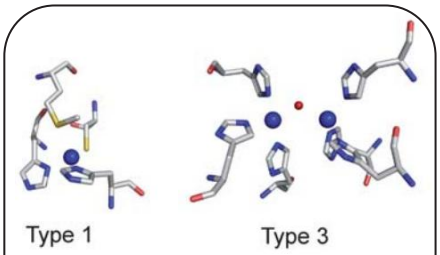
Tuning copper enzyme activity using an exogenous carbene ligand

Nathalie Ségaud, [a,b] Matteo Planchestainer[b], Francesca Paradisi[b], Martin Albrecht[a]

[a] *Dept. Chemistry & Biochemistry, Universität Bern, Freiestrasse 3, 3012 Bern, Switzerland ;* [b] *School of Chemistry, University of Nottingham, Nottingham, NG7 2RD, UK ; nathalie.segaud@unibe.dcb.ch*

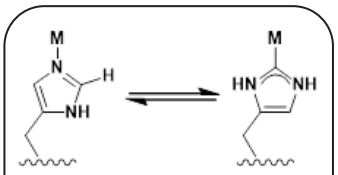
Abstract

Copper enzymes are efficient catalysts for electron transfer and dioxygen activation.[1] Histidine is the common amino acid in the different active site types composition (Type 1, 2, 3 and CuA), and binds copper through a well-established N-bonding mode.[2]

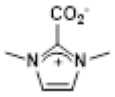


Copper enzyme active sites

In synthetic complexes, C-bonding to different metal centers has been observed by tautomerization processes.[3–5] We were interested in studying C-bonding over N-bonding of an imidazole and its effect on the activity of selected copper enzymes. For this purpose, N,N'-dimethylimidazolium-2-carboxylate (DMI-CO_2) has been used as an N-heterocyclic carbene (NHC) precursor. In this presentation, NHC binding to Type 1 and Type 3 copper active sites will be described spectroscopically, and its effect on electron transfer and oxidation catalysis by the selected enzymes will be demonstrated.



Imidazole bonding tautomerization



DMI-CO_2 NHC precursor

References

1. Holm, R. H., Kennepohl, P. & Solomon, E. I. Structural and Functional Aspects of Metal Sites in Biology. *Chem. Rev.* **96,** 2239–2314 (1996).
2. Solomon, E. I. *et al.* Copper Active Sites in Biology. *Chem. Rev.* 3659–3853 (2014).
3. Hsieh, C.-H., Pulukkody, R. & Darensbourg, M. Y. A dinitrosyl iron complex as a platform for metal-bound imidazole to N-heterocyclic carbene conversion. *Chem. Commun.* **49,** 9326–9328 (2013).
4. Huertos, M. A., Pérez, J., Riera, L. & Menéndez-Velázquez, A. From N-Alkylimidazole Ligands at a Rhenium Center: Ring Opening or Formation of NHC Complexes. *J. Am. Chem. Soc.* **130,** 13530–13531 (2008).
5. Ruiz, J. & Perandones, B. F. Base-Promoted Tautomerization of Imidazole Ligands to N-Heterocyclic Carbenes and Subsequent Transmetalation Reaction. *J. Am. Chem. Soc.* **129,** 9298–9299 (2007).

Artificial metalloenzymes as heterogeneous catalyst

Sarah Lopez,[a] Laurianne Rondot,[a] Chloé Leprêtre,[a] Caroline Marchi-Delapierre,[a] Christine Cavazza,[a] and Stéphane Ménage[a]

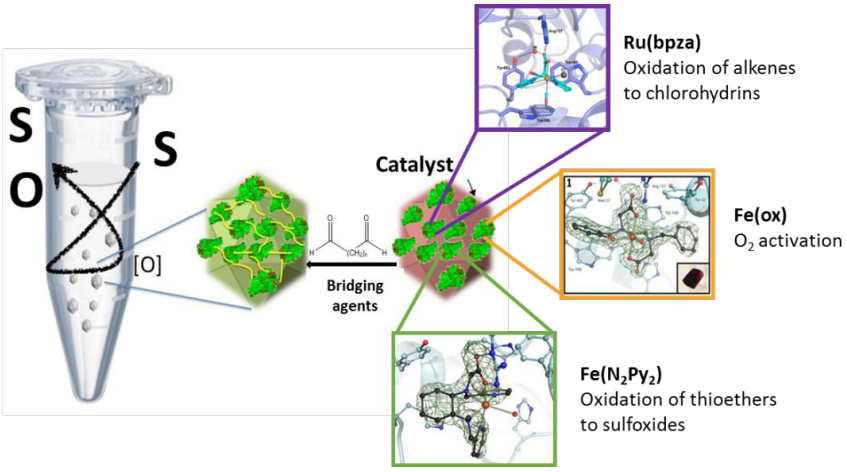
[a] *Laboratoire de Chimie et Biologie des Métaux, 17 avenue des matyrs 38000 Grenoble ; sarah.lopez@cea.fr*

Abstract

Catalytic processes are at the cutting-edge of the development of green chemistry. One solution is based on the combination of homogenous catalysis and biocatalysis through the design of artificial metalloenzymes.[1] The hybrids, formed by the embedment of an inorganic complex (playing the role of the active site) into a protein (driving the selectivity of the reaction) should deploy new properties, taking advantages of the two fields.

In this context, our original strategy relies on the design of a heterogeneous crystal/solution version of the already demonstrated artificial enzymes technology. Our approach is based on the setting up of oxidation reactions catalyzed "in cristallo" using Fe or Ru complexes mimicking the active site of non-heme iron oxygenases, incorporated into the nickel-binding protein NikA. The Cross-linked Enzyme Crystals[2] (CLEC) technology has been used to increase the stability of our NikA-based hybrid crystals in order to scan various reactions conditions (solvent, temperature, pH), that will permit infinite oxidation reactions.

In a first step, NikA/Fe-N_2Py_2 and NikA/Fe-ox cross-linked crystals were used for sulfoxidation and alkene oxychlorination, respectively (See figure). The two systems efficiently oxidize a large scope of substrates in organic solvents with good yields (up to 90 % for sulfoxidation using NaOCl as oxidant). Our promising approach will be extended to new reactivities.



References

1. Lopez, S.; Rondot, L.; Cavazza, C.; Iannello, M.; Boeri-Erba, E.; Burzlaff, N.; Strinitz, F.; Jorge-Robin, A.; Marchi-Delapierre, C.; Menage, S., Efficient conversion of alkenes to chlorohydrins by a Ru-based artificial enzyme. *Chemical Communications* **2017**.
2. St. Clair, N. L.; Navia, M. A., Cross-linked enzyme crystals as robust biocatalysts. *J. Am. Chem. Soc.* **1992,** *114* (18), 7314-7316.

Redox enzyme mimicking by copper(II) complexes of tripodal peptide ligands

Ágnes Dancs,[a,b] Katalin Selmeczi,[a] Tamás Gajda[b]

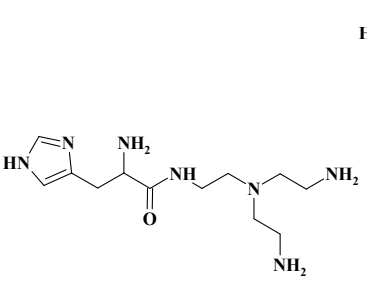
[a] *Laboratoire SRSMC UMR 7565, CNRS - Université de Lorraine, Nancy, France*

[b] *Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary*

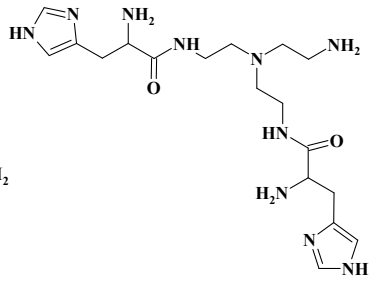
email : agnes.dancs@univ-lorraine.fr

Abstract

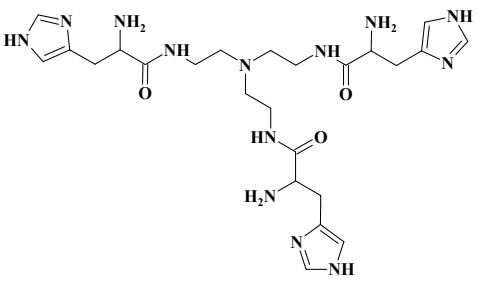
Tripodal ligands are powerful chelating agents used in coordination chemistry; their enhanced chelate effect provides exceptional stability for their transition metal complexes. Suitable modification of such tripods allows to implement special features in the corresponding metal complexes, resulting useful properties such as *e.g.* self-assembly, catalysis or molecular recognition.
Our intentions are to functionalize symmetrical tripodal molecules with amino acids, resulting terminally free tripodal peptides. Peptides have a well described background with respect to coordination chemistry, and their metal complexes are targets of many enzyme mimetic studies. However, due to their lack of fixed three dimensional structure, the biologically relevant metal binding modes are often not robust enough for catalytic applications. Tripodal peptides are capable of enhancing metal binding in an advantageous way, and so, allowing their application as catalysts.



tren1his



tren2his



tren3his

In this work, we present the catalytically active copper(II) complexes of three N-terminal histidine containing ligands, based on tren (*tris*(2-aminoethyl amine)) tripodal scaffold. Two of the presented ligands are capable of inducing the formation of multicopper(II) complexes. Such multinuclear species are advantageous in *e.g.* catechol oxidase mimicking; kinetic study and substrate binding regarding 3,5-di-*tert*-butyl catechol (H_2DTBC) substrate will be presented. Furthermore, copper(II) species of all ligands were investigated as superoxide dismutase (SOD) mimics, possessing notable catalytic activity.

The activation of carbon monoxide dehydrogenase: CooT accessory protein for nickel insertion

Marila Alfano,[1] Julien Perard,[1] Giulia Veronesi,[1] Barbara Zambelli,[2] Stefano Ciurli [2] and Christine Cavazza [1].

[1]*Laboratoire de Chimie et Biologie des Métaux, 38054 Grenoble, France*
[2]*Laboratory of Bioinorganic Chemistry, University of Bologna, 40127 Bologna, Italy*
marila.alfano@cea.fr

The products of *cooCJT genes* are accessory proteins involved in the *in vivo* insertion of nickel into carbon monoxide dehydrogenase (CODH). CODH plays a central role in hydrogenogenic carboxydotrophs (such as *Rhodospirillum rubrum* or *Carboxydothermus hydrogenoformans*), determining their ability to use CO oxidation as sole carbon and energy source. In this case, the oxidization of CO to CO_2 catalysed by CODH is coupled with the production of hydrogen, catalysed by [NiFe]-hydrogenase in the overall reaction $CO + H_2O \rightarrow CO_2 + H_2$. The crystal structures of CODH from *C. hydrogenoformans* (*Ch*CODH) and *R. rubrum* (*Rr*CODH) had been solved, revealing as enzyme active site the unique $[NiFe_3S_4]$ cubane coordinated to a mononuclear iron site, known as C-cluster. While the reaction mechanism of CODH is well established[1], very little is known about the activation of the C-cluster. Ni insertion is essential for the enzyme activation and requires the intervention of the accessory proteins CooC, CooJ and CooT.

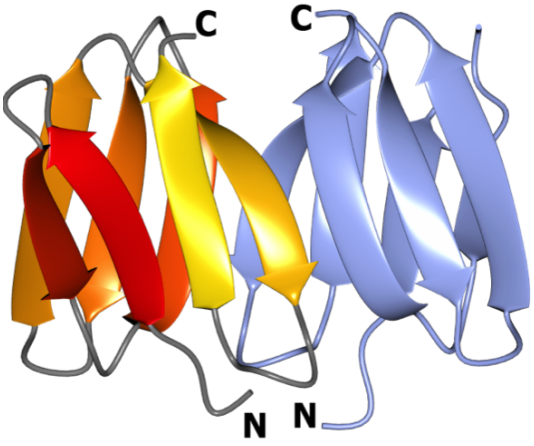


Figure 1 CooT [2] structure at 1.9 Å resolution (pdb: 5N76)

Here we present our recent structural and biochemical characterization of CooT, highlighting the existence of a novel Ni-binding protein family. The specificity of this protein towards nickel is assessed comparing the wild type strain with different protein's mutants, using different biophysical techniques such as circular dichroism (CD), isothermal titration calorimetry (ITC), nuclear magnetic resonance (NMR) and extended x-ray absorption fine structure (EXAFS). These results move us a step forward towards the full understanding of the CODH's C-cluster activation.

1. M.W. Ribbe **(2015)**, Angew. Chem. Int. Ed., 54, 8337.
2. J. Timm, C. Cavazza, C. Brochier-Armanet, J. Perard, B. Zambelli, S. Ollagnier-de- Choudens, S. Ciurli . " The CO dehydrogenase accessory protein CooT is a novel nickel-binding protein" (article currently submitted for publication)

Tyrosinase dicopper active site as a target to develop new inhibitors

Catherine Belle,[a] Elina Buitrago,[a,b] Ahcène Boumendjel,[b] Romain Haudecoeur,[b] Renaud Hardré,[c] Marc Maresca,[c] Jalila Simaan,[c] Marius Réglier,[c] Hélène Jamet [a]

[a] DCM (UMR-CNRS 525, Equipe Cire), [b]DPM (UMR-CNRS 5063), [a,b]Université Grenoble Alpes, Grenoble (France) and [c]Aix Marseille Université, UMR-CNRS 7313, ISM2/BioSciences, Marseille (France)

email : catherine.belle@univ-grenoble-alpes.fr

Abstract

Tyrosinases (Ty) are metalloenzymes belonging to the type-3 copper protein family which contain two copper ions in the active site.[1] They are found in various prokaryotes as well as in plants, fungi, arthropods and mammals and are responsible for pigmentation. Tyrosinases perform two sequential enzymatic reactions: hydroxylation of monophenols and oxidn. of diphenols to form quinones which polymerize spontaneously to melanin. In humans, Tyrosinases are an important class of targets that are relevant to depigmentation and diseases such as cutaneous hyperpigmentation or melanoma.[2] Although numerous inhibitors of Ty have been reported, there is a lag in the discovery of the new functional compounds. To control the Ty activity, a strategy is to target the Ty binuclear copper active site by chelators, which have structural analogies to natural substrate but are not oxidizable. In this presentation, our efforts to understand the binding of selected chelator will be discussed from structural and spectroscopic studies associated with theoretical modeling, using functional models of the Ty active site.[3]

Then, with the aim to develop more effective and selective human tyrosinase inhibitors, we investigated derivatives whose a chelator moiety is embedded.[4] This approach gives us the means to improve inhibition activity on isolated enzyme and confirmed by measuring the melanogenesis suppression ability in melanoma cells. Further investigations are being used to reveal insights into the influence of the protein active site on inhibitor binding.

Acknowledgments: Supports from the French ANR (Program ANR-09-BLAN-0028), Labex ARCANE (ANR-11-LABX-0003-01) and European COST Program (action CM1003).

References

[1] Solomon E. I, Heppner D., Johnston E., Ginsbac J., Cirera J., Qayyum M., Kieber-Emmons M., Kjaergaard C., Hadt R. and Tian L.*Chem. Rev.* **2014**, *114*, 3659.

[2] a) Yaar, M. J. *Invest. Dermatol.* 2013, 133, 11 ; b) Buitrago, E.; Hardré, R.; Haudecoeur, R.; Jamet, H.; Belle, C.; Boumendjel, A.; Bubacco, L.; Réglier, M. *Curr. Top. Med. Chem.* **2016**, *16*, 3033.

[3] a) M. Orio, C. Bochot, C. Dubois, G. Gellon, R. Hardré, H. Jamet, D. Luneau, C. Philouze, M. Réglier, G. Serratrice, C. Belle, *ChemEurJ.* **2011**, *17*, 13482 ; b) C. Bochot, E. Favre, C. Dubois, B. Baptiste, L. Bubacco, P-A. Carrupt, G. Gellon, R. Hardré, D. Luneau, Y. Moreau, A. Nurisso, M. Réglier, G. Serratrice, C. Belle, H. Jamet, *ChemEurJ.* **2013**, *19*, 3655.

[4] a) C. Dubois, R. Haudecoeur, M. Orio, C. Belle, C. Bochot, A. Boumendjel, R. Hardré, H. Jamet, M. Réglier, *ChemBioChem* **2012**, *13*, 559 ; b) R. Haudecoeur, M. Carotti, A. Gouron, M. Maresca,, E. Buitrago, R. Hardré, E. Bergantino, H. Jamet, C. Belle, M. Réglier, L. Bubacco, A. Boumendjel, *ACS ChemMedLett,* **2017**, *8*, 55.

Development of New Binuclear Mixed Valence Cu(II)/Cu(III) Complexes: Combination of Theoretical and Experimental Methods

Felix Bacher,[a] Aurore Thibon-Pourret,[a] Helene Jamet,[a] James Isaac,[a] Rolf David,[a] and Catherine Belle.[a]

[a] *Universite Grenoble Alpes, DCM, CNRS, F-38000 Grenoble, France*

E-mail: felix.bacher@univ-grenoble-alpes.fr

Abstract

The oxidation of methane to methanol is a process of industrial interest. Unfortunately, this reaction requires high temperatures, leading to undesired side reactions. Until now there is a lack of catalysts that sufficiently lower the activation energy of this reaction [1, 2]. However, methanotroph bacteria are able to perform this reaction at ambient temperature and pressure. In particular, the reaction is catalyzed by particulate methane monooxygenase (pMMO) [3]. The X-ray structure of pMMO suggested that the active feature of the enzyme could be a dicopper center and a mixed valence Cu(II)/Cu(III) species has been proposed as intermediate [4-6]. Our aim is to synthesize and characterize dinuclear complexes which are able to stabilize the Cu(II)/Cu(III) oxidation state. The synthesis of such systems is not trivial due to the instability of the Cu(III) oxidation state, examples in the literature are scarce and only partly characterized [7]. Our general synthesis rationale is to prepare ligands containing one arm that stabilizes the Cu(II) oxidation state and another arm stabilizing the Cu(III) state, linked together by a spacer (see Figure 1). Modifications on all the three mentioned features are possible, leading to a large variety of possible ligands. To reach our goal more efficiently we use DFT calculations to find complexes with a chemically accessible Cu(II)/Cu(III) redox potential and stable ligands. The accuracy of our calculations has been proved before with the calibration of the methods on some experimental data [8]. The most promising compound series to be synthesized is a series of complexes containing a naphthyridine spacer [9] and a phenoxo-arm, see Figure 1. Preliminary experimental data will be presented.

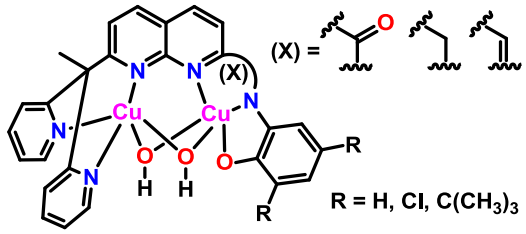


Figure 1: Compound series in development.

References

[1] M. C. Alvarez-Galvan, J. L. G. Fierro et al., *Catal. Today* **2011,** *171*, 15-23.

[2] H. Schwarz *Angew. Chem., Int. Ed.* **2011,** *50*, 10096-10115.

[3] M. A. Culpepper, and A. C. Rosenzweig *Crit. Rev. Biochem. Mol. Biol.* **2012**, *47*, 483-492.

[4] M. A. Culpepper, A. C. Rosenzweig et al., *J. Am. Chem. Soc.* **2012**, *134*, 7640-7643.

[5] Y. Shiota, Y. Kazunari et al., *Inorg. Chem.* **2013**, *52*, 7907-7917.

[6] V. C.-C. Wang, S. I. Chan et. al. *Chem. Rev.* **2017**, Ahead of Print, DOI:10.1021/acs.chemrev.6b00624

[7] M. R. Halvagar, W. B. Tolmann et al., *J. Am. Chem. Soc.***2014**, *136*, 7269-7272.

[8] F. Gennarini, H. Jamet, N. Le Poul et al., *Inorg. Chem.* In Submission

[9] J. A. Isaac, C. Belle et al., *Inorg. Chem.* **2016**, *55*, 8263-8266.

Entatic state models - influence of the solvent on the electron transfer of copper complexes

Julia Stanek, [a] Nina Sackers, [a] Fabian Fink, [a] Melanie Paul, [a] Alexander Hoffmann, [a] Sonja Herres-Pawlis [a]

[a] *RWTH Aachen University, Institute for Inorganic Chemistry, Landoltweg 1, 52074 Aachen ;*
email : julia.stanek@ac.rwth-aachen.de, sonja.herres-pawlis@ac.rwth-aachen.de

Abstract

The concept of the entatic state describes the influence of the surrounding protein matrix on the geometry of the active site of metalloproteins. The ligand sphere of the metal centre highly affects the electron transfer ability of enzymes and their model complexes. [1,2]

In $[Cu(GUAqu)_2]^{+/2+}$ complexes, the metal centre is coordinated by two different nitrogen donors (a guanidine and an aromatic imine unit) of two hybrid guanidine ligands. The molecular structures of the complexes in both oxidation states are highly distorted from the ideal tetrahedral (Cu(I)) or square planar (Cu(II)) form and resemble each other strongly. Therefore, they could function as a static entatic state model. [3,4]

To obtain a deeper insight into the electron transfer abilities of the copper guanidine complexes, we examined their electron self-exchange rate k_{11} (the reaction rate of the redox reaction of a complex with itself) in different solvents. An electron of the reduced form of the complex is transferred to its oxidised form. The rate constant k_{11} of this reaction is determined via the *Marcus cross relation* based on the *Marcus theory*. [5]

Therefore, the cross reaction rate k_{12} of the redox reaction of the copper complex with a suitable oxidant or reductant was determined via stopped flow techniques based on the increase or decrease of the corresponding charge transfer bands of the regarded copper complexes (Fig. 1).

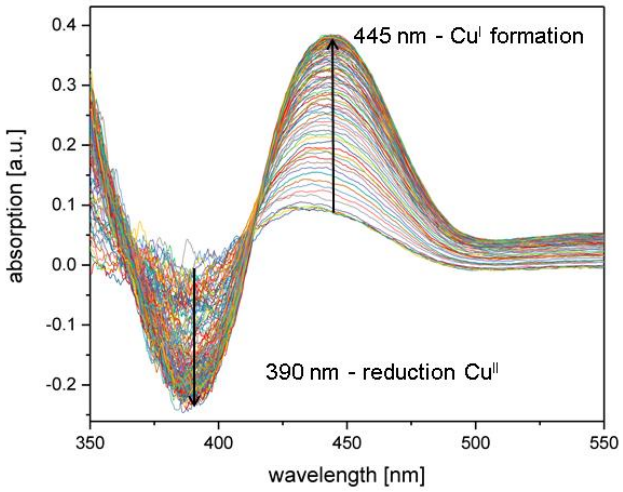


Figure 1: UV/Vis spectra of the reaction of $[Cu(TMGqu)_2]^{2+}$ with $[Fc(Cp^*)_2]$ with 1:5 ratio in propionitrile.

References

1) B.L. Vallee, R.J.P. Williams, Proc. Natl Acad. Sci. USA, 1968, 59, 498.
2) P. Comba. Coord. Chem. Rev., 2000, 200, 217.
3) A. Hoffmann, J. Stanek, B. Dicke, L. Peters, A. Wetzel, B. Grimm-Lebsanft, A. Jesser, M. Bauer, M. Gnida, W. Meyer-Klaucke, M. Rübhausen, S. Herres-Pawlis, Eur. J. Inorg. Chem., 2016, 4731.
4) A. Hoffmann, S. Binder, A. Jesser, R. Haase, U. Flörke, M. Gnida, M. Salomone Stagni, W. Meyer-Klaucke, B. Lebsanft, L.E: Grünig, S. Schneider, M. Hashemi, A. Goos, A. Wetzel, M. Rübhausen, S. Herres-Pawlis, Angew. Chem. Int. Ed. 2014, 53, 299.
5) R.A. Marcus, N. Sutin, Biochim. Biophys. Acta, 1985, 811, 265.

Bifunctional KatGs and *de novo* designed mini-heme proteins: Ferryl heme-Trp$^{\bullet}$ as catalytic intermediates and long-range e^{-} transfer pathways.

Karl Koebke [a], Peter C. Loewen [b], Vincent L. Pecoraro [a], Anabella Ivancich [c]

[a] *Department of Chemistry, University of Michigan, Ann Arbor, USA.* [b] *Department of Microbiology, University of Manitoba, Winnipeg, Canada.* [c] *Laboratoire de Bioénergétique et Ingénierie des Protéines, UMR 7281, CNRS & Aix-Marseille Université, Marseille, France.*

email : aivancich@imm.cnrs.fr

Abstract

Protein-based radicals, reacting in a concerted way with the metal active site of metalloproteins, are important for biocatalysis (1). In heme enzymes, Tyr and Tyr can play crucial roles, both as true intermediates in their catalytic cycles and as transient radical relays, facilitating electron transfer between redox partners (2). Certain monofunctional peroxidases and catalases, including cytochrome c peroxidase, lignin peroxidase and lactoperoxidase stabilize [Fe(IV)=O Trp$^{\bullet+}$] and/or [Fe(IV)=O Trp$^{\bullet}$], [Fe(IV)=O Tyr$^{\bullet}$] species as catalytically-active intermediates (3,4,5,6), formed subsequently to the primary oxidizing intermediate, the [Fe(IV)=O Por$^{\bullet+}$]. Bifunctional peroxidases (KatGs) constitute a challenging family of enzymes to possibly understand putative evolutionary strategies in fine-tuning the oxidation reactions catalyzed by heme-Trp$^{\bullet}$ intermediates and related e^{-} transfer processes mediated by Trp$^{\bullet}$/Tyr$^{\bullet}$ (7,8,9). The crystal structure of a cavity mutant (D141A), that is very disruptive of the fine-tuned H-bonding network of the heme distal side, shows that only in this case the pro-drug isoniazid can also to bind close to the heme (10). Our recent spectroscopy studies clearly show that even in this case, the natural binding site of INH (at more than 20 Å away from the heme) and its reaction with the [Fe(IV)=O Trp139$^{\bullet}$] is retained. Interestingly, although the oxidation reaction with the INH molecule bound at the engineered site proceeds via the [Fe(IV)=O Por$^{\bullet+}$], it does not impede the natural reaction occurring via the Trp139 radical intermediate with the distant natural site (11). Moreover, our recent studies on naturally-occurring mutants of *M. tuberculosis* KatG conferring resistance to the activation of the pro-drug isoniazid show that allosteric effects related to the long-range electron transfer pathways are crucial for the oxidation of INH (12). Our recent efforts using *de novo* design metalloproteins (13) in order to conceive mini-heme proteins to better understand the physicochemical properties enabling formation of the high-valent intermediates in bifunctional peroxidases will be discussed.

References

1) J. Stubbe and W. van der Donk. *Chem. Rev.* **1998**, *98*, 705−762. 2) *Reviewed in*: G. Smulevich, A. Feis, B.D. Howes, A. Ivancich. *Handbook of Porphyrin Science* **2010**, vol. 6, chap. 31, 367-453. 3) K.D. Miner, T.D. Pfister, P. Hosseinzadeh, N. Karaduman, L.J. Donald, P.C. Loewen, Y. Lu, A. Ivancich *Biochemistry* **2014**, *53*, 3781-3789. 4) A. T. Smith, W. A. Doyle, P. Dorlet, A. Ivancich *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16084-16089. 5) A. J. Fielding, R. Singh, B. Boscolo, P. C. Loewen, E. M. Ghibaudi, A. Ivancich *Biochemistry* **2008**, *47*, 9781-9792. 6) P.C. Loewen, J. Villanueva, J. Switala, L.J. Donald, A. Ivancich *Proteins: Structure, Function, and Bioinformatics* **2015**, *83*, 853-866. 7) R. Singh, J. Switala, P.C. Loewen, A. Ivancich, *J Am Chem Soc.* **2007**, *129*, 15954-15963. 8) J. Colin, B. Wiseman, J. Switala, P. C. Loewen, A. Ivancich, A. *J. Am. Chem. Soc.* **2009**, *131*, 8557–8563. 9) A. Ivancich A, L.J. Donald, J. Villanueva, B. Wiseman, I. Fita, P.C. Loewen **2013**, *Biochemistry*, *52*, 7271−7282. 10) P. Vidossich, P. C. Loewen, X. Carpena, G. Fiorin, I. Fita, C. Rovira *J. Phys. Chem. B* **2014**, *118*, 2924-2931. 11) J. Switala, P.C. Loewen, A. Ivancich (*manuscript in preparation*). 12) F. Brossier, J. Switala, P. C. Loewen, W. Sougakoff, A. Ivancich (manuscript in preparation). 13) *Reviewed in*: C.S. Mocny, V.L. Pecoraro *Acc. Chem. Res.* **2015**, *48*, 2388-2396.

Metal ions interactions with amphiphiles supramolecular assemblies

Bruno ALIES, Mohamed Amine OUELHAZI, Angéline NOIREAU, Karen GAUDIN, Philippe BARTHELEMY,

ChemBioPharm - Université de Bordeaux - Inserm U1212 - UMR CNRS 5320

146 rue Léo Saignat 33076 Bordeaux Cedex France

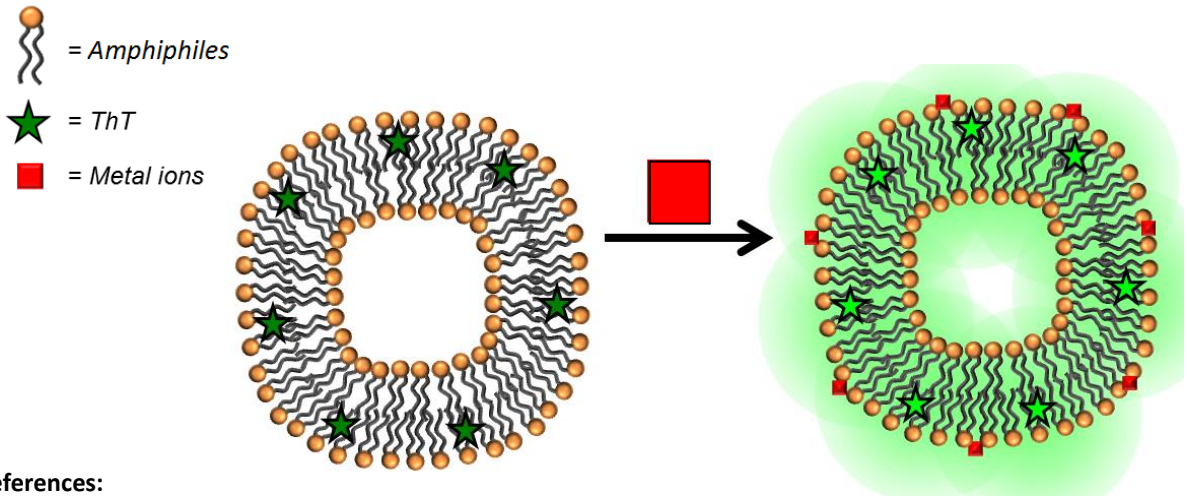
email : bruno.alies@u-bordeaux.fr

Abstract

It has been shown using metal ions-specific DNAs, that metal ions can be incorporated between DNA bases to form metal ions base pairs.[1,2] In this work, we have utilized a common fluorescence dye, Thioflavin T (ThT), combined with nano-objects formed by amphiphiles to interact with metal ions highlighted by fluorescence spectroscopy.

Nano-object have been prepared by hydration of a lipid film with an aqueous solution. They were then sized in DLS and electron microscopy. The interaction of metal ions was investigated by fluorescence spectroscopy. Amphiphiles form spontaneously nano-objects in water. UV-vis and fluorescence measurements showed that ThT interacts with nano-objects leading to an enhancement of fluorescence likely due to the charged interaction in binding.[3] Addition of Ag^{+} or Hg^{2+} induce a further increase of ThT fluorescence with nano-object form by amphiphiles.

Even at low concentration (nM range) of metal ions (such as Ag^{+} and Hg^{2+}) interaction could be seen in a simple and rapid fluorescence assay with a high selectivity against other metal ions.



References:

[1] Akira Ono et al., Chem. Soc. Rev. 2011; 40: 5855-5866.

[2] Shenshan Zhan et al., Biosensors and Bioelectronics, 2016; 86: 353-368.

[3] Satish Kumar et al., J Fluoresc. 2008 ; 18:1199-1205.

As^{III}-binding of a peptide bearing two cysteine residues

L. Szekeres,[a] B. Gyurcsik,[a] T. Kiss,[a,b] S.V. Hoffmann,[c] N.C. Jones,[c] A. Jancsó[a]

[a] Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary ; [b] MTA-SZTE Bioinorganic Chemistry Research Group, Hungary ; [c] ISA, Department of Physics and Astronomy, Aarhus University, Denmark ; email : slevente@chem.u-szeged.hu

Abstract

Although arsenic induced toxic effects are well-known for many years, their biochemical mechanisms on molecular level are not clear. Under reductive intracellular conditions arsenic is stabilized as arsenous acid (H_3AsO_3). According to the most accepted theories, the coordination of H_3AsO_3 to various enzymes leads to the indirect induction of toxicity.[1] However, only certain Cys-rich motifs can bind H_3AsO_3 efficiently. In order to identify such binding sites and to reveal the mechanistic details of the toxic effects of arsenic, a deeper understanding of the thiolate-arsenic interactions would be essential.

H_3AsO_3 can form three As-S bonds via condensation reactions. If all of the three hydroxyl functional groups of H_3AsO_3 are substituted, the maximum number of the forming thioesther bonds is considered to be three. Accordingly, tris complexes are the dominant products of the reaction between H_3AsO_3 and monothiol ligands like reduced gluthatione. In addition, stable adducts with a As(III):lig = 2:3 composition may also form if the interacting ligands are dithiols.[2]

In the presented work, the interaction of H_3AsO_3 with a hexapeptide, Ac-DCSSCY-NH_2, was studied by various spectroscopic methods at 3 different pH values applying different H_3AsO_3-peptide ratios. A series of SRCD (synchrotron radiation circular dichroism) spectra recorded at pH = 7.5 indicates the increase of helicity in the average conformation of the peptide by the addition of H_3AsO_3. As complementary experiments, UV absorption and ^{1}H-NMR spectra were also recorded with samples under similar conditions. The results obtained with all of the applied techniques undoubtedly suggest the existence of mono- and bis-complexes. Numerical evaluation of the CD and spectrophotometric data provided nearly the same conditional stability constants for these species. Comparison of the logβ values determined for certain complexes showed that the binding of the second ligand is almost as preferred as that of the first one, which is clearly bound via two thioesther bonds. Moreover, the calculated molar absorption and ellipticity spectra of the mono- and bis-complexes are also very similar. According to these findings, the peptide seems to adopt analogous helical-like structure in the two species suggesting a bidentate type binding of both ligands in the bis-complexes. Indeed, all of the above observations may reflect a unique trigonal bipyramidal coordination sphere around the arsenic atom which was also supported by molecular modelling methods.

References

1) M.F. Hughes, Toxicology Letters., 2002, 133, 1-16.
2) A.M. Spuches, H.G. Kruszyna, A.M. Rich, D.E. Wilcox, Inorg. Chem. 2005, 44, 2964-2972.

Acknowledgements: Hungarian National Research, Development and Innovation Office (K_16/120130 and GINOP-2.3.2-15-2016-00038) and COST (CA15126 MOBIEU

Metalation pathway of a plant metallothionein

Alma Salim, Eva Freisinger

University of Zurich, Department of Chemistry, Zurich, Switzerland ; email : alma.salim@chem.uzh.ch

Abstract

The metallothionein 2 protein from the plant *Cicer arietinum* (cicMT2) is a low molecular weight (~8kDa) protein that features two cysteine-rich regions with eight and six cysteine residues, respectively, which are separated by a 41 amino acids long, Cys-free linker region. It can coordinate five divalent metal ions *in vitro*. Previous results strongly suggest that the coordinated metal ions are arranged in a single metal-thiolate cluster resulting in a hairpin-like fold of the protein backbone.[1,2] However, so far, the metalation pathway leading to the fully metal-loaded protein is unknown. In the presented study we focus on the identification of the binding region for each metal ion, an endeavor never performed with any plant MT so far. The cluster formation process upon stepwise metal ion reconstitution is monitored by ESI-MS aided by limited proteolytic digestion with *Tritirachium album* proteinase K, which cleaves the protein backbone mainly in the Cys-free region. Identification of the binding region of the metal ion is complemented and corroborated by investigations with size exclusion chromatography, atomic absorption spectroscopy, and dynamic light scattering.

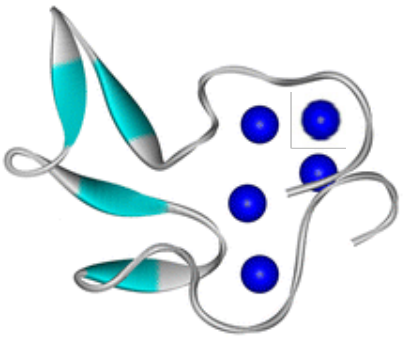


Figure 1. Schematic presentation of the hairpin-like backbone arrangement of Cd_5cicMT2. (adapted from 2)

References

1) Wan, X., Freisinger, E., Metallomics, 2009, 1, 489-500.
2) Freisinger, E., J. Biol. Inorg. Chem., 2011, 16, 1035-1045.

De novo design of metal-binding peptides for use in solar energy capture and storage

P. Teare,[a] A.F.A. Peacock[a]

[a] *School of Chemistry, University of Birmingham, Edgbaston, B15 2TT, United Kingdom ; pxt517@bham.ac.uk*

Abstract

We have developed a peptide system to bind three different metal cofactors, with the intent to mimic the photoinduced electron transfer that occurs in biological photosystems.[1] Work within the group has shown peptides can be constructed to bind two different lanthanides,[2] so the existing sequence has been modified to add a third histidyl site designed to bind copper.[3] Circular dichroism shows that the peptide self assembles to form a trimer in the presence of lanthanide ions. Each peptide trimer can bind up to two lanthanide ions, where the first ion bound induces a significant folding event and the second a more minor change.

Binding of Cu^{2+} to the pre-assembled peptide has been observed by UV-vis spectroscopy, and the sensitisation of luminescent lanthanides such as Eu^{3+} and Tb^{3+} by tryptophan enabled the characterisation of lanthanide binding by fluorescence spectroscopy.[4] We aim to establish whether these systems can act as a scaffold to encourage photoinduced charge separation across the length of the metal triad to give a long lived charge separated state.

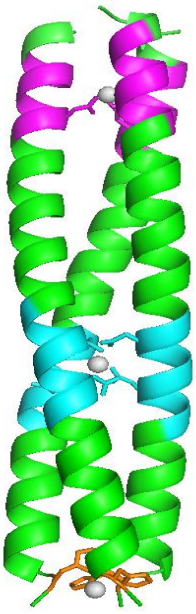


Figure 1: Representation of the peptide and its three binding sites, top and middle are designed for lanthanides, bottom site designed to bind Cu(II).

References

1) D. Gust, T.A. Moore and A.L. Moore, Acc. Chem. Res., 2001, 34 (1), 40–48
2) L. Slope, unpublished work.
3) M. R. Ghadiri and M. A. Case, Angew. Chem., 1993, 105, 1663-1667
4) M. R. Berwick, D. J. Lewis, A. W. Jones, R. A. Parslow, T. R. Dafforn, H. J. Cooper, J. Wilkie, Z. Pikramenou, M. M. Britton and A. F. A. Peacock, J. Am. Chem. Soc., 2014, 136, 1166-1169.

Selective Lanthanide Binding Sites Engineered within a *De Novo* Designed Coiled Coil

Louise N. Slope, [a] and Anna F. A. Peacock[a]

[a] *University of Birmingham, School of Chemistry, Birmingham, B15 2TT, UK ; email : lxs998@bham.ac.uk*

Abstract

We present a trimeric coiled coil sequence that is designed to embed a lanthanide binding site within its interior.[1] Translating the binding site along the coiled coil, at 1 nm intervals, generates a series of four peptide complexes, that although similar, behave differently and bind terbium in different coordination environments.[2]

Using these designs we have shown, for the first time, that protein sites can now be engineered that are selective for different lanthanide ions based on their size, thereby achieving unprecedented lanthanide discrimination and selectivity. These findings have been used to generate a coiled coil capable of accommodating two different lanthanide ions, selected for their size and differing chemical properties. More importantly the binding sites are designed so as to selectively bind these two different lanthanide ions to the two distinct sites, with a high degree of control. This represents the first example of a lanthanide selective binding site utilising a protein scaffold, and the first dual lanthanide binding coiled coil. We have also been able to control the proximity between the two lanthanide ions (Figure 1), and are currently investigating the opportunities that might be afforded by hetero-lanthanide complexes and if it is possible to determine if the two lanthanide ions can communicate with each other, and whether this is distance dependent.

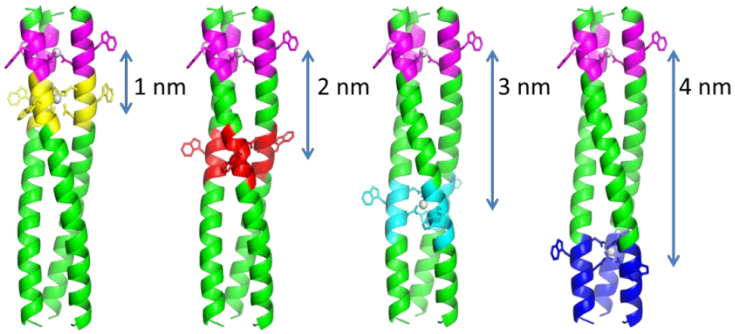


Figure 1. Pymol images of the double binding site coiled coils, showing the proximity between the two lanthanide ions.

References

1) M. R. Berwick, D. J. Lewis, A. W. Jones, R. A. Parslow, T. R. Dafforn, H. J. Cooper, J. Wilkie, Z. Pikramenou, M. M. Britton and A. F. A. Peacock, J. Am. Chem. Soc., 2014, 136, 1166.
2) M. R. Berwick, L. N. Slope, C. F. Smith, S. M. King, S. L. Newton, R. B. Gillis, G. G. Adams, A. J. Rowe, S. E. Harding, M. M. Britton and A. F. A. Peacock, Chem. Sci., 2016, 7, 2207.

Exploring the uniqueness of histidine-rich metallothioneins

Jelena Habjanič,Oliver Zerbe,Eva Freisinger

Department of Chemistry, University of Zurich,Winterthurerstrasse 190, 8057 Zurich, Switzerland;
email: jelena.habjanic@uzh.ch

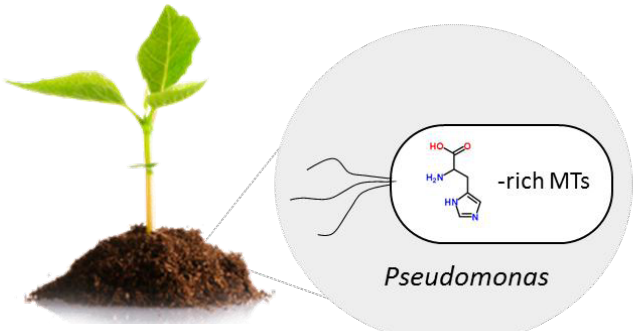
Abstract

Metallothioneins (MTs) are ubiquitous metalloproteins characterized by a high amount of cysteine residues, a low molecular weight and the formation of unique metal clusters. As a consequence of their metal binding abilities and abundance of Cys residues, they are important partakers in various physiological processes (e.g. metal homeostasis, metal detoxification, oxidative stress). While all MTs share these properties, across the different kingdoms of life a high diversity in amino acid sequences, 3D structures and functionalities has evolved.

Although bacterial MTs (bacMTs) have resided for a long time in the shadows of their mammalian counterparts, they have several unique features that break withmany MT paradigms, i.e. the contribution of histidine residues to metal ion binding, a higher percentage of secondary structure elements, as well as the presence of aromatic amino acids.[1]

The presence of MTs in *Pseudomonas* species has become more apparent after sequencing of numerous bacterial strains in the last decade. These MT sequences reveal unusually high amounts of histidine residues and a high diversity in the primary structure. At the same time they show a rather conserved Cys distribution pattern consisting of an N-terminal CxCxxCxC motif, a central YCC/SxxCA stretch, as well as an C-terminal Cxxxx(x)CxC part.

We are investigating, how differences found in the primary structure of these novel bacterial MTs influence function and 3D structure, including protein fold and the metal clusters.



http://www.agrylnovagryl.com/assets/images/crop-types/large/young_plants.png

Financial support by the Forschungskredit of the University of Zurich (JH) is gratefully acknowledged.

References

1) C. A. Blindauer, M. D. Harrison, J. A. Parkinson, A. K. Robinson, J. Cavet, N. J. Robinson, P. J. Sadler, Proc. Natl. Acad. Sci. 2001, 98, 9593.

Hydrolysis of the amide bond in L-methionine- and L-histidine-containing peptides catalyzed by various dinuclear Pt(II) complexes: Dependence of the hydrolysis rate on the nature of the bridging ligand

Snežana Rajković[a], Marija D. Živković[b], Biljana Đ. Glišić[a], Miloš I. Djuran[a]

[a]*University of Kragujevac, Faculty of Science, Department of Chemistry, R. Domanovića 12, 34000 Kragujevac, Serbia*

[b]*University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Pharmacy, Svetozara Markovića 69, 34000 Kragujevac, Serbia, e-mail:* snezana@kg.ac.rs

Abstract

Dinuclear Pt(II) complexes were shown as very effective hydrolytic reagents in the cleavage of the amide bond in L-methionine- and L-histidine-containing peptides.[1] Recent findings showed that inhibition of the hydrolytic cleavage of the amide bond involving the carboxylic group of L-methionine or L-histidine could be achieved by structural modification of the dinuclear platinum(II) complex.[1] As a continuation of this investigation, in the present study, three new dinuclear complexes, $[\{Pt(en)Cl\}_2(\mu\text{-qx})]Cl_2\cdot 2H_2O$ **(1)**, $[\{Pt(en)Cl\}_2(\mu\text{-qz})](ClO_4)_2$ **(2)** and $[\{Pt(en)Cl\}_2(\mu\text{-phtz})]Cl_2\cdot 4H_2O$ **(3)**, (qx is quinoxaline (1,4-benzodiazine); qz is quinazoline (1,3-benzodiazine); phtz is phthalazine (2,3-benzodiazine); en is bidentate coordinated ethylenediamine) have been synthesized and characterized by elemental microanalysis, NMR (^1H and ^{13}C) spectroscopy and UV-Vis spectrophotometry. The square planar geometry of platinum(II) ions in these complexes has been predicted by DFT calculations. The chlorido Pt(II) complexes were converted into the corresponding aqua species, $[\{Pt(en)(H_2O)\}_2(\mu\text{-L})]^{4+}$ (L is qx, qz and phtz) and their reactions with Ac-L-Met-Gly and Ac-L-His-Gly were studied by ^1H NMR spectroscopy. All reactions were performed in 1 : 1 molar ratio, at $2.0 < pH < 2.5$ in D_2O as solvent and 37 °C. It was found that in all investigated reactions with the Ac-L-Met-Gly dipeptide regioselective cleavage of the Met-Gly amide bond has occurred. Complex **1** had the best catalytic activity, while complexes **2** and **3** showed almost two and three times lower reactivity in these reactions. However, in the reactions with Ac-L-His-Gly dipeptide, only when complex **1** was used as catalyst, the hydrolysis of the amide bond involving the carboxylic group of histidine can be observed. In case of reactions of this dipeptide with complexes **2** and **3**, as a consequence of the absence of Pt(II) coordination to the imidazole ring, no hydrolysis of any of the amide bond has occurred. Difference in the reactivity of these complexes with imidazole ring of the Ac-L-His-Gly strongly depends from the position of nitrogen atoms in the bridging qx, qz and phtz ligands of these complexes.

References

1) S. Rajković, M. D. Živković, M. I. Djuran, Curr. Protein Pept. Sc. 2016, 17, 95.

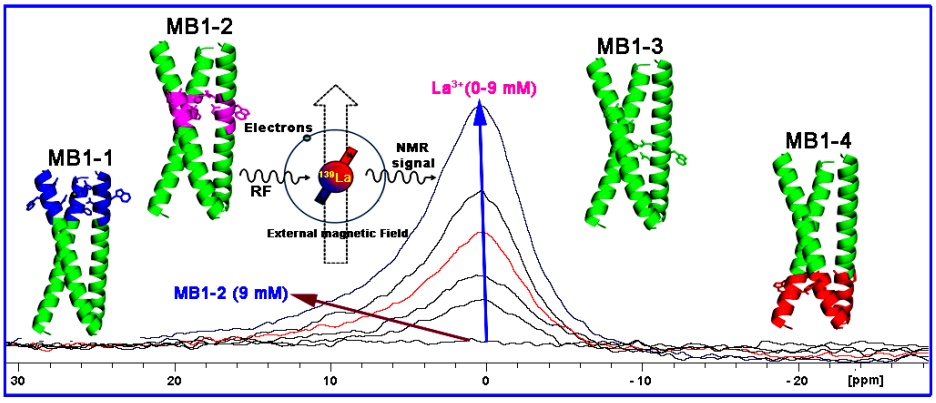
Acknowledgements. Financial support by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 172036) is gratefully acknowledged.

La^{3+} binding to a series of *de novo* designed coiled coils-probed by ^{139}La-NMR

Sellamuthu Anbu,[a] Cecile Stephanie Le Duff,[a] Anna F. A. Peacock[a]

[a]*School of Chemistry, University of Birmingham, Edgbaston, B15 2TT, UK. email : SellamuA@adf.bham.ac.uk*

Development of *de novo* (from scratch) designed coiled coils offers the possibility of incorporating metal binding sites into miniature protein scaffolds so as to achieve new function, and reactivity, or to advance biological understanding.[1,2] Engineering lanthanide binding sites into *de novo* designed coiled coil peptides, is the promising approach to distinct the behaviour of multiple alternate folding domain in natural protein architectures[3] via non-biological or *xeno* metal—ligand bonding interactions.[4] Herein we report the lanthanide binding capability of a small family of coiled coils by ^{139}La NMR and CD spectroscopy. ^{139}La NMR is a powerful tool for the study of hydrodynamic structure of protein.[5] This also has been used to ascertain the exchange rate of La^{3+} between the free and protein bound states[6] and the spin-spin relaxation of ^{139}La nuclide provides the information about the symmetry of ligand bound Lanthanum and the primary coordination sphere.[7] In this context, we have developed a series of coiled coils (MB1-1-4) and investigated their La^{3+} binding capability by ^{139}La NMR (see Figure) and CD spectroscopy. The CD spectra of these four peptides reveal an increase in folding on addition of up to an equivalent of La^{3+} per trimer without substantial folding. The ^{139}La NMR titration profiles clearly discriminate the symmetry and dynamic nature of MB1-1-4 coiled coils in solution.



References

1) J. T. Welch, W. R. Kearney, and S. J. Franklin, PNAS, 2003, 100, pp 3725.
2) M. R. Berwick, L. N. Slope, C. F. Smith, S. M. King, S. L. Newton, R. B. Gillis, G. G. Adams, A. J. Rowe, S. E. Harding, M. M. Britton and A. F. A. Peacock, Chem. Sci., 2016, 7, pp 2207.
3) D. Ghosh, and V. L. Pecoraro, Inorg. Chem., 2004, 43, pp 7902.
4) M. R. Berwick, D. J. Lewis, A. W. Jones, R. A. Parslow, T. R. Dafforn, H. J. Cooper, J. Wilkie, Z. Pikramenou, M. M. Britton and A. F. A. Peacock, J. Am. Chem. Soc., 2014, 136, 1166.
5) L. E. Kay, Journal of Magnetic Resonance, 2005, 173, pp 193.
6) S.-J. Lin, T. Wakagi, H. Matsuzawa, E. Yoshimura, J. Inorg. Biochem., 1999, 77, pp 205.
7) T. Yaita, D. Ito, and S. Tachimori, J. Phys. Chem. B 1998, 102, pp 3886.

Poly(HEMA-GMA)@Nic-Fe cryogels for pesticide removal

Kazım Köse, [a]Kadir Erol,[b]Murat Yüce,[c] Dursun Ali Köse,[c]

[a]*Hitit University, Department of Food Processing, Alaca Avni Çelik Vocational School, Çorum, Turkey,*

[b]*Hitit University, Osmancık Ömer Derindere Vocatinal School,Çorum, Turkey,*

[c]*Hitit University, Faculty of Science and Arts, Çorum, Turkey,*

email : murattyc@gmail.com

Abstract

Pesticides are very dangerous chemicals for plant and animal life. Although, use of pesticide is forbidden in many countries, they are still used or they are persistent in environment even two or three decades. The removal of pesticides is attracted great care recently because of very deep effects especially in endocrine system. The study of under concern, nicotinamide modified iron chelating will be the solution for the removal of pesticides. The iron anchored poly (2-hydroxyethylmethacrylate-glycidyl methacrylate)-Nictonaminde cryogelsis the adsorbent for effective removal of target ppesticide with positively charged cation against negatively charged pesticides in alcoholic medium.To be sure abut the synthesized structure, FTIR (Fourier Transform Infrared Spectroscopy), SEM (ScanningElectron Microscopy), AFM (Atomic Force Microscopy), elemental analysisand surfacearea measurement are performed. The adsorption performanceof cryogels is estimated to be sure about the feasibility for removal [1].

References

1) Kose, K. and Köse, DA, Removal of DDE by exploiting the alcoho-phobic interactions, 2017, pp. 1-7.

Polyethyleneimine assisted-two-step polymerization to develop surface imprinted cryogels for lysozyme purification

Kadir Erol[a,b], Kazım Köse[a,c], Lokman Uzun[a], Rıdvan Say[d], Adil Denizli[a]

[a]Hacettepe University, Department of Chemistry, Ankara, Turkey

[b]Hitit University, Osmancık Ömer Derindere Vocational School, Department of Property Protection and Safety, Çorum, Turkey

[c]Hitit University, Alaca Avni Çelik Vocational School, Department of Food Processing, Çorum, Turkey

[d]Eskişehir University, Department of Chemistry, Eskişehir, Turkey

Abstract

Surface imprinting strategy is one of the promising approaches to synthesize plastic antibodies while overcoming the problems in the protein imprinting research (1-2). In this study, we focused our attentions on developing two-step polymerization to imprint on the bare surface employing polyethyleneimine (PEI) assisted-coordination of template molecules, lysozyme. For this aim, we firstly synthesized poly(2- hydroxyethyl methacrylate-glycidyl methacrylate), poly(HEMA-GMA) cryogels as a bare structure. Then, we immobilized PEI onto the cryogels through the addition reaction between GMA and PEI molecules. After that, we determined the amount of free amine (NH_2) groups of PEI molecules, subsequently immo-bilized methacrylate functionalities onto the half of them and another half was used to chelate Cu(II) ions as a mediator between template, lysozyme and PEI groups. After the characterization of the materials developed by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and the micro-computed tomography (μCT), we optimized the lysozyme adsorption conditions from aqueous solution. Before performing lysozyme purification from chicken egg white, we evaluated the effects of pH, interaction time, the initial lysozyme concentration, temperature and ionic strength on the lysozyme adsorption. Moreover, the selectivity of surface imprinted cryogels was examined against cytochrome c and bovine serum albumin (BSA) as the competitors. Finally, the mathematical modeling, which was applied to describe the adsorption process, showed that the experimental data is very well-fitted to the Langmuir adsorption isotherm.

References

1) G. Ozaydin Ince, E. Armagan, H. Erdogan, F. Buyukserin, L. Uzun, G. Demirel, 2013, One-dimensional surface-imprinted polymeric nanotubes for specific biorecognition by ınitiated chemical vapor deposition (iCVD), ACS Appl. Mater. Interfaces, 5,6447–6452.

2) Y. Lv, T. Tianwei, F. Svec, Molecular imprinting of proteins in polymers attached to the surface of nanomaterials for selective recognition of biomacromolecules, 2013, Biotechnol. Adv., 31, 1172–1186.

The effect of distant cysteinyl residues on the complex formation processes of peptides containing other binding sites

Norbert Lihi, Ágnes Grenács, Katalin Várnagy and Imre Sóvágó

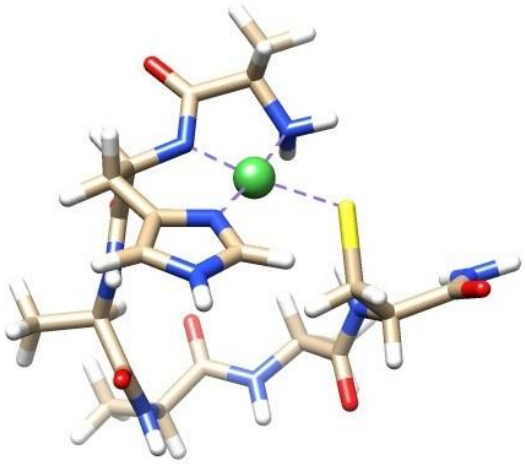
Department of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary

email : lihi.norbert@science.unideb.hu

Peptides are effective ligands to bind metal ions. However, this metal binding affinity is rather selective and largely depends on the characteristic of the metal ion and the quality of the donor group. As a consequence, the peptide sequence can significantly determine the metal binding ability. For studying these effects the investigation of short peptides with multiple binding sites is indispensable and the results may contribute for better understanding to metal – peptide interactions and the fine tuning effects of the side chain donor groups[1].

Based on this fact, in our lab, systematic studies have been performed to synthesize N-terminally free peptides containing Asp, His or Cys residues at the N-terminal domain of the peptide and another cysteine at the C-terminal part[2,3,4]. These peptides were the following: AAASSC-NH_2, ADAAAC-NH_2, AADAAC-NH_2, AHAAAC-NH_2, AAHAAC-NH_2, CSSACS-NH_2 and ACSSACS-NH_2. The complex formation processes with copper(II), nickel(II), cadmium(II), zinc(II) and palladium(II) were characterised using equilibrium, spectroscopic and theoretical (DFT) methods.

Our results indicated that the contribution of the distant cysteine to the complex formation largely depends on the position of the other donor group of the N-terminal region. As a representative example, in the case of nickel(II) complexes of AAHAAC-NH_2 the primary binding site is the N-terminal region to form albumin-related structure and the non-coordinated thiolate is an independent metal binding site for other nickel(II)ion. In the case of copper(II), this albumin-like coordination is able to hinder the redox reaction between the metal ion and thiolate group. In contrast, the replacement of the His to the secondary position (AHAAAC-NH_2) results in unsaturated coordination sphere with (NH_2,N^-,N_{im}) donor set that can be completed by macrochelation of the distant thiolate of cysteine.



Acknowledgement

This work was supported by the Hungarian Scientific Research Fund (K 115480), Richter Gedeon Talentum Foundation and EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008.

References

1) I. Sóvágó, K. Várnagy, N. Lihi, Á. Grenács, *Coord. Chem. Rev.*, **327-328** (2016) 43-54.
2) N. Lihi, Á. Grenács, S. Timári, I. Turi, I. Bányai, I. Sóvágó, K. Várnagy, *New J. Chem.*, **39** (2015) 8364-8372.
3) M. Raics, N. Lihi, A. Laskai, Cs. Kállay, K. Várnagy, I. Sóvágó, *New J. Chem.*, **40** (2016) 5420-5427.
4) N. Lihi, D. Sanna, I. Bányai, K. Várnagy, I. Sóvágó, *New. J. Chem.*, **41** (2017) 1372-1379.

The Toxicity of Lead(II) and Its Close Relationship to Copper(II)

Astrid Sigel and Helmut Sigel

University of Basel, Department of Chemistry, Inorganic Chemistry,
Spitalstrasse 51, CH-4056 Basel, Switzerland
E-mail: Helmut.Sigel@unibas.ch

Abstract

Lead is known to mankind for thousands of years and its toxicity was recorded already by Greek and Arab scholars (for refs see [1]). Considering this and its wide distribution in the environment it is surprising to find that information about the interaction of lead(II) with bio-ligands is scarce. Martin applied the so-called *Stability Ruler* [2] to Pb(II) and he observed that in a first approximation the stabilities of Pb(II) complexes with N-donor ligands correspond stability-wise to those of Fe(II) [3]. This contrasts with O-donor ligands where the stability of Pb(II) complexes strongly moves up the *Ruler* and equals that of the corresponding Cu(II) complexes. For example, the stabilities of the 1:1 complexes with methyl phosphate (= MPO^{2-} = $CH_3OPO_3^{2-}$) are log $K^{Pb}_{Pb(MPO)}$ = 2.98 ± 0.10 and log $K^{Cu}_{Cu(MPO)}$ = 2.94 ± 0.03, and there are more such examples [1]. This provides the confidence to use Cu(II) complexes as mimics for Pb(II) complexes. For example, with hydroxyacetate (= $HOAc^-$ = $HO–CH_2–COO^-$) and Cu(II) it was shown that an intramolecular equilibrium between an "open" isomer with carboxylate coordination only exists, next to a "closed" or chelated species in which a 5-membered chelate involving also the HO^- group is formed [1, 4]; this chelated isomer has a formation degree of 84 ± 3% and it is concluded that the one for $Pb(HOAc)_{cl}^+$ is similar. Moreover, the formation degree for $Ca(HOAc)_{cl}^+$ of 83 ± 3% illustrates the relationship between Ca(II) and Pb(II) [note, 95% of the body burden of Pb(II) is stored in bones; note further, the absolute stabilities of the $Ca(HOAc)^+$ and $Pb(HOAc)^+$ complexes differ, but the intramolecular equilibria regarding chelate formation are the same] [1]. -- Furthermore, the stability constants of nucleoside 5'-monophosphates (NMP^{2-}) with non-coordinating nucleobase residues [and pK_a values of about 6.20 for the deprotonation of the $–P(O)_2^-(OH)$ group] are log $K^{Pb}_{Pb(NMP)}$ = 2.93 ± 0.08 and log $K^{Cu}_{Cu(NMP)}$ = 2.87 ± 0.06, where NMP^{2-} = cytidine 5'-monophosphate, uridine 5'-monophosphate, etc.. This equality allows to make sophisticated estimates [1] for the stabilities of Pb(II) complexes, e.g., with nucleoside 5'-di- and 5'-triphosphates, for which no stability constants have yet been measured.

Supported by the Department of Chemistry of the University of Basel, Switzerland

References

[1] A. Sigel, B. P. Operschall, H. Sigel, *Met. Ions Life Sci.* 2017, *17,* 319-401.
[2] R. B. Martin, *Met. Ions Biol. Syst.* 1986, *20*, 21-65.
[3] H. Sigel, C. P. Da Costa, R. B. Martin, *Coord. Chem. Rev.* 2001, *219-221*, 435-461.
[4] F. M. Al-Sogair, B. P. Operschall, A. Sigel, H. Sigel, J. Schnabl, R. K. O. Sigel, *Chem. Rev.* 2011, *111*, 4964-5003.

Nucleotide Incorporated Magnetic Microparticles for Isolation of DNA

Kazım Köse

Hitit University, Department of Food Processing, AlacaAvniÇelik Vocational School, Çorum, Turkey,
email : kazimkose1903@gmail.com

Abstract

For the comprehensive understanding of many diseases, especially genetic disease, the highly purified deoxyribonucleic acid (DNA) is needed to perform in-detail studies. There are various methods to obtain highly purified DNA, but low in number. In this study, Fe&Ni-mc-poly (2-hydroxyethyl methacrylate-adenine methacrylate) [poly(HEMA-AdeM)] microparticles were synthesized to get high purification ratio because of natural and thus selective interaction between adenine on the microparticles and thymine on the DNA molecule and also high amount of DNA purified. For the characterization of microparticles scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), elemental analysis, swelling ratio and zeta size analysis methods were performed. All experiments were performed batch-wise for the determination of optimum conditions such as pH, initial DNA concentration, temperature and interaction time. At the end of the all study, the desorption yield was obtained as 97.7% and after 5 adsorption-desorption cycle, the adsorption capacity was decreased only 3%[1].

References

1) Kose, K., Nucleotide incorporated magnetic microparticles for isolation of DNA, 2016, 51, pp. 1644-1649.

Development of peptide-conjugate ligands for selective delivery of anticancer Titanium(IV) compounds

Lauren V. Fernandez Vega, Arthur D. Tinoco, Mallesh Pandrala, Frances Serrano

[a] *University of Puerto Rico-Río Piedras, San Juan, PR 00931; email : lauren.fernandez@upr.edu*

Abstract

Ti(IV) compounds have demonstrated terrific potential as anticancer drugs to attack a wide spectrum of tumors. It has been observed that human transferrin (hTf), a serum metal binder protein, is able to stabilize the hydrolysis prone Ti(IV) ion and successfully transport it into cells even when hTf is known to bind Fe(III) preferentially. This transporting role is believed to be central to Ti(IV)'s anticancer function, but efforts to synthesize Ti(IV) compounds targeting transferrin have not produced a drug possibly due to poor Ti(IV) release in cells. However, the Ti(IV) stabilizing hTf binding site offers insights to develop a new Ti(IV)-based drug design strategy: the structural characteristics of the hTf binding site moiety serves as a valuable template for designing biomimicking ligands capable of stably chelating Ti(IV) and facilitating the transport of the metal into cancer cells. Currently, peptides, proteins and smalls molecules have been used to improve the selective drug delivery to cancer cells over normal cells forming drug-carrier conjugates. The principal idea is to take advantage of this metal binding site by using ligands that mimic it to effectively bind Ti(IV). In addition, we will incorporate a bioactive peptide moiety on the ligand. The choice of peptide is governed by those that have a transport receptor overexpressed in cancer cells relative to healthy cells, which will permit transport of our compounds into cancer cells. This strategy would lead to significantly more specificity of the Ti(IV) complexes towards cancer cells than normal cells. We are currently working on the synthesis of the iron chelator deferasirox (a drug used to treat iron-overload diseases) conjugated to substance P (SP), a bioactive peptide whose primary receptor is the neurokinin 1 receptor. Standard purification and spectral characterization methods will be used to confirm successful synthesis of different versions of the conjugate. Ti(IV) coordination by the conjugates will then be performed followed by typical work up protocols to yield pure Ti(IV) compound. Selective delivery of our conjugates will be examined using cell lines with and without an overexpression of different receptors.

References

(1) Kostova, I. Titanium and vanadium complexes as anticancer agents. **2009**, *9*, 827.

(2) Harding, M. M.; Mokdsi, G. Antitumour metallocenes: structure-activity studies and interactions with biomolecules. **2000**, *7*, 1289.

(3) M. Miller, et al., Insights into molecular mechanism of action of salan titanium(IV) complex with in vitro and in vivo anticancer activity, J. Inorg. Biochem. **2016**,163, 250–257

(4) Sumit Majumdar and Teruna J. Siahaan. Peptide-MediatedTargeted Drug Delivery. **2010**. Published online.

New efficient ligands for phosphate anions: formation and complex stability studies.

Joanna I. Lachowicz,[a] Valeria M. Nurchi,[a] Guido Crisponi,[a] Claudia Caltagirone,[a] Sergio Murgia[a]

[a] *University of Cagliari ; email : lachowicz@unica.it*

Abstract

Due to the crucial roles played by anions in biological, environmental and industrial fields, one of the main topics of supramolecular chemistry nowadays is anion recognition and sensing.[1–3] In particular, phosphates are among the most important anions in biological systems as they play a central role in the building of two fundamental molecules in the living systems, DNA and RNA. Phosphates are also involved in various processes such as energy storage, signal transduction, gene regulation and muscle contraction, and, in the form of phospholipids, they are essential constituents of lipid membranes.[4,5] Moreover, they are important components of medicinal drugs and fertilizers and their increasing presence in natural water sources is related to the eutrophication of the aquatic ecosystems.[6] For these reasons, a great effort has been made to design receptors highly selective for phosphorylated species.[7–9]

Four new kojic acid derivatives have been synthesized as phosphate anion receptors. Their binding properties towards different phosphate anions (PO_4^{3-}, $P_2O_7^{4-}$, $P_3O_{10}^{5-}$, ATP, ADP and AMP) have been studied by means of potentiometry, ^{1}H and ^{14}P-NMR, UV-Vis and Fluorescence spectroscopy. Moreover, mixed metal-anion-ligand complexes were studied by means of ^{1}H, ^{27}Al and ^{14}P-NMR, and single crystal X-ray diffraction.

References

1) P. A. Gale, N. Busschaert, C. J. E. Haynes, L. E. Karagiannidis and I. L. Kirby, Chem. Soc. Rev., 2014, 43, 205–241.
2) P. A. Gale and C. Caltagirone, Chem. Soc. Rev., 2015, 44, 4212–4227.
3) N. Busschaert, C. Caltagirone, W. Van Rossom and P. A. Gale, Chem. Rev., 2015, 115, 8038–8155.
4) R. L. P. Adams, J. T. Knowler and D. P. Leader, The Biochemistry of the Nucleic Acids, Chapman and Hall, New york, 10th edn, 1986.
5) W. Saenger, Principles of Nucleic Acid Structure, Springer-Verlag, New York, 1984.
6) H. Tiessen, Phosphorus in the Global Environment: Transfers, Cycles, and Management, Wiley, New York, 1995.
7) C. Bazzicalupi, A. Bencini and V. Lippolis, Chem. Soc. Rev., 2010, 39, 3709–3728.
8) A. Bencini, F. Bartoli, C. Caltagirone and V. Lippolis, Dyes Pigm., 2014, 110, 169–192.
9) A. E. Hargrove, S. Nieto, T. Zhang, J. L. Sessler and E. V. Anslyn, Chem. Rev., 2011, 111, 6603–6782.
10) Nurchi, V.M., Crisponi, G., Arca, M., Crespo-Alonso, M., Lachowicz, J.I., Mansoori, D., Toso, L., Pichiri, G., Santos, M.A., Marques, S.M., Niclos-Gutierrez, J., Gonzalez-Perez, J.M., Dominguez-Martin, A., Choquesillo-Lazarte, D., Szewczuk, Z., Antonietta Zoroddu, M., Peana, M., 2014. J. Inorg. Biochem. 141, 132-143.
11) Lachwicz, J.I., Nurchi, V.M., Crisponi, G., Pelaez, G.J., Stefanowicz, P., Zoroddu, M.A., Peana, M., 2014. J. Biol. Inorg. Chem. 19, S776-S776.
12) Lachowicz, J.I., Nurchi, V.M., Crisponi, G., Jaraquemada-Pelaez, M.G., Arca, M., Pintus, A., Santos, M.A., Quintanova, C., Gano, L., Szewczuk, Z., Zoroddu, M.A., Peana, M., Dominguez-Martin, A., Choquesillo-Lazarte, D., 2016. Dalton Trans 45, 6517-6528.

Lactose intolerance treatment: silica carriers

Ileana-Alexandra Pavel, Nadia Canilho, Andreea Pasc

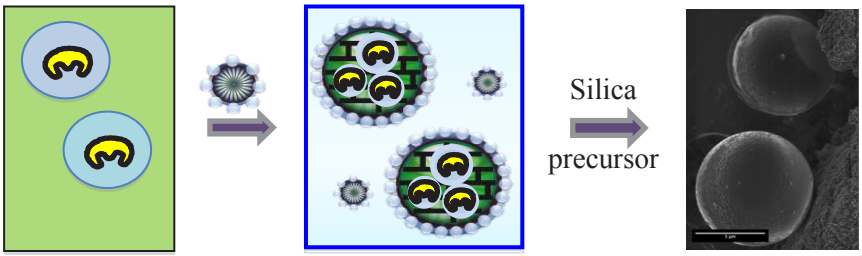
SRSMC UMR 7565, Université de Lorraine, F-54506 Vandoeuvre-lès-Nancy cedex, France

email : ileana-alexandra.pavel@univ-lorraine.fr

Abstract

Protein drug delivery and drug targeting have been proposed for medical treatments due to their selectivity and catalytic activity. Oral administration of exogenous β-galactosidase is one way to tread lactose intolerance but enzyme efficiency is limited by human body conditions such as gastric pH and enzymatic inhibition. Thus enzyme encapsulation in silica shell is a food grade method to preserve lactase up to the intestine. Porous silica materials are being studied for oral drug delivery systems (ODDS) due to their numerous advantages as good biocompatibility, low toxicity, generally regarded as safe (GRAS), resistant to microbial attack. Moreover their intrinsic properties such as high pore volume and surface area, adjustable pore size and facile tuneable properties afford to design bioinorganic carriers pH sensitive.

Herein we present the formulation of nanostructured lipid carries, coated with silica, having a double trigger response. In fact, recently Solid Lipid Nanoparticles (SLN) showed to be good porous silica templates[1] and thus promising drug carriers[2]. β-Galactosidase is encapsulated in protective solid lipid carrier prepared by double emulsion. At low temperatures, the diffusion of enzyme from the carriers is limited, while above the melting point of the lipid a progressive release occurs when the lipid chains undergo a phase transition. This thermal responsive behaviour makes these particles interesting from the point of view of a controlled release of enzyme. Moreover, the possibility of selective mineralization of the solid lipid nanoparticles[3] towards silica should insure a pH response and higher storage stability due to protective inorganic shell.



The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° 606713.

References

1) R. Ravetti-Duran, J.-L. Blin, M.-J. Stébé, C. Castel and A. Pasc, J. Mater. Chem., 2012, 22, 21540–21548
2) S. Kim, M.-J. Stébé, J.-L. Blin and A. Pasc, J. Mater. Chem. B, 2014, 2, 7910–7917.
3) J.-L. Blin, J. Jacoby, S. Kim, M.-J. Stébé , N. Canilho and A. Pasc, Chem. Commun. , 2014, 50, 11871–11874

Protein Engineering Of FhuA And Nitrobindin For Biohybrid Catalysts

Alexander Grimm[1], Marcus Arlt[1], Stephanie Mertens[1], Daniel Sauer[2], Takashi Hayashi[3], Jun Okuda[2], Ulrich Schwaneberg[1]

[1] *Institute of Biotechnology, RWTH Aachen University, Worringerweg 3, 52074 Aachen, Germany.*
[2] *Institute of Inorganic Chemistry, RWTH Aachen University, Landoltweg 1, 52074 Aachen, Germany*
[3] *Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*
u.schwaneberg@biotec.rwth-aachen.de

Abstract

Efforts to develop biocatalysts with new chemical reactivity and selectivity not found in nature have led to the construction of biohybrid catalysts, such as metalloenzymes[1]. Biohybrid catalysts have the potential to overcome limitations characteristic to both enzymes and synthetic catalysts. On the one hand, the use of artificial catalytic centers in biohybrid catalysts increases the range of applicable substrates that might not be catalyzed by enzymes due to their strict substrate-specificity. On the other hand though, the amino acid side chains in the protein scaffolds provide a second ligand sphere which enables selectivity towards substrates, e.g. enantioselectivity. These structural features are difficult to obtain in low-molecular synthetic catalysts[2].

A number of biohybrid catalysts have been developed capable of catalyzing non-natural C-C bond formation reactions, which belong to the most basic reactions in organic synthesis. These include a Hoveyda-Grubbs-type complex conjugated to the FhuA ΔCVF^{tev} ß-barrel protein capable of performing ring-opening olefin metathesis (ROMP), and a Rh-nitrobindin (Rh-NB) hybrid that catalyzes the stereoselective polymerization of phenylacetylene[3, 4, 5].

References

1) Onoda, A. *et al.*, A rhodium complex-linked β-barrel protein as a hybrid biocatalyst for phenylacetylene polymerization, 2012, 48, 9756-9758.
2) Matsuo, T. *et al.*, Artificial enzymes with protein scaffolds: Structural design and modification, 2014, 22, 5638-5656.
3) Fukumoto, K. *et al.*, Rhodium-complex-linked hybrid biocatalyst: Stereo-controlled phenylacetylene polymerization within an engineered protein cavity, 2014, 6, 1229-1235.
4) Philippart, F. *et al.*, A hybrid ring-opening metathesis polymerization catalyst based on an engineered variant of the β-barrel protein FhuA, 2013, 19, 13865-13871.
5) Sauer, D. *et al.*, Hybrid ruthenium ROMP catalysts based on an engineered variant of beta-barrel protein FhuA ΔCVF^{tev}: Effect of spacer length, 2015, 10, 177-182.

Financial support by DFG and SeleCa is gratefully acknowledged.

Study of TtcA-TtuA enzyme family involved in tRNA thiolation

Bimaï Ornella [1], Simon Arragain[1], Pierre Legrand[2], Sylvain Caillat[3], Jean-Luc Ravanat[3], Nadia Touati[4], Laurent Binet[4,5], Mohamed Atta[6], Marc Fontecave[1*], Béatrice Golinelli-Pimpaneau[1*]

[1] Laboratoire de Chimie des Processus Biologiques, UMR 8229 CNRS, Collège de France, Université Pierre et Marie Curie, 11 Place Marcelin Berthelot, 75231 Paris cedex 05, France
[2] Synchrotron SOLEIL, L'Orme des Merisiers, Saint Aubin BP48, Gif-sur-Yvette 91198, France
[3] University of Grenoble Alpes, CEA, iNAC, SyMMES, F-38054 Grenoble, France
[4] IR CNRS Renard, Chimie-ParisTech, 11 rue Pierre et Marie Curie, 75005 Paris, France
[5] PSL Research University, Chimie-ParisTech, Institut de Recherche de Chimie-Paris, 11 rue Pierre et Marie Curie, 75005 Paris, France
[6] University of Grenoble Alpes, CEA, DRF-BIG-LCBM, UMR5249, F-38000 Grenoble, France
[1]ornella.bimai@college-de-france.fr;[1]simon.arragain@college-de-france.fr; [1]marc.fontecave@cea.fr; [1]béatrice.golinelli@college-de-france.fr

Abstract

Transfer RNAs are essential components of cellular translation machinery [1]. To achieve their function they possess several post-transcriptional chemical modifications (MODOMICS: a database of RNA modification pathways). These modifications improve recognition between tRNA and its partners during translation and thus ensure translation fidelity and efficiency. Sulfur is present in several of these modified nucleosides: thiouridine and derivatives (s^4U8, s^2U34, m^5s^2U54), 2-thioadenosine derivatives (ms^2i^6A37, ms^2t^6A37) and 2-thiocytidine (s^2C32)[2]. However, mechanisms of sulfur insertion into tRNAs are largely unknown and the enzymes responsible for these reactions incompletely characterized.
My project consists in the structural and functional study of TtcA/TtuA enzyme family that is responsible for the thiolation of transfer RNAs (tRNAs). The reaction consists in the substitution of an oxygen atom by a sulfur on the C2 atom of a pyrimidine (cytosine or uridine). At first glance, the mechanism of this non-redox reaction should involve persulfides, as previously observed for the biosynthesis of other thiolated nucleosides [3]. However, the TtcA enzyme responsible for the thiolation of s^2C_{32} was shown to contain a [4Fe-4S] center that is essential for its catalytic activity [4]. In fact all these enzymes belong to the same superfamily, whose sequence displays conserved cysteines and ATP-binding motif.
The projects aims at determining if the two other enzyme sub-classes that target positions 54 (TtuA) and 34 (Ncs6/Ctu1/NcsA) also use an iron-sulfur cluster cofactor and at elucidating their biochemical and structural mechanisms. TtuA enzymes catalyze the C2-thiolation of uridine 54 in the T-loop of thermophilic tRNAs, allowing stabilization of tRNAs at high temperature in thermophilic microorganisms [5]. We report the first detailed biochemical and structural characterization of TtuA enzymes that demonstrates the presence of a [4Fe-4S] cluster essential for activity (**submitted on PNAS**). The crystal structures of *P. horikoshii* TtuA show that the cluster, chelated by only three cysteines, is adjacent to the ATP-binding site. Electron density near the fourth iron, non-bonded to the protein, can be assigned to an exogenous sulfide, which likely acts as the sulfurating agent. TtuA is the first structurally characterized member of the nucleoside non-redox thiolation enzymes superfamily in which

Surface-engineered gold nanoparticles to inhibit beta-amyloid aggregation

A. B. Caballero, [a] Alba Espargaró,[b] Guillem Vázquez,[a] Raimon Sabaté,[b] Patrick Gamez [a,c]

[a)] Department of Inorganic and Organic Chemistry, University of Barcelona, Martí i Franquès 1–11, 08028 Barcelona (Spain); [b)] Department of Physical Chemistry, Faculty of Pharmacy, University of Barcelona, Avda. Joan XXIII 27-31, 08028 Barcelona (Spain); [c)] Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona (Spain) ; email : ana.caballero@qi.ub.es

Abstract

Alzheimer's disease (AD) represents the most common form of dementia. In 2015, the number of people suffering from AD was estimated to 46 million and this number is expected to triplicate within the next three decades. The existing treatments only alleviate the symptoms and the disease is currently incurable. Therefore, the search for novel approaches to lessen the toxicity associated to the prevalence of amyloid deposits in AD brains remains a scientific challenge.

Our group is starting to develop potential drugs that are based on gold nanoparticles (AuNPs), with the objective to overcome the main drawbacks commonly shown by small molecules as anti-AD drugs. The new nanoconjugates are intended to exhibit the following properties: (i) permanent slow-down or inhibition of beta-amyloid aggregation, (ii) scavenging and clearance of extracellular beta-amyloid deposits and (iii) permeability through the blood-brain barrier.

In this communication, we will describe our approach and present the first results showing the effect of the designed conjugates on the aggregation kinetics of beta-amyloid fragments.

Acknowledgements

Financial support by the MINECO (CTQ2014-55293-P and CTQ2015-70371-REDT), European Commission (Marie Skłodowska-Curie grant of A.B.C) and IN2UB is kindly acknowledged.

Lead complexes of cysteine containing peptides

György Szunyog, Katalin Várnagy

Department of Inorganic and Analytical Chemistry, University of Debrecen,
Egyetem tér 1, Debrecen, Hungary, 4032; email: szunyog.gyorgyi@science.unideb.hu

Abstract

The lead is serious environmental toxin, because it has accumulated in the environment much above the natural level due to the human activity during thousands of years. Based on the soft character of the metal ion, it can bind to the proteins and first of all the side chain thiol groups provide effective binding site for them. As a consequence, this metal ion is able to substitute the essential metal ions (e.g. zinc(II), nickel(II), calcium(II)) in the metalloenzymes and metalloproteins resulting drastic change in their biochemical functions.[1] The accumulation of toxic metal ions can cause carcinogenic effect or physiological change in the bones, kidneys or indirectly might take part in the development of neurodegenerative disorders.

The goal of our research was the designing and synthesis of peptides with high lead(II) binding affinity. We synthesized a series of peptides containing one or two cysteine residues. One group of the ligands have free N-terminal amino group and amide group on the C-termini: Ser-Ser-Cys-Ser-Ser-Ala-Cys-Ser-NH_2 (SSCSSACS-NH_2), Ala-Cys-Ser-Ser-Ala-Cys-Ser-NH_2 (ACSSACS-NH_2) and Cys-Ser-Ser-Ala-Cys-Ser-NH_2 (CSSACS-NH_2), while the other group of them are terminally protected peptides: Ac-Ser-Cys-Cys-Ser-NH_2 (Ac-SCCS-NH_2), Ac-Cys-Ser-Ser-Cys-NH_2 (Ac-CSSC-NH_2) and Ac-Cys-Ser-Ser-Ala-Cys-Ser-NH_2 (Ac-CSSACS-NH_2).

We studied the complex formation processes of these molecules in the presence of toxic lead(II) ions and compared to those of toxic cadmium(II) and essential zinc(II) ions.[2] The stoichiometry and stability constants of the metal complexes were determined by potentiometry, while their structures were supported by means of UV-, MS- and NMR-spectroscopy.

In the case of three peptides containing free terminal amino group the thiolate groups are the main binding site, but the coordination of terminal amino group contributes to the formation of stable complexes.

For the terminally protected tri-, tetra- and hexapeptides the thiolate group is the primary binding site. The di-cysteine containing peptides bind metal ions through both thiolate groups resulting in mono- and/or bis(ligand) complexes with high stability. This coordination mode are able to prevent the hydrolysis of lead(II) ions and stable mixed hidroxido complexes are formed in equimolar solutions at high pH range.

References

1) E. Farkas, B. Bóka, B. Szőcs, A. Godó, I. Sóvágó, Inorganica Chimica Acta, 2014, 423, 242.
2) N. Lihi, A. Grenács, S. Timári, I. Turi, I. Bányai, I. Sóvágó, K. Várnagy, New J. Chem., 2015, 39, 8364.

This work was supported by the Hungarian Scientific Research Fund (K 115480), EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008 and Campus Mundi Scholarship Fund.

Session B

Friday June 9th

HD	**Metals in health and disease**
DG	**Metal-based drugs**
ME	**New methods around metals in biology**
IM	**Metals in imaging and sensing**
EZ	**Metalloenzymes, inspiration, mimics function and inhibition**
BM	**Metals and biomolecules**

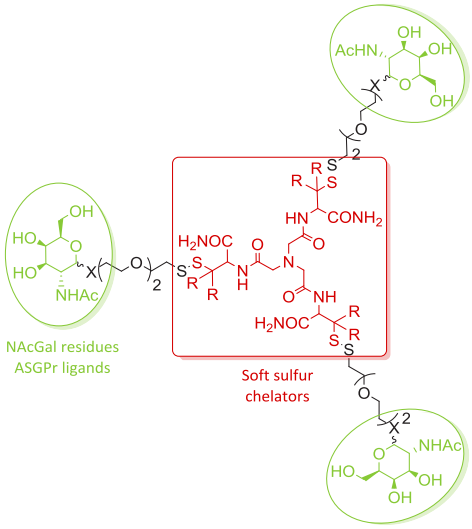
Design of IntraHepatocyte Copper(I) Chelators as Drug Candidates for Wilson's Disease

Christelle GATEAU,[a] Marie MONESTIER,[a] Faustine ROBERT,[a] Colette LEBRUN,[a] Peggy CHARBONNIER,[b] Elisabeth MINTZ,[b] Pascale DELANGLE[a]

[a]SyMMES (UMR 5819 CEA CNRS UGA), INAC CEA-Grenoble, 17, rue des Martyrs F-38054 Grenoble, France ;
[b]LCBM (UMR 5249 CEA CNRS UGA), BIG CEA-Grenoble, 17, rue des Martyrs F-38054 Grenoble, France ;
email : christelle.gateau@cea.fr

Wilson's disease is an autosomal recessive disease caused by mutations on the ATP7B gene. Since the corresponding ATPase is in charge of copper (Cu) distribution and excretion, its malfunctioning leads to Cu overload mainly in liver and brain. The current drugs used to treat this disease (D-Penicillamine and trientine) are based on Cu chelation. Although this drugs induces many side effects.

To develop more efficient and specific treatment for Wilson's disease, we propose to target the pool of intracellular Cu in hepatocytes.



As the intracellular Cu pool is mainly in the +1 oxidation state, soft sulfur chelators inspired from binding sites in metallothioneins were developed. Their functionalization with N-cetylgalactosamine residues, known ligands of the asialoglycoprotein receptors led to their hepatocyte incorporation. Here we presented the rational design, synthesis and evaluation of these new intrahepatocyte copper(I) chelators as drug candidates for Wilson's disease, namely the influence of several structural parameters on the targeting of human hepatocytes.

This research was supported by the Labex ARCANE (Grant ANR-11-LABX-0003-01) the Fondation pour la Recherche Médicale (grant DCM20111223043), the Agence Nationale pour la Recherche (grant ANR-11-EMMA-025 "COPDETOX")

References

1) A. Pujol, C. Gateau, C. Lebrun, P. Delangle, *J. Am. Chem. Soc.*, **2009**, 131 (20), pp 6928–6929 ; A. M. Pujol, C. Gateau, C. Lebrun and P. Delangle *Chem. Eur. J.,* **2011**, 17(16), pp 4418-4428 ; A.-S. Jullien, C. Gateau, C. Lebrun, I. Kieffer, D. Testemale, P. Delangle *Inorg. Chem.,* **2014,** 53 (10), pp 5229–5239
2) A. M. Pujol, M. Cuillel, A-S. Jullien, C. Lebrun, D. Cassio, E. Mintz, C. Gateau, P. Delangle *Angew. Chem. Int. Ed.,* **2012,** 51(30), pp 7445-7448 ; P. Delangle, C. Gateau *Ann. N.Y. Acad. Sci.,* **2014,** 1315, pp 30-36 ; M. Monestier, P. Charbonnier, C. Gateau, M. Cuillel, F. Robert, C. Lebrun, E. Mintz, O. Renaudet, P. Delangle *ChemBioChem,* **2016,** 17(7), pp 590-594

Cu(II) chelation as a therapeutic approach against Alzheimer's disease: impact of kinetic aspects

Amandine Conte-Daban, [a,b] **Maryline Beyler,** [c] **Raphaël Tripier** [c] **and Christelle Hureau** [a]

[a] Laboratoire de Chimie de Coordination, CNRS, 31077 Toulouse Cedex 4, France; [b] Université Paul Sabatier, 31077 Toulouse Cedex 4, France; [c] Université de Bretagne Occidentale, UMR-CNRS 6521, UFR des Sciences et Techniques, 29238 Brest Cedex 3, France; email : amandine.conte-daban@lcc-toulouse.fr

Abstract

Alzheimer's disease is the most common neurodegenerative disease, with no known cure. One of the hallmarks of the disease is the extracellular senile plaques consisting of insoluble fibrillar and amorphous aggregates of the amyloid-β peptide (Aβ). These aggregates also contain different metal ions such as Cu(II) and Zn(II).[1] Aβ-Cu(II) complexes are considered to be the most toxic form due to their capability to produce Reactive Oxygen Species (ROS).[2]

One therapeutic strategy in tackling Alzheimer's disease is to reduce the formation of these toxic species, by promoting the retrieval of Cu(II) from Aβ, through the use of Cu(II) chelators.[3,4,5] The ideal chelator should at least be able to (i) effectively remove Cu(II) from Aβ, (ii) stop (or significantly reduce) the production of ROS, (iii) reduce the formation of Cu-Aβ oligomeric species and (iv) show a sufficiently high Cu over Zn selectivity to target Cu and not Zn.[6] In the present study, three macrocycles from the cyclam family (Fig 1) have been tested: cyclam, Hte1pa and H_2te2pa incorporating one or two picolinate pendant arm(s), respectively.[7] The kinetic of the Cu(II) chelation by the macrocycle ligands has been studied (with or without the presence of Zn(II) ions) as well as the impact on the arrest of ROS production. The addition of such pendant moiety can be efficient from a thermodynamic point of view (stability and Cu over Zn selectivity) but also from a kinetic point of view. Such a kinetic effect of Cu(II) removal from Aβ has never been studied so far within the context of Alzheimer's disease.

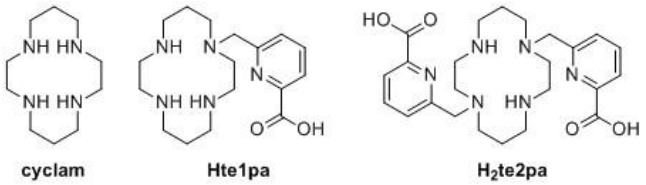


Figure 1. cyclam and derivatives chelators.

Financial supports by ERC-2014-StG-638712 aLzINK grant and by the "Société Chimique de France Midi-Pyrénées" grant are gratefully acknowledged.

References

1) P. Faller, *et al*, Inorg. Chem., **2013**, 52, 12193−12206.
2) C. Hureau, *Coord. Chem. Rev.*, **2012**, 256, 2164– 2174.
3) N. Xia, *et al, Mini Rev. Med. Chem.*, **2014**, 14, 271-281.
4) A. Robert, *et al, Acc. Chem. Res.*, **2015**, 48, 1332−1339.
5) A. Santos, *et al, Coord. Chem. Rev.*, **2016**, 327 –328, 287–303.
6) A. Conte-Daban, *et al, Dalton Trans.*, **2016**, 45, 15671-15678.
7) L. M. P. Lima, *et al, Inorg. Chem.*, **2012**, 51, 6916−6927.

Synthesis and Structure Characterization of Neuromelanin Analogues Suitable for Modeling Parkinson's Disease

Andrea Capucciati[a], Emanuele Ferrari[b], Enrico Monzani[a], Luigi Zecca[b], Luigi Casella[a]

[a] *Department of Chemistry, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy.*
[b] *Institute of Biomedical Technologies, National Research Council of Italy, Via Fratelli Cervi 93, 20090 Segrate (Milan), Italy.*

email : andrea.capucciati@gmail.com

Abstract

Neuromelanins (NMs) are dark pigments that accumulate during aging in various brain areas and are particularly concentrated in dopaminergic neurons of *substantia nigra* and in noradrenergic neurons of *locus coeruleus,* which selectively degenerate in Parkinson's Disease [1]. NMs are characterized by three types of organic moieties covalently linked to each other (melanin, protein, and lipid), and bind iron(III) and other metals (mainly copper and zinc) [2].

X-ray powder analysis of NMs showed the presence of a motif possibly due to fibrillar aggregates with typical cross-β structure. This observation suggests that the initial step of NM formation relies on the presence of fibrillar protein seeds. In order to understand if this motif is due to the presence of fibrillar protein in NM core, we have synthesized NM models containing a fibrillated protein.

The melanic portion of the conjugates has either eumelanic or mixed eumelanic/pheomelanic composition, the latter better simulating natural NMs. In addition, the conjugates contain dosable amounts of iron(III), mostly bound to the melanin component and associated in multinuclear clusters, as occurs in NMs.

The melanin-fibrils conjugates have been characterized with different techniques (NMR, LC-MS, CD, ICP-MS, EPR), which showed that upon melanization conjugates maintain the amyloid cross-β protein core as the only structurally organized element, similarly to human NMs.

Furthermore, the synthetic models are able to activate microglia cells in vitro through up-regulation of the typical pro-inflammatory genes, similarly to human NM[3]. These results suggest that melanin-protein conjugates can be used to study NM-induced neuroinflammation [4] and to conceive new *in vitro* and *in vivo* models of Parkinson's Disease.

References

[1] Zecca, L. *et al.* New melanic pigments in the human brain that accumulate in aging and block environmental toxic metals. *Proc. Natl. Acad. Sci. U. S. A.* **2008a,** 105**,** 17567–17572.

[2] Zucca, F. A. *et al.* Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease. *Prog. Neurobiol.* **2015**, doi:10.1016,j.pneurobio.2015.09.012.

[3] Zhang, W. *et al.* Neuromelanin Activates Microglia And Induces Degeneration Of Dopaminergic Neurons: Implications For Progression Of Parkinson's Disease. *Neurotox. Res*. **2011**, 19**,** 63–72.

[4] Emanuele Ferrari°, Andrea Capucciati° *et al*. Synthesis, Structure Characterization, and Evaluation in Microglia Culture of Neuromelanin Analogues Suitable for Modeling Parkinson's Disease. *ACS Chemical Neuroscience.* **2017**, 8 (3)

Complete conception of radiopharmaceuticals to target hepatocellular carcinoma metastases

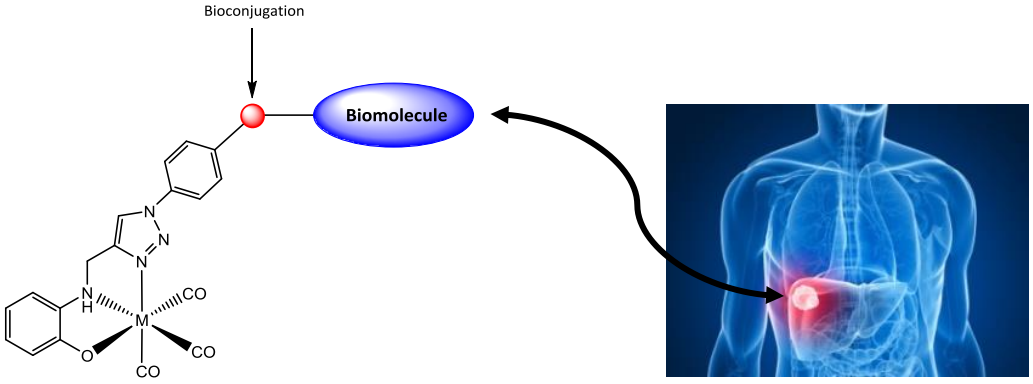
Romain Eychenne, [a] Jinhui Wang, [a] Pascal Loyer, [b] Nicolas Lepareur, [b, c] Eric Benoist [a]

[a] *Université de Toulouse III, UPS, Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR CNRS 5068, 118 Route de Narbonne, F-31062 Toulouse Cedex 9, France ;*
email : eychenne@chimie.ups-tlse.fr
[b] *INSERM UMR 1241 « Nutrition, Métabolismes et Cancer », F-35033, Rennes, France*
[c] *Centre Eugène Marquis, Nuclear Medicine Department, F-35042, Rennes, France*

Hepatocellular carcinoma (HCC) is one of the most important cancer in terms of mortality. By 2020, forecasts indicate a threefold increase of new HCC cases worldwide. Nowadays, the large majority of diagnosed cases are advanced and the only existing treatments showed some drawbacks (expensive costs, low efficiency…). Different alternatives have been developed, especially for intrahepatic metastasis (radioembolization). But when there is also an extrahepatic dissemination, as in many cases, very few possibilities are available.

That's precisely these advanced cases we would like to target using the recognition properties of somatostatin receptors, well-known to be largely overexpressed by this kind of tumor tissues. [1] For it, the aim is to develop a radiopharmaceutical. This agent consists of a three-part system including a biomolecule, a bifunctional chelating agent (BCA) and a radioactive isotope which delivers γ or β^- emission. To be efficient, this system must be stable *in vivo* in order to image and/or irradiate selectively the targeted tumour mass. [2]

In this communication, we report our first results related to each step of the development of the targeting radiopharmaceutical. From the synthesis of the chelating cavity, to the first in vitro studies, by way of all the bioconjugation work and the radiolabelling with ^{99m}Tc and ^{188}Re. [3]



M = ^{99m}Tc, ^{188}Re/Re

References:
[1] H. Reynaert, K. Rombouts, A. Vandermonde, D. Urbain, U. Kumar, P. Bioulac-Sage, M. Pinzani, J. Rosenbaum, A. Geerts, *Gut*, **2004**, 53, 1180-1189
[2] C.F. Ramogida, C. Orvig, *Chem. Commun.*, **2013**, 49, 4720-4739
[3] R. Eychenne, S. Guizani, J.H. Wang, C. Picard, N. Malek, P.L. Fabre, M. Wolff, B. Machura, N. Saffon, N. Lepareur, E. Benoist, *Eur. J. Inorg. Chem.*, **2017**, 1, 69-81.

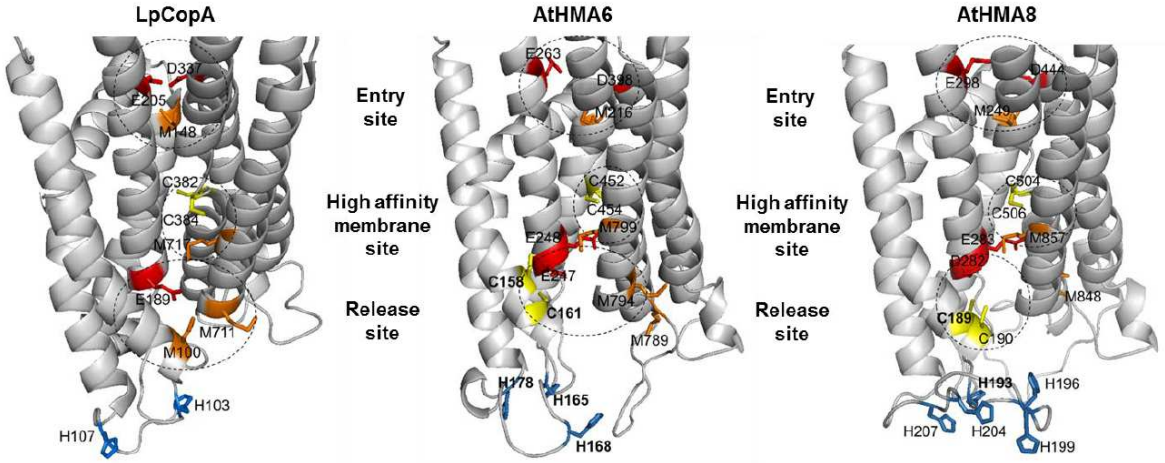
Identification of Two Conserved Residues Involved in Copper Release from Chloroplast P_{IB-1}-ATPases.

Emeline Sautron*,†,‡,§, Cécile Giustini*,†,‡,§, ThuyVan Dang*,†,‡,§, Serge Crouzy†,‡, , Norbert Rolland*,†,‡,§, Patrice Catty†,‡, ,1, Daphné Seigneurin-Berny*,†,‡,§,1

**CNRS, Laboratoire de Physiologie Cellulaire et Végétale, UMR 5168, 17 rue des Martyrs, F-38054 Grenoble, France. †Univ. Grenoble Alpes, F-38054 Grenoble, France. ‡CEA, DSV, BIG, F-38054 Grenoble, France. INRA, LPCV, UMR 1417, 17 rue des Martyrs, F-38054 Grenoble, France. CNRS, Laboratoire de Chimie et Biologie des Métaux, UMR 5249, 17 rue des Martyrs, F-38054 Grenoble, France.*

Abstract

Copper is an essential transition metal for living organisms. In the plant model *Arabidopsis thaliana*, half of the copper content is localized in the chloroplast, and as a cofactor of plastocyanin, copper is essential for photosynthesis. Within the chloroplast, copper delivery to plastocyanin involves two transporters of the PIB-1-ATPases subfamily: HMA6 at the chloroplast envelope and HMA8 in the thylakoid membranes. Both proteins are high affinity copper transporters but share distinct enzymatic properties. In the present work, the comparison of 140 sequences of PIB-1-ATPases revealed a conserved region unusually rich in histidine and cysteine residues in the TMA-L1 region of eukaryotic chloroplast copper ATPases. To evaluate the role of these residues, we mutated them in HMA6 and HMA8. Mutants of interest were selected from phenotypic tests in yeast and produced in *Lactococcus lactis* for further biochemical characterizations using phosphorylation assays from ATP and Pi. Combining functional and structural data, we highlight the importance of the cysteine and the first histidine of the CX3HX2H motif in the process of copper release from HMA6 and HMA8 and propose a copper pathway through the membrane domain of these transporters. Finally, our work suggests a more general role of the histidine residue in the transport of copper by PIB-1-ATPases.



Detection of beta-amyloid aggregates with fluorescent copper(II)-chelating peptides

Guillem Vázquez, [a] Ana B. Caballero, [a,b] Ernesto Nicolás, [a] Patrick Gamez [a,b,c]

[a] Departament de Química Inorgànica i Orgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona; [b] Institute of Nanoscience and Nanotechnology (IN2UB), Universitat de Barcelona; [c] Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona; email: guillem.vazquez@qi.ub.es

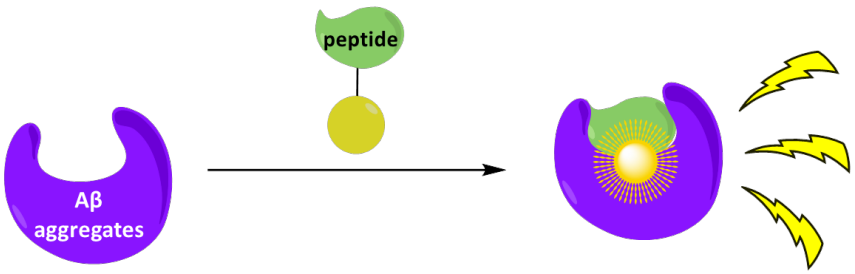
Abstract

There is an urgent necessity to improve our understanding of the processes involved in the development of Alzheimer's disease since the number of affected people is expected to greatly increase within the next years. Consequently, it will entail a huge socio-economical burden for the health-care system and families.

Accumulating evidence has highlighted the interaction of extracellular copper(II) ions with amyloid beta (Aβ) peptide, promoting its aggregation and generating oxidative stress. [1] Therefore, we have tested the capacity of different fluorescent peptides to ameliorate the production of Reactive Oxygen Species (ROS) and prevent the copper-induced formation of toxic Aβ oligomers. [2]

The development of early diagnostic techniques is also expected to be of paramount importance for efficiently treating the disease. In this line, solvatochromic fluorophores are a promising tool for the detection of amyloid deposits, which could thus be used as biomarkers of the pathology. Among such probes, the fluorophores of the dimethylaminonaphthalimide family possess excellent photophysical properties. [3]

In this communication, we present our studies on the interaction of two peptides encompassing a solvatochromic fluorescent probe with fibers of the Aβ(1-40) fragment. In the presence of these aggregates, the peptides exhibit an enhanced blue-shifted emission. Experiments with metal ions, which modulate the aggregation process and the nature of the species formed, were also conducted.



References

1) Gamez, P.; Caballero, A.B., Copper in Alzheimer's disease: Implications in amyloid aggregation and neurotoxicity, 2015, 5, 092503-1.
2) Caballero, A. B., Terol-Ordaz, L., Espargaró, A., Vázquez, G., Nicolás, E., Sabaté, R., Gamez, P., Histidine-Rich Oligopeptides To Lessen Copper-Mediated Amyloid-β Toxicity, 2016, 22, 7268.
3) Loving, G.; Imperiali, B., A Versatile Amino Acid Analogue of the Solvatochromic Fluorophore 4-*N,N*-Dimethylamino-1,8-naphthalimide: A Powerful Tool for the Study of Dynamic Protein Interactions, 2008, 130, 13630.

How the metal ions induce the different aggregation's pathway in the human and murine Amyloid-β peptide.

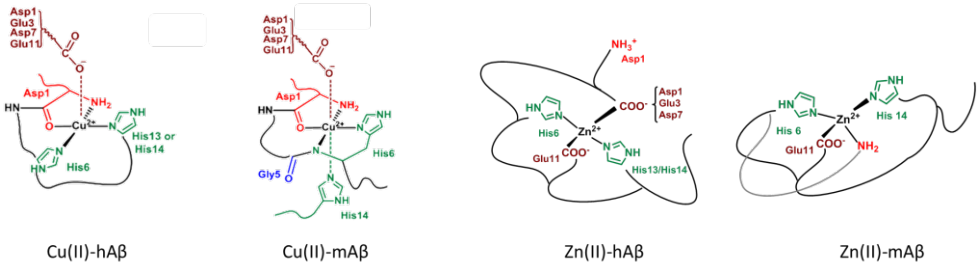
Valentina Borghesani,[a,b] Bruno Alies,[a,b,c] Stéphanie Sayen,[d] Emmanuel Guillon,[d] Peter Faller ,[e] Christelle Hureau[a,b]

a) CNRS, Laboratoire de Chimie de Coordination, 205 Route de Narbonne, 31400 Toulouse b) Université Toulouse III Paul Sabatier, 31077 Toulouse, France c) Current address: Université de Bordeaux, ChemBioPharm INSERM U1212 CNRS UMR 5320, Bordeaux, France d) CNRS Institut de Chimie Moléculaire de Reims (ICMR), Groupe de Chimie de Coordination, Université de Reims Champagne-Ardenne, France e) Institut de Chimie, Biometals and Biological Chemistry Université de Strasbourg Le Bel, 8eme etage, 4 rue B. Pascal 67081 Strasbourg, France. valentina.borghesani@lcc-toulouse.fr

Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disease, with no known cure. In the brain of Alzheimer's patients are present intracellular neurofibrillary tangles and extracellular senile plaques, consisting of insoluble fibrillar aggregates of the amyloid-β peptide (Aβ). The amyloid cascade hypothesis describes this aggregation; where monomeric Aβ aggregates first into oligomers and later into fibrils. In the presence of metal ions, this aggregation is modified.[1] Coordination of hAβ with Cu(II) generally favors the formation of oligomeric species, whereas Zn(II) generally induces the formation of fibrils of the peptide.

The *in vivo* studies are often performed on transgenic mice or rats that produce the human Aβ (hAβ) peptide in addition to their own peptide (mAβ). In fact, the not transgenic mice, who produce only the mAβ, do not show amyloid deposition.[2] Murine Aβ peptide differs from the human Aβ peptide by three point mutations (R5G, Y10F and H13R).



Cu(II)-hAβ Cu(II)-mAβ Zn(II)-hAβ Zn(II)-mAβ

Figure 1. Proposed binding modes of Cu(II) to hAβ and mAβ (major component at physiological pH) [2] and Zn(II) [3] coordination to hAβ and mAβ.

Here, after having determined the Zn(II) coordination site in the human and murine peptides, we want to evaluated how the different coordination sites of Zn(II) and Cu(II) in murine peptides versus the human Aβ impact on the amyloid-β peptide aggregation. The aggregation of the peptide is evaluated by ThT fluorescence and the morphology of the aggregates probes by AFM and TEM, while the different coordination is evaluated by XANES, 1H-NMR and determination of the affinity constant.

Financial support by ERC-2014-StG-638712 aLzINK grant is gratefully acknowledge.

References

1) Hureau, C., Coord. Chem. Rev., 2012, 256, 2164-2174.
2) Eury, H. et al.; Angew. Chem. Int. Ed., 2011, 50, 901-905.
3) Alies, B. et al.; Inorg. Chem. 2016, 10499-10509.

A tunable chimera of two Cu (II) binding peptides: expanding the two monohistidine canonical binding motifs.

Paulina Gonzalez[1,2], Bertrand Vileno[1], Karolina Bossak, Youssef El Khoury, Petra Hellwig, Wojciech Bal, Christelle Hureau[2], Peter Faller[1,2]

[1] Institut de Chimie, UMR 7177, CNRS-Université de Strasbourg, 4 rue Blaise Pascal, 67000, Strasbourg, France.

[2] University of Strasbourg Institute for Advanced Study (USIAS), Strasbourg, France

Peptides containing a multi-histidine sequence are characteristic Cu (II) chelator motifs of several proteins like serum albumin (DAHK), or the wound healing factor (GHK). The electronic properties of these two peptides differ considerably due to the histidine position. Nonetheless the properties of a consecutive dihistidine motif has not been fully explored. The work here presented focuses in the spectroscopic characterization and coordination tuning of a dihistidine motif. UV-Vis, circular dichroism, and EPR show that the chosen peptide sequence AHH, is able to combine the electronic properties of the two main types for Cu (II) coordination sphere (3N and 4N) by means of pH tuning. More over upon addition of PO_4^{-2} or imidazole to the system one can force the equilibrium towards the 3N type coordination sphere. The results obtained from the different spectra suggest that these properties result specifically from the vicinity of the histidines in the peptide. This could be useful for future applications for chelation involved therapies.

References

(1) Hureau, C; *Coord. Chem. Rev*., **2012**, 256, 2164-2174.

(2) Hureau, C; Faller, P. Peptides as biological shuttles for copper ions. USIAS research proposal. **2015**

(3) Hureau, C; Eury, H; Guillot, R; Bijani, S; Solari, P; Guillon, E; Faller, P; Dorlet, P. *Chem. Eur. J*. **2011**, 17, 10151 – 10160

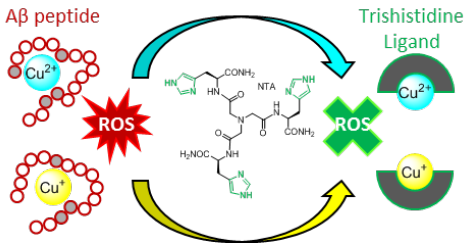
A trishistidine pseudopeptide with ability to remove both Cu(I) and Cu(II) from the Alzheimer's peptide and to stop the associated ROS formation

A. Conte-Daban,[b,c] B. Boff,[a] A. Candido Matias,[a,e] C. N. Montes Aparicio,[b,c] C. Gateau,[a] C. Lebrun,[a] G. Cerchiaro,[e] I. Kieffer,[f,g] S. Sayen,[d] E. Guillon,[d] C. Hureau[b,c] and P. Delangle[a]

[a] SyMMES (UMR 5819 - CEA, CNRS, UGA), CIBEST, CEA Grenoble, INAC, 17 rue des martyrs, 38 054 Grenoble, [b,c] CNRS, LCC (Laboratoire de Chimie de Coordination), 205 route de Narbonne,BP 44099 31077 Toulouse Cedex 4, University of Toulouse, UPS, INPT, 31077 Toulouse Cedex 4. [d]Institut de Chimie Moléculaire de Reims (ICMR, UMR CNRS 7312), Université de Reims Champagne-Ardenne, F-51687 Reims Cedex 2, France. [e]Center for Natural Sciences and Humanities, Federal University of ABC – UFABC, 09210-580, Santo André, SP, Brazil, [f]BM30B/FAME beamline, ESRF, F-38043 Grenoble cedex 9, [g]Observatoire des Sciences de l'Univers de Grenoble, UMS 832 CNRS Université Grenoble Alpes, F-38041 Grenoble, France

christelle.hureau@lcc-toulouse.fr

The pseudopeptide L, derived from a nitrilotriacetic acid scaffold and functionalized with three histidine moieties, is reminiscent of the amino acid side chains encountered in the Alzheimer's peptide (Aβ). Its synthesis and coordination properties for Cu(I) and Cu(II) are described. A combination of ESI-MS, potentiometry and EPR demonstrates that L efficiently complex Cu(II) in a square-planar geometry involving three imidazole nitrogen atoms and an amidate-Cu bond. By contrast, Cu(I) is coordinated in a tetrahedral environment as deduced from EXAFS. The redox behavior is irreversible and follows an ECEC mechanism in accordance with the very different environments of the two redox states of the Cu center. This is in line with the observed resistance of the Cu(I) complex to oxidation with oxygen and the Cu(II) complex reduction by ascorbate. The affinities of L for Cu(II) and Cu(I) at physiological pH are larger than that reported for the Aβ peptide. The removal of Cu from Aβ, whatever Cu oxidation state is directly demonstrated by EPR and XANES spectroscopies for Cu(II) and Cu(I), respectively. Therefore, due to its Cu coordination properties, the ligand L is able to target both redox states of Cu and redox silence them. This ligand thus represents a new strategy in the long route of finding new coordination concepts for fighting Alzheimer's disease.



Acknowledgments. Funding from the ERC (StG-638712) is warmly acknowledged.

References

1. C. Hureau, *Coord. Chem. Rev.*, 2012, **256**, 2164..
2. K. J. Barnham and A. I. Bush, *Chem. Soc. Rev.*, 2014, **43**, 6727.
3. M. A. Santos, K. Chand and S. Chaves, *Coord. Chem. Rev.*, 2016, **327-328**, 287.
4. A. M. Pujol, C. Gateau, C. Lebrun and P. Delangle, *J. Am. Chem. Soc.*, 2009, **131**, 6928
5. A. M. Pujol, M. Cuillel, A.-S. Jullien, C. Lebrun, D. Cassio, E. Mintz, C. Gateau and P. Delangle, *Angew. Chem. Int. Ed.*, 2012, **51**, 7445.
6. A. Conte-Daban, A. Day, P. Faller and C. Hureau, *Dalton Trans.*, 2016, **45**, 15671.
7. E. Atrian-Blasco, E. Cerrada, A. Conte-Daban, D. Testemale, P. Faller, M. Laguna and C. Hureau, *Metallomics*, 2015, **7**, 536.

Studies of selective copper removal from the Aβ peptide : towards therapeutic proof-of-concept to fight Alzheimer's disease

Sabater Laurent, [a,b] Hermann Petr,[c] and Hureau Christelle[a,b]

[a,b] *CNRS, LCC (Laboratoire de Chimie de Coordination), 205 route de Narbonne,BP 44099 31077 Toulouse Cedex 4, University of Toulouse, UPS, INPT, 31077 Toulouse Cedex 4. France and* [c] *Department of Inorganic Chemistry, Faculty of Science, Universita Karlova (Charles University), Hlavova 2030, 12840 Prague 2,Czech Republic.*

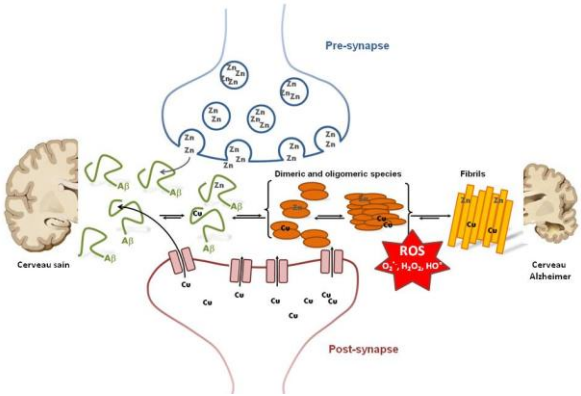
laurent.sabater@lcc-toulouse.fr

Abstract

The aetiology of Alzheimer's disease is linked to the aggregation of the amyloid-β (Aβ) peptide. Cu and Zn ions can bind to the Aβ peptide and modulate its aggregation. In addition, Cu can catalyze the production of Reactive Oxygen Species (ROS) from dioxygen and ascorbate, an highly concentrated physiological reductant.[1]

While the role of both ions in the Aβ aggregation process is still controversial, the higher toxicity of the redox competent Cu ions (compared to the redox inert Zn ions) in ROS production is acknowledged.[2,3] Cu ions are thus seen as the main therapeutic targets and we are currently developping several therapeutic strategies accordingly. In this poster communication we will detail the interference of Zn ions in Cu removal. Indeed because Zn ions are present in higher quantity than Cu ions in the synaptic cleft, they can interfere and even preclude detoxification of Cu by chelators.[4]

The results of such proof-of-concept studies contribute to delineate the importance of several key players and of their interactions in the aetiology of the disease at a molecular level. They are benchmark data for the development of new therapeutics against Alzheimer.



Acknowledgments. Funding from the ERC (StG-638712) is warmly acknowledged.

References

[1] C. Hureau C. Coord. Chem. Rev., **256**, 2164 (2012).
[2] Alies B, Sasaki I, Proux O, Sayen S., Guillon E, Faller P, Hureau C. Chem. Commun. **49**, 1214 (2013).
[3] Chassaing, S.; Collin, F.; Dorlet, P.; Gout, J.; Hureau, C.; Faller, P., Curr. Topics Med. Chem., **12**, 2573 (2012).
[4] Conte-Daban A, Day A, Faller P, Hureau C. Dalton Trans., **45**, 15671 (2016).

Neutral NHC-gold(I) complexes for antileishmanial activity

Alvaro FERNANDEZ ALVAREZ,[a,b] Chen Zhang,[a,b] Sandra Bourgeade Delmas,[d] Alexis Valentin,[c,d] Catherine Hemmert,*[a,b] Heinz Gornitzka*[a,b]

[a] *Laboratoire de Chimie de Coordination du CNRS, 205 Route de Narbonne, 31077 Toulouse Cedex 04, France.*

[b] *Université de Toulouse, UPS, INPT, F-31077 Toulouse Cedex 04, France.*

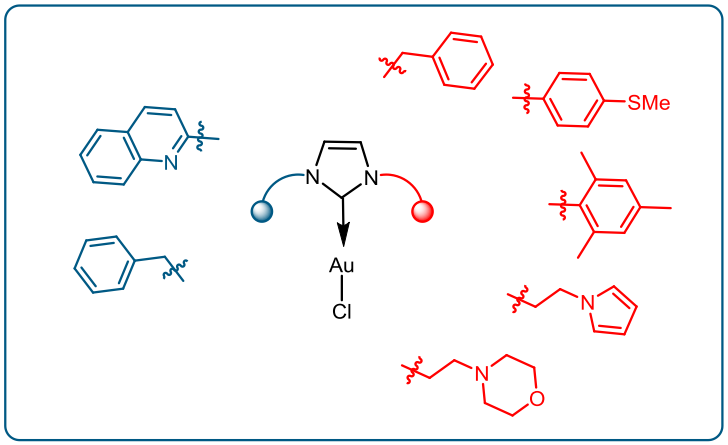
[c] *Université de Toulouse; UPS; UMR 152 Pharma-DEV; Université Toulouse 3; Faculté des sciences pharmaceutiques; 35 Chemin des maraîchers, F-31062 Toulouse cedex 9, France.*

[d] UMR 152 PHARMA-*DEV*, Université de Toulouse, IRD, UPS, France

email: alvaro.fernandez@lcc-toulouse.fr

Abstract

In the last years, gold organometallic complexes have been studied for several biomedical applications and within them, for their applications against *leishmania*. Mononuclear neutral gold(I) complexes containing functionalized *N*-heterocyclic carbenes (NHCs) have been synthesized, fully characterized by spectroscopic methods, and evaluated *in vitro* towards *Leishmania infantum*.



These complexes were tested against both, promastigote and amastigote stages of *Leishmania infantum*. Moreover, their cytotoxicity was studied on the murine macrophage cells J774A. All gold compounds show an important activity against *Leishmania infantum* and some of them also demonstrated a high selectivity index.

References

1) C. Hemmert, H. Gornitzka, *Dalton Trans*., 2016, 45, 440–447.
2) L. Boselli, M. Carraz, S. Mazeres, L. Paloque, G. Gonzalez, F. Benoit-Vical, A. Valentin, C. Hemmert, H. Gornitzka, *Organometallics*, 2015, 34, 1046−1055.
3) C. Hemmert, A. Valentin, H. Gornitzka, L. Paloque, *Eur. J. Med. Chem*., 2015, 94, 22-29.
4) Chen Zhang, Alvaro Fernandez Alvarez, Sandra Bougeade Delmas, Alexis Valentin, Catherine Hemmert, Heinz Gornitzka, *Eur. J. Med. Chem*., submitted.

New coordination compounds of bencimidazole and imidazole derivatives with possible citotoxic activity

Guillermo Durán-Solares, Silvia Flores-Leyva, Irvin Rodríguez-Virto, Mónica P. Aguilar-Herrera and Norah Barba-Behrens

[1]*Departamento de Química Inorgánica y Nuclear, División de Estudios de Posgrado, Facultad de Química, Av. Universidad 3000, Col. Universidad Nacional Autónoma de México, C. U., C.P. 04510, Coyoacán, México, Ciudad de México; email: dsg.chem@gmail.com*

Abstract

The pharmaceutical properties and synthetic intermediates of imidazole and benzimidazole derivatives have had an important relevance. The imidazole and benzimidazole motif is a fundamental part of the main structure of some molecules essential to life, i.e., the amino acid histidine and cobalamin respectively. These moieties can readily be modified by adequate substituents to influence their cytotoxic, antihelmintic, anti-inflammatory, antifungal and antibacterial activity. Otherwise, sulfone compounds may present a broad spectrum of bioactivities.[1] Lone pair···π interactions appear to be importance for the stabilization of biological macromolecules, as well as for the binding of inhibitors. Intra lone pair S=O···π interactions play an important role in the stabilization of biologically active complexes.[2] We have been interested in the investigation of coordination compounds with higher selectivity and less side effects than those anti-cancer drugs commercially available. Different coordination compounds were synthesized with benzimidazole and imidazole derivatives and metallic centers of cobalt(II), nickel(II), copper(II), zinc(II), ruthenium(II) and palladium(II). They were characterized by IR, UV–Vis–NIR, elemental analysis, mass spectrometry, magnetic susceptibility and X-ray diffraction analysis. For some compounds the cytotoxic activity was assessed through a human cancer cell-growth inhibitory assay, on carcinoma cell-lines HCT-15 (colon), HeLa (cervix-uterine) and MCF-7 (breast).

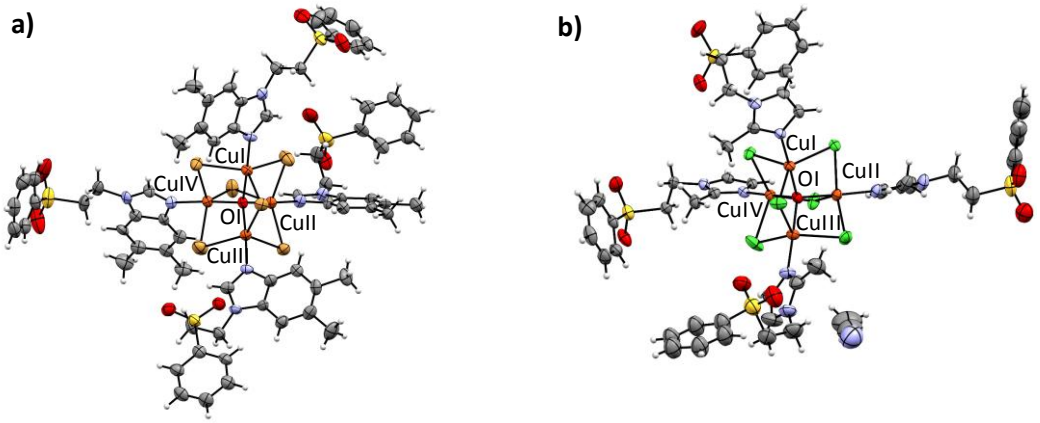


Figure 1. Tetranuclear copper(II) complexes a) $[Cu_4(\mu^4\text{-}O)(\mu^2\text{-}Br)_6(dmsbz)_4]$ b) $[Cu_4(\mu^4\text{-}O)(\mu^2\text{-}Cl)_6(2\text{-}mfsi)_4]\cdot MeCN$

Acknowledgments: To CONACyT's project CB2012-178851

References

1) Archana S., et al. *Current Pharmaceutical Design*, 22, 21,**2016**,3265-3301
2) Tiddo J. M., Patrick G., Jan R. *CrystEngComm*, **2008**, 10, 1501-1515

Improving cytotoxicity in cisplatin-resistant ovarian cancer cells with new hydroxy- and sulfonamide-azobenzeneplatinum(II) complexes

Katia G. Samper,[a,b] Sierra C. Marker,[b] Pau Bayón,[a] Samantha N. MacMillan,[b] IvanKeresztes,[b] Òscar Palacios,[a] Justin J. Wilson,[b]

[a]*Departament de Química, Facultat de Ciències, UniversitatAutònoma de Barcelona, Cerdanyoladel Vallès, E-08193 Barcelona, Spain ;* [b]*Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853 ; email : Katia.Ghannadzadeh@uab.cat*

Abstract

Since the serendipitous discovery of the anticancer activity of cisplatin, platinum-basedcomplexeshave becomesomeof the most important anticancer drugs used in the clinic. Although new promising complexes have been developed, they have not yet been able to match the efficacy ofcisplatin. However, the disadvantages of cisplatin, such as toxicside effects and drug resistance, require the development of novel platinum-drugs with improved tumour selectivity. Moreover, the interaction of platinum drugs with proteins may be related to theirtoxic sideeffects.

Photoactivatable Chemotherapy (PACT) is a potential alternative to the conventional platinum-drugs.The use of light to modulate biological activity of complexes is a goal that is actively being pursued by researchers worldwide. Although severalstudies show the different behavior of the activeand non-active complexes, the *in situ* activation by irradiation of the complex performed by the modification of the complexes' structure is still under study.(1)Azobenzenesare on such photoreactive groups that can be switched between E and Z isomers with UV-vis light. This configurational change can provokea strong modification of the azobenzene-metallic complexes formed. While azobenzenes isomerization is widely studied, the isomerization of Pt(II)complexes with azobenzene as ligand is a promising research direction that has not been pursued. Tuning the functionalization of suchazobenzene ligandsmay be crucial to modulate the reactivity of the drug.

Here we present three platinum-complexes bearing azobenzene-deriveds bidentate ligands.These complexes formsix-membered chelate ringswith theazobenzene ligands, which inhibitphotoisomerization. However, the anticancer activity of these complexes in wild-type (A2780) and cisplatin-resistant (A2780CP70) ovarian cancer cellsis reflected by IC_{50} values below 10 µM.They displaysimilar activity in both A2780 and A2780CP70 cell lines, indicating that they are not cross-resistant with cisplatin.The interaction of these platinum-complexes with some proteins is also evaluated by ESI-MS, showing different behaviour for each complex.(2)

Acknowledgments

The financial support received from Cornell University and MINECO-FEDER (Projects BIO2015-67358-C2-2-P and CTQ2015-70371-REDT) is acknowledged. K.G.S. acknowledges Departament de Química from UAB for PIF scholarship and UAB for the grant "short stay for research PIF-UAB 2016".

References

1)A. Presa, R.F. Brissos, A.B. Caballero, I. Borilovic, L. Korrodi-Gregório, R. Pérez-Tomás, O. Roubeau, P. Gamez, Photoswitching the cytotoxic properties of platinum(II) compounds, Angew. Chemie - Int. Ed. 2015, 54, 4561–4565.

2)K. G. Samper, S. C. Marker, P.Bayón, S. N. MacMillan, I.Keresztes, Ò. Palacios, J. J. Wilson, Anticancer Activity of Hydroxy- and Sulfonamide-Azobenzene Platinum(II) Complexes in Cisplatin-Resistant Ovarian Cancer Cells, JIB, 2017. (Submitted)

Synthesis and characterization of Ir(III) and Rh(III) bis-cyclometalated complexes. Study of their photophysical and cytotoxicity properties

Jairo FIDALGO, Begoña GARCIA, José M. LEAL, Natalia BUSTO and Gustavo ESPINO

Plaza Misael Bañuelos, 09001, BURGOS, Spain
University of Burgos, Burgos, Spain
e-mail: jfzorrilla@ubu.es

It is known that Ru(II), Ir(III) and Rh(III) bis-cyclometalated complexes show good chemical stability and photophysical properties, and can interact with proteins and DNA in order to act as luminiscent probes with potential anticancer activity. One example of this is their use in photodynamic therapy[1].

In this field, one of the pharmacological targets in the fight against cancer that has aroused interest in recent years is telomerase, whose enzymatic function can be inhibited by the stabilization of the telomeres. These regions of DNA (rich in guanine) may adopt G-quadruplex structural forms. Complexes of Ru(II) or Ir(III) with heteroaromatic ligands with a very extended planarity could recognize and stabilize G-quadruplex form by π-stacking interactions and may inhibit the enzymatic function of telomerases in tumor cells[2].

In this work, we present a series of cationic mono and di-nuclear Ir(III) and Rh(III) complexes with general formula $[M(ppy)_2(N\hat{}N)]X$ and $[(ppy)_2M(N\hat{}N)Ph(N\hat{}N)M(ppy)_2]$ where N^N = ligands derived from phenanthrolindione to spread the planarity of phenanthroline[3] (see structures). The complexes were fully characterised by NMR, IR, mass spectrometry and elemental analysis. Besides that, cytotoxic activity was evaluated by MTT assay and the interaction with DNA was studied by circular dichroism and absorbance spectrophotometry, differential scanning and isothermal titration calorimetry, agarose gel electrophoresis and viscosity measurements.

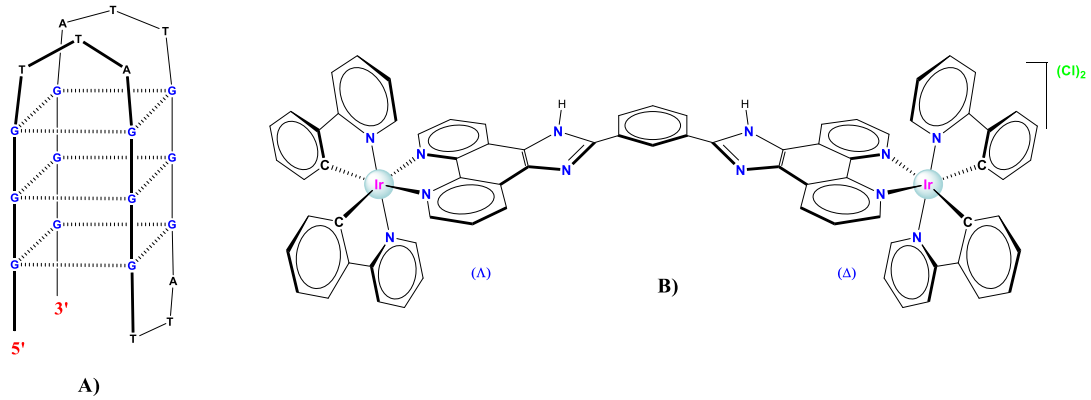


Figure 1. A) G-quadruplex structural form of a guanine-rich DNA region. **B)** Example of one Ir(III) di-nuclear complex that could interact with human antiparallel telomeric DNA sequences in their G-quadruplex form.

References

[1] Wen Lv, Zhang Zhang, Kenneth Yin Zhang, Huiran Yang, Shujuan Liu, Aqiang Xu, Song Guo, Qiang Zhao, and Wei Huang, *Angew. Chem. Int. Ed.*, **2016**, *55*, 9947 –9951

[2] Georgiades, S. N.; Abd Karim, N. H.; Suntharalingam, K.; Vilar, R., *Angew. Chem.-Int. Edit*. **2010**, 49, (24), 4020-4034.

[3] Qiong Wu, Kangdi Zheng, Siyan Liao, Yang Ding, Yangqiu Li, and Wenjie Mei, *Organometallics* **2016**, *35*, 317–326.

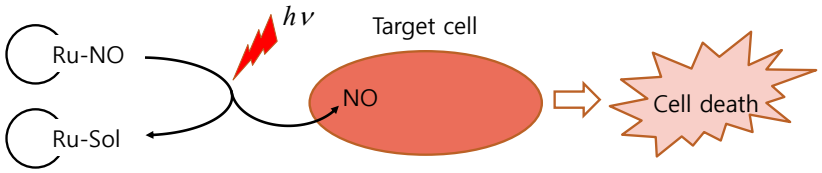
Synthesis and Characterization of NO-releasing Ruthenium Complexes for Photodaynamic Therapy Agent

Hong-In Lee, Minyeong Kim

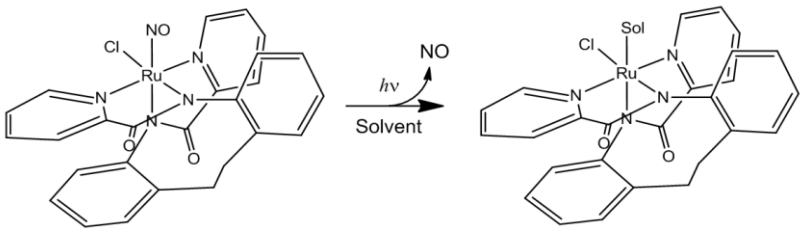
Department of Chemistry, Kyungpook National University, Daegu, Republic of Korea ; email : leehi@knu.ac.kr

Abstract

Since the discovery of Nitric Oxide (NO) as one of the major signal-transduction molecules in cells, there have been many attempts to devise acute NO-delivering systems for the purpose of developing disease therapies as well as studying cell functions.[1] Metal-nitrosyl complexes are often releasing NO by light activation. This ability can be adapted to killing cancer cells with high specificity because high concentration of NO in cells induces apoptosis. Ru-NO complexes have been studied for the usages of these photodynamic therapy (PDT) agents for a time.[2]



This research aims at developing Ru-NO complexes which absorb long wavelength visible light to release NO with high quantum yield. Previously, we have developed a new Ru-NO bis-pyridyl/biscarboxamide compound, [Ru(III)(ebpp)(Cl)(NO)], where H_2ebpp = *N*,*N*'-(ethylene-di-*p*-phenylene)bis(pyridine-2-carboxamide), to be tested as a model NO-releasing agent. Here, we present the strategies to develop visible-light sensitive NO-releasing Ru complexes with higher quantum yield. We introduce new series of ligands, salophen and naphophen, to develop Ru-NO complexes. In this poster, we present UV-VIS and EPR data which can be interpreted as that the diamagnetic [Ru-NO]^6 electronic state of the complex becomes low-spin Ru(III) (d^5, S=1⁄2) state upon losing NO by photoactivation.



References

1) Ignarro, L. J., *Nitric Oxide: Biology and Pathobiology*, Academic Press, San Diego, **2000**.
2) Fry, N. L. and Mascharak, *Accounts of Chemical research*, **2010**, 44, 289-298.

Influence of the geometry and structural conformation of tinidazole complexes on their biological properties.

Rodrigo Castro-Ramírez, [a] Israel Alfaro-Fuentes, [a] Julia Brumaghim, [b] Patrick Gámez, [c] Noráh Barba-Behrens, [a]

[a] Depto. Química Inorgánica, Facultad de Química, Universidad Nacional Autónoma de México, C.U., Coyoacán, México D. F., 04510, México. ; [b] Chemistry Department, Clemson University, Clemson, SC 29634-0973, U.S.A; [c] Departament de Química Inorgànica, Universitat de Barcelona, Martí i Franquès 1-11 Barcelona 08028, España;

email : rozurigokas@gmail.com

Abstract

Tinidazole (tnz), a 5-nitroimidazole derivative, is widely used as an anthelmintic agent due to the ability to generate reactive oxygen species [1]. In this work, complexes with tinidazole and transition metals found in biological systems, such as Co^{II}, Cu^{II} and Zn^{II}, are presented. X-ray structures of these compounds depict different geometries and novel weak interactions known as lone pair···π (Figure 1). These interactions appeared as a stabilizing factor in tnz complexes, allowing the assessment of their biological properties [2,3]. Initially, we studied how the anthelmintic activity of tnz could be modified by being coordinated to a metal ion. *In vitro* and *in vivo* (two different methodologies, i. e. intubation and bath) studies were performed to study the ability of tnz complexes to cure parasite-infected fish. The *in vivo* bath technique showed $[Cu(tnz)_2Br_2]$ to be the most effective with 95% of parasites eliminated at 8h of treatment [3]. Through electrochemical techniques we found Cu^{II} complexes to effectively damage plasmid DNA. Alongside with this, cytotoxic studies of these complexes showed good antineoplastic activity, with $[Cu(tnz)_2(\mu\text{-}Cl)Cl]_2$ being the most active and selective, as it affects cancerous cell more effectively. The results presented here showed how coordinating the tinidazole to different metal ions enhanced, modified or even allowed them to present a different biological activity as an antineoplastic agent.

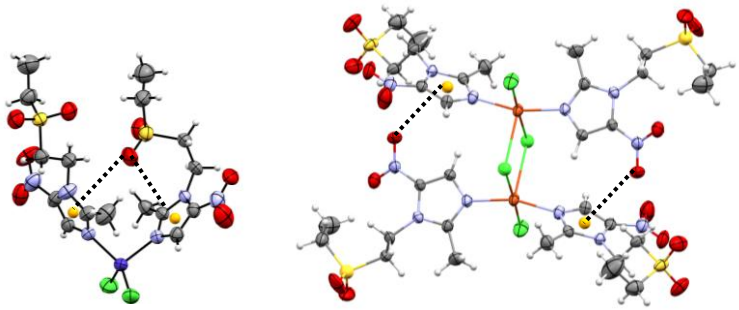


Figure 1. X-structures (50% ellipsoids) of (left) $[Co(tnz)_2Br_2]$
and (right) dinuclear $[Cu(tnz)_2(\mu\text{-}Cl)Cl]_2$ depicting intramolecular S=O···π contacts

We acknowledge CONACyT CB2012-178851 for the financial support.

References

1) G. Reysset. Anaerobe. 1996, 2, 59.
2) I. Alfaro-Fuentes, H. López-Sandoval, E. Mijangos, A. M. Duarte-Hernández, G. Rodríguez-López, M. I. Bernal-Uruchurtu; R. Contreras, Angelina Flores-Parra, N. Barba-Behrens, Polyhedron, 2014, 67, 373.
3) I. Alfaro-Fuentes, R. Castro-Ramírez, N. Ortiz-Pastrana, R. M. Medina-Guerrero, L. C. Soler-Jiménez, I. Martínez-Rodríguez, M. Betancourt-Lozano, L. Ibarra-Castro, N. Barba-Behrens, E. J. Fajer-Ávila, J. Inorg. Biochem. (submited).

Interaction of thiolato-bridged dinuclear arene ruthenium complexes with phospholipids and model membranes

Hedvika Primasová, Martina Vermathen, Julien Furrer

Departement für Chemie und Biochemie, Universität Bern, Freiestrasse 3, CH-3012 Bern, Switzerland;
email : hedvika.primasova@dcb.unibe.ch

Abstract

Thiolato-bridged dinuclear arene ruthenium complexes are highly cytotoxic against various cancer cell lines with IC_{50} values of up to 30 nM.[1] A recent *in vivo* study has demonstrated that these complexes have potential as anticancer drugs, as one complex significantly prolongs the survival of tumor-bearing mice.[2] Interestingly, these complexes are very stable under physiological conditions as well as acidic and basic conditions, and they are particularly inert toward substitution. Only sulfur containing biomolecules such as cysteine and glutathione undergo catalytic oxidation in their presence.[3]

Since many aspects of cellular uptake and of the tumor-inhibiting action displayed by these complexes are still largely unknown, we have studied the interactions of three trithiolatho complexes with different degrees of lipophilicity $[(\eta^6\text{-}p\text{-}MeC_6H4Pr^i)_2Ru_2(R^1)_2(R^2)]^+$ ($R^1 = SC_6H_4\text{-}m\text{-}Pr^i$:**1**; $R^2 = SC_6H_4\text{-}m\text{-}Pr^i$:**1**; $R^1 = SC_6H_4\text{-}p\text{-}OMe$:**2**; $R^2 = SC_6H_4\text{-}p\text{-}OH$:**2**; $R^1 = SCH_2C_6H_4\text{-}OMe$:**3**; $R^2 = SC_6H_4\text{-}p\text{-}OH$:**3**) and of one dithiolato complex $[(\eta^6\text{-}p\text{-}MeC_6H4Pr^i)_2Ru_2(SCH_2C_6H_5)_2Cl_2]$:**4** with lipid membrane models in form of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) vesicles, 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) micelles and sodium dodecyl sulfate (SDS) micelles by nuclear magnetic resonance (NMR) spectroscopy and other techniques. 1D ^{1}H NMR spectra, 2D ^{1}H diffusion ordered spectroscopy (DOSY) spectra and T2 (spin-spin) relaxation time measurements together with electrospray ionization mass spectrometry (ESI-MS) suggest noncovalent interaction between the vesicles and the three trithiolato complexes. As expected, the strength of the interaction with the vesicles parallels the lipophilicity of the complexes. The results with the dithiolato complex **4**, on the other hand, suggest that none or only very weak interaction takes place. **1** was further studied with DOPC in presence of the lanthanide shift reagent $PrCl_3$ for estimating if the complex remains at the vesicle surface, is inserted between the fatty acid chains or is localized inside the DOPC vesicle.

References

1) Furrer, J., Süss-Fink, G. Thiolato-bridged dinuclear arene ruthenium complexes and their potential as anticancer drugs. Coord. Chem. Rev. **2016**, *309*, 36-50.

2) Tomšík, P.; Muthná, D.; Řezáčová, M.; Mičuda, S.; Ćmielová, J.; Hroch, M.; Endlicher, R.; Červinková, Z.; Rudolf, E.; Hann, S.; et al. [(P-MeC6H4Pri)2Ru2(SC6H4-P-But)3]Cl (Diruthenium-1), a Dinuclear Arene Ruthenium Compound with Very High Anticancer Activity: An in Vitro and in Vivo Study. *J. Organomet. Chem.* **2015**, *782*, 42–51.

3) Giannini, F.; Süss-Fink, G.; Furrer, J. Efficient Oxidation of Cysteine and Glutathione Catalyzed by a Dinuclear Areneruthenium Trithiolato Anticancer Complex. *Inorg. Chem.* **2011**, *50* (21), 10552–10554.

Photodynamic Therapy in Iridium(III) Complexes bearing Hydroxyphenylbenzazole Ligands

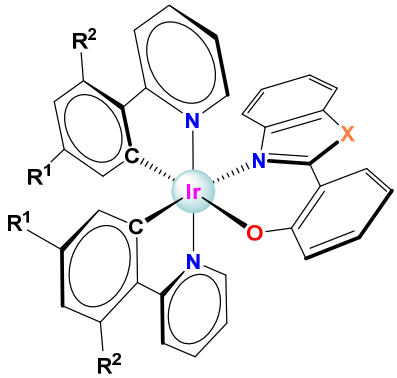
M. Martínez-Alonso,[a] G. Espino,[a] N. Busto,[a] M.C. Carrión,[b] J. V. Cuevas,[a] A. Carbayo,[a] B. García,[a] F. A. Jalón,[b] B. R. Manzano,[b] A. M. Rodríguez.[b]

[a]Department of Chemistry, Faculty of Science, University of Burgos, Plaza Misael Bañuelos s/n, 09001, Burgos, Spain,[b]Department of Inorganic, Organic Chemistry and Biochemistry, Faculty of Chemistry, University of Castilla-La Mancha, Avda. Camilo J. Cela 10, 13071 Ciudad Real, Spain; email: mmalonso@ubu.es

Abstract

The O_2-sensitive photoluminescence of bis-cyclometalated iridium(III) complexes is widely known.[1–3] Molecular oxygen ($^3O_2$) is a potent quencher of phosphorescent Ir(III) complexes, which undergo a deactivation of their excited state through an energy transfer (ET) process. This process simultaneously turns molecular oxygen into singlet oxygen ($^1O_2$), an extremely reactive species, able to oxidize other biomolecules. Photodynamic therapy (PDT) uses the activation of singlet oxygen to cause cell death through oxidation of different biotargets. The aim of this new technique is to generate cytotoxic species *in situ*, such as $^1O_2$, after irradiation of a photosensitizer with a selected wavelength.

In this work, we present the synthesis and characterization of six neutral Ir(III) bis-cyclometalated complexes bearing 2-phenylpyridinate as the cyclometalating ligand and hydroxyphenyl-benzazolate O^N donors as ancillary ligands. In addition, the catalytic activity of these derivatives was tested in the photooxidation of thioanisole with UV and blue lights. All of them behaved as efficient photosensitizers of $^3O_2$ but exhibited C^N and O^N depending catalytic effects. The cytotoxic activity of the complexes was also tested in the SW480 cell line, and the phototoxic index (PI = ratio of its IC_{50} in the dark to its IC_{50} upon light irradiation) was determined. One of the complexes displayed a PI above 200, which highlights the complex as a promising drug for cancer therapy.



[1a]: $R^1 = R^2 =$ H, C^N = **ppy**; X = NH, O^N = **hPhbim**
[2a]: R^1 = Me, R^2 = H, C^N = **tpy**; X = NH, O^N = **hPhbim**
[3a]: $R^1 = R^2$ = F, C^N = **dfppy**; X = NH, O^N = **hPhbim**
[1b]: $R^1 = R^2 =$ H, C^N = **ppy**; X = S, O^N = **hPhbtz**
[2b]: R^1 = Me, R^2 = H, C^N = **tpy**; X = S, O^N = **hPhbtz**
[3b]: $R^1 = R^2$ = F, C^N = **dfppy**; X = S, O^N = **hPhbtz**

References

(1) Jiang, X.; Peng, J.; Wang, J.; Guo, X.; Zhao, D.; Ma, Y. *ACS Appl. Mater. Interfaces* **2016**, *8*, 3591–3600.
(2) You, Y.; Nam, W. *Chem. Soc. Rev.* **2012**, *41*, 7061–7084.
(3) Nakagawa, A.; Hisamatsu, Y.; Moromizato, S.; Kohno, M.; Aoki, S. *Inorg. Chem.* **2014**, *53*, 409–422.

Iridium(III) complexes bearing new N-Benzyl-N,N-Dipyridylamine ligands: Properties and application in photodynamic therapy.

A. Ruiz-Riaguas,[a] M. Vaquero,[a] J. Fidalgo,[a] G. Espino,[a] A. Carbayo,[a] B. García,[a] N. Busto,[a] F.A. Jalón,[b] B. R. Manzano.[b]

[a] *Department of Chemistry, Faculty of Sciences, University of Burgos, Plaza Misael Bañuelos s/n, 09001, Burgos, Spain;* [b] *Department of Inorganic, Organic Chemistry and Biochemistry, Faculty of Chemistry, University of Castilla-La Mancha, Avda. Camilo J. Cela 10, 13071 Ciudad Real, Spain.; email : mvaquero@ubu.es*

Abstract

Iridium (III) complexes with phenylpyridinate and N-unsubstituted N,N-dipyridylamine ligands (as ancillary ligands) have been described and applied as photoredox catalysts.[1] Inspired by this studies, a new family of N-benzyl-N,N-dipyridylamine ligands have been synthesized and characterized, and cationic Iridium(III) complexes with phenylpyridinate, as cyclometalated ligand, were prepared with the aim to be applied in photodynamic therapy (PDT) (see Fig. 1). PDT uses the activation of singlet oxygen to cause cell death through oxidation of different biotargets. The aim of this new technique is to generate cytotoxic species *in situ*, such as $^1O_2$, after irradiation of a photosensitizer with a selected wavelength.[2]

Photophysical properties of the new Ir(III) complexes have been studied, as well as the ability of the new derivatives as O_2 photo-sensitizers; being the photooxidation of thioanisole with ultraviolet light the benchmark reaction for checking the catalytic activity of Ir(III) complexes.

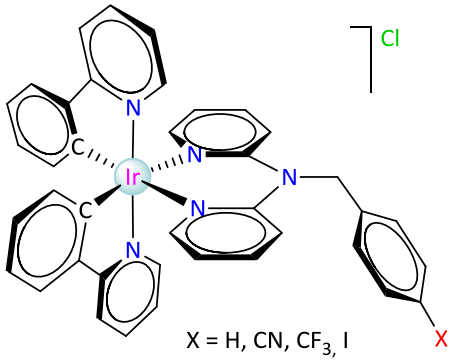


Fig. 1. Molecular structures of the synthesized bis-cyclometalated Ir(III) complexes.

References

1) Sauvageot, E.; Marion, R.; Sguerra, F.; Grimault, A.; Daniellou, R.; Hamel, M.; Gaillard, S.; Renaud, J.-L., *Org. Chem. Front*., **2014**, *1*, 639-644.

2) a) You, Y.; Nam, W. *Chem. Soc. Rev.* **2012**, *41*, 7061–7084; b) Jiang, X.; Zhu, N.; Zhao, D.; Ma, Y., *Sci. China Chem*. **2016**, *59*, 40-52.

Unconventional Platinum(IV) Complexes as Anticancer Agents

Krishant M. Deo,[a] Benjamin J. Pages,[a] Paul Wormell,[a] Jack K Clegg,[b] Yingjie Zhang,[c] Feng Li,[a] Gang Zheng,[a] Jennette Sakoff,[d] Jayne Gilbert[d] and Janice R. Aldrich-Wright[a]

[a]*Nanoscale Organisation and Dynamics Group, School of Science and Health, Western Sydney University, Australia*

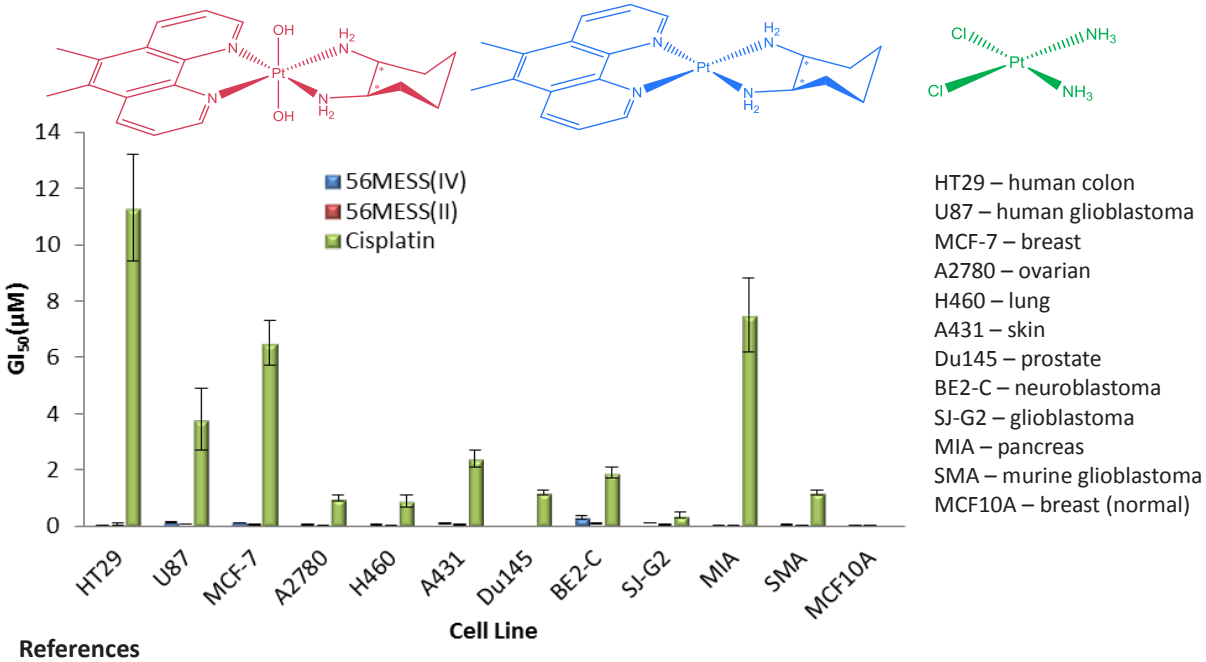
[b]*School of Chemistry and Molecular Biosciences, The University of Queensland, Australia;*

[c]*Australian Nuclear Science and Technology Organisation, Australia*

[d]*Calvary Mater Newcastle, Australia ; email: K.Deo@westernsydney.edu.au*

Abstract

Clinically used platinum(II) anticancer drugs are limited in their efficacy due to acquired and intrinsic resistance and severe systemic toxicities.[1] To overcome these issues, we have recently developed platinum(IV) di-hydroxo compounds with cytotoxic potential. Each was derived from the active platinum(II) complexes and are composed of a heterocyclic ligand (H_L) and ancillary ligand (A_L) in the form $[Pt(H_L)(A_L)(OH)_2]^{2+}$.[2] Several crystal structures have been obtained during the synthesis. We have confirmed that the activity previously seen with the platinum(II) species is demonstrated with the analogous platinum(IV) complex.[3] The reduction kinetics and half-lives of the platinum(IV) complexes were also determined.



References

1) Wang, X, Guo, Z, The Role of Sulfur in Platinum Anticancer Chemotherapy, *Anti-Cancer Agents Med. Chem.* 2007, 7, 19-34.
2) N. J. Wheate, R. I. Taleb, A. M. Krause-Heuer, R. L. Cook, S. Wang, V. J. Higgins and J. R. Aldrich-Wright, Novel platinum(II)-based anticancer complexes and molecular hosts as their drug delivery vehicles, *Dalton Trans.*, 2007, 5055.
3) F. J. Macias, K. M. Deo, B. J. Pages, P. Wormell, J. K. Clegg, Y. Zhang, F. Li, G. Zheng, J. Sakoff, J. Gilbert and J. R. Aldrich-Wright, Synthesis and Analysis of the Structure, Diffusion and Cytotoxicity of Heterocyclic Platinum(IV) Complexes, Chem. Eur. J., 2015, 21, 16990-17001.
4) B. W. J. Harper, A. Friedman-Ezra, R. Sirota, E. Petruzzella, J. R. Aldrich-Wright, D. Gibson, Probing the Interactions of Cytotoxic [Pt(1*S*,2*S*-DACH)(5,6-dimethyl-1,10-phenanthroline)] and Its Pt^{IV} Derivatives with Human Serum, *ChemMedChem*, 2017, 12, 510-519.

Investigation of Structure Activity Relationships of new Rhodium(I) *N*-Heterocyclic Carbene Complexes as Possible Anticancer Drugs

Jessica Wölker,[a,b] Ingo Ott[a,b]

[a] *Institute of Medicinal and Pharmaceutical Chemistry, Technische Universität Braunschweig, Beethovenstrasse 55, 38106 Braunschweig, Germany,* [b] *Center of Pharmaceutical Engineering (PVZ), Technische Universität Braunschweig, Franz-Liszt Strasse 35a, 38106 Braunschweig, Germany;*
email : j.woelker@tu-braunschweig.de

Abstract

Rhodium complexes with *N*-heterocyclic carbene (NHC) ligands have been generally used in catalytic chemistry. Although the most extensively investigated derivatives of this class of compounds contain gold(I) and silver(I) metal ions, rhodium(I) NHC complexes with 1,5-cyclooctadiene (COD) and a halido ligand (X, Figure 1, left) have also shown a high potential as antitumor agents.[1,2] The complexes of the type Rh(I)(NHC)(COD)Cl trigger antiproliferative effects against MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cells and inhibit the enzyme thioredoxin reductase (TrxR)[1,2] which is overexpressed in tumor cells. For complexes with iodido ligands (X = I) higher biological activity in comparison to their chlorido (X = Cl) counterparts were confirmed.[2] Rhodium complexes were also proven to interact with DNA.[3,4]

Figure shows the newly synthesized rhodium complexes with a benzimidazole backbone or a halogen-containing phenylimidazole backbone. These phenylimidazole ligands in combination with gold(I) have demonstrated a better activity against different tumor cell lines than the benzimidazole gold(I) complexes.[5]

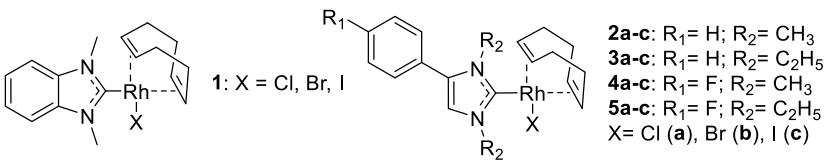


Figure: new series of rhodium(I) NHC complexes. left: Rh(I)(NHC)(COD)X with benzimidazole backbone; right: Rh(I)(NHC)(COD)X with phenylimidazole backbone.

Chemical and biological properties of the new complexes with variations in the X ligand and the backbone will provide new insights on the structure activity relationships (SARs).

Acknowledgments

The financial support within a Georg Christoph Lichtenberg fellowship by the State of Lower Saxony (graduate program µ-Props, "Processing of poorly soluble drugs at small scale") is gratefully acknowledged.

References

1) Oehninger, L.; Küster, L.-N.; Schmidt, C. et al., A Chemical–Biological Evaluation of Rhodium (I) N-Heterocyclic Carbene Complexes as Prospective Anticancer Drugs. Chem. Eur. J. (2013), 19, 17871 – 17880.
2) Oehninger, L.; Spreckelmeyer, S.; Holenya, P. et al., Rhodium (I) N-heterocyclic carbene bioorganometallics as in vitro antiproliferative agents with distinct effects on cellular signaling. J. Med. Chem. (2015), 58, 9591–9600.
3) McConnell, J. R.; Rananaware, D. P.; Ramsey, D. M.et al., A potential rhodium cancer therapy - Studies of a cytotoxic organorhodium (I) complex that binds DNA. Bioorg. Med. Chem. Lett. (2013), 23, 2527–2531.
4) Kielkopf, C. L.; Erkkila, K. E.; Hudson, B. P. et al., Structure of a photoactive rhodium complex intercalated into DNA. Nature Struct. Biol. (2000), 7, 117-121.
5) Schmidt, C.; Karge, B.; Misgeld, R. et al., Gold (I) NHC Complexes - Antiproliferative Activity, Cellular Uptake, Inhibition of Mammalian and Bacterial Thioredoxin Reductases, and Gram-Positive Directed Antibacterial Effects. Chem. Eur. J. (2017), 8, 1869–1880.

Cationic Gold(I) bisNHC Complexes for Anticancer Application

Chen Zhang,[a,b] Catherine Hemmert,*[a,b] Heinz Gornitzka,*[a,b]

Olivier Cuvillier[c,d], Raymond Wai-Yin Sun[e,f]

[a] *CNRS, Laboratoire de Chimie de Coordination, 205 Route de Narbonne, 31077 Toulouse Cedex 04, France*

[b] *Université de Toulouse, UPS, INPT, F-31077 Toulouse Cedex 04, France*

[c] *CNRS, Institut de Pharmacologie et de Biologie Structurale, Toulouse, France*

[d] *Université de Toulouse, UPS, IPBS, Toulouse, France*

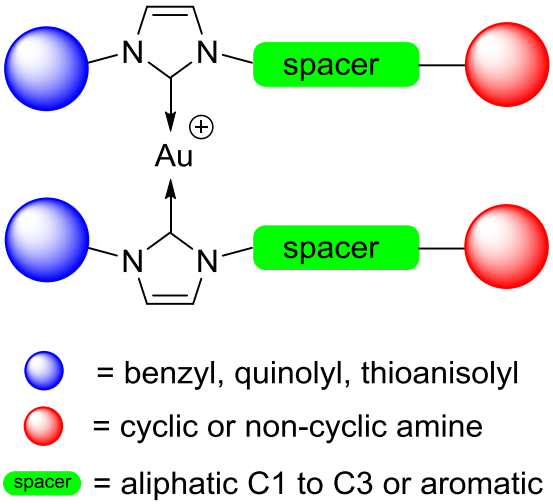
[e] *Department of Chemistry and Key Laboratory for Preparation and Application of Ordered Structural Materials of Guangdong Province, Shantou University, 243 Da Xue Road, Shantou, Guangdong 515063, PR China*

[f] *Guangzhou Lee & Man Technology Company Limited, Guangzhou, P. R. China*

email: chen.zhang@lcc-toulouse.fr

Abstract

Gold-*N*-heterocyclic carbenes (NHCs) complexes have shown interesting properties for biomedical applications. New cationic gold(I)-bisNHC complexes with nitrogen-containing side arms have been synthesized and fully characterized. These complexes have been investigated *in vitro* on a representative panel of cancer cell lines. The results show interesting cytotoxic activities.



References

1) L. Boselli, I. Ader, M. Carraz, C. Hemmert, O. Cuvillier, H. Gornitzka, *Eur. J. Med. Chem.*, 2014, 85, 87-94.
2) L. Boselli, M. Carraz, S. Mazère, L. Paloque, G. Gonzalez, F. Benoit-Vical, A. Valentin, C. Hemmert, H. Gornitzka, *Organometallics*, 2015, 34, 1046-1055.

Antiplasmodial activities of functionalized N-heterocyclic carbenes gold(I) complexes

Catherine Hemmert, *[a,b] Arba Pramundita Ramadani, [a,b] Luca Boselli, [a,b] Lucie Paloque, [a,b] Heinz Gornitzka, *[a,b] Françoise Benoit-Vical, [a,b]

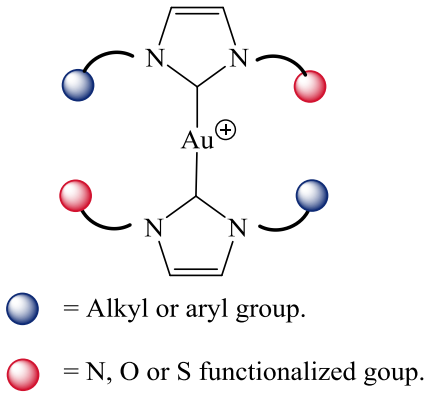
[a] *CNRS, LCC (Laboratoire de Chimie de Coordination), 205 route de Narbonne, BP 44099, F-31077 Toulouse Cedex 4, France,* [b] *Université de Toulouse, UPS, INPT, F-31077 Toulouse Cedex 4, France;*

email : Catherine.Hemmert@lcc-toulouse.fr

Abstract

Malaria is an infectious disease caused by the parasite *Plasmodium* and transmitted to humans by bite of a female mosquito. 3.2 Billion people are at risk with 214 million cases of malaria and 438 000 deaths in 2015. Recommendations of the WHO as first-line treatment for *Plasmodium falciparum* are the use of ACTs (Artemisinin-based Combination Therapies) which combine artemisinin derivatives with a partner drug. However, ART resistance recently emerged in the entire Greater Mekong region and ACTs resistance appeared in some parts of Cambodia and Thailand. In this context, new drugs with alternative chemical structures are urgently needed.

Mononuclear cationic, anionic or neutral gold(I) complexes, including N-, O- or S-functionalized *N*-heterocyclic carbene (NHC) ligands, were tested *in vitro* for their antimalarial potency against chloroquine-resistant *P. falciparum* strains. The pharmaco-modulation of the key substituents on the NHC ligands, allowed to obtain gold-based antiplasmodial agents with promising activity and selectivity and highlighted the interest for gold cationic compounds which can be considered as a good scaffold for the optimisation of a new antimalarial chemistry.



References

1) C. Hemmert, A. Fabié, A. Fabre, F. Benoit-Vical, H. Gornitzk, Eur. J. Med. Chem., 2013, 60, 64-75.

2) C. Hemmert, A. Pramundita Ramadani, L. Boselli, A Fernández Álvarez, L. Paloque, J.M. Augereau, H. Gornitzka, F. Benoit-Vical, Bioorg. Med Chem., 2016, 24, 3075-3082.

Palladium(II) complexes as potential anticancer agents: cytotoxicity, apoptosis, cell-cycle and topoisomerase II inhibition

[a] Fillipe V. Rocha, [a] Mauro A. Lima, [b] Renata N. Goto, [b] Andrea M. Leopoldino, [c] Adriano B. de Oliveira, [d] Saulo S. Garrido, [d] Renan L. de Farias, [d] Adelino V. G. Netto.

[a] Department of Chemistry, Federal University of São Carlos, São Carlos, SP CEP 13.565-905, Brazil ; [b] Department of Clinical Analyses, Toxicology and Food Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil; [c] Federal University of Sergipe, UFS, São Cristovão, SE, Brazil; [d] Chemistry Institute, Sao Paulo State University, UNESP, Campus Araraquara, Araraquara, SP 14800-900, Brazil; email : fillipe@ufscar.br

Abstract

DNA-Topoisomerase II (topoII) is an enzyme that participates of several nuclear processes as repair and replication of DNA. Besides, their expression in tumour cells is greater than normal cells[1]. Due these facts topo II is considered to be the primary pharmacological target for some of the most active drugs currently available for the treatment of human malignancies[2].

Thiosemicarbazones (TSC) are compounds that have been studied for their anticancer activity[3]. Recently Huang *et. all*[4] showed the capacity of thiosemicarbazones in inhibit the action of topo II. Furthermore, when the TSC is coordinated to the metal ion, in general, increases the activity or contributes to decrease the side effects[3]. Some examples in the literature showed the relationship between the inhibition of action of the enzyme and the cytotoxicity of square planar metal compounds[5,6].

Therefore, in this work we design twelve new palladium (II) compounds bearing thiosemicarbazones and triphenylphosphine as ligands(Figure 1). The compounds present few alterations in their structures to determine which parts are important to the cytotoxicity and enzyme interaction. Palladium(II) complexes were fully characterized by elemental analysis, mass spectrometry, NMR ^{1}H, ^{13}C and ^{31}P spectroscopy, IR spectroscopy and X-ray diffraction.

Cytotoxicity of TSC ligands and the new complexes were evaluated against the cell line, MCF-7 (human breast cancer) and their cytotoxicity were compared with cisplatin. All the compounds showed better results than standard drug since 2-fold to 25-fold more cytotoxics. The two most promising compounds (**2** and **8**) were evaluated against another two cell lines Cal27 (oral adenosquamous carcinoma) and HEK293 (Human Embryonic Kidney 293 cells) to investigated their selectivity. Complex **8** presented a greater value of selectivity index compared to **2** and cisplatin, 7.4, 1,3 and 0.53, respectively. Continuing the toxicity trials the capacity of the two compounds in performing hemolisys was investigated, only the compound **2** demonstrated lisys of erythrocytes, 0,9, 3,3, 7,5 and 24% in the concentrations of 3,12, 6,25, 12,5 e 25 µM. Ultimately complex **8** was tested against macrophages cells with IC_{50} value of 5.73 µM, also larger than to cancer cells.

Parp cleavage assay was performed to understand the pathway of cell death. Compound 8 realized the Parp cleavage in the tumor cells (CAL27) but not in the normal cells (HEK293) in the IC50 value. All the complexes inhibit the action of topoisomerase II in the range of 3.25-25 µM, comparable to the standard drugs doxorubicin (5 µM) and etoposide (32 µM)[7]. In general, compounds that interact with topoisomerase II performed a cell-cycle arrest in phase S. A similar result was found to the complex 8, when the percentage of the cells increased 20% in the S phase to

Comparative solution equilibrium studies of antitumor α-*N*-pyridyl thiosemicarbazones and their Cu(II) complexes: effect of methyl substitution

Orsolya Dömötör,[a,b] Nóra V. May,[c] Christian R. Kowol,[d,e] Bernhard K. Keppler,[d,e] Éva A. Enyedy,[a]

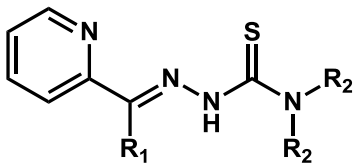
[a)] Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7. H-6720 Szeged, Hungary ; [b)] MTA-SZTE Bioinorganic Chemistry Research Group, Dóm tér 7. H-6720 Szeged, Hungary ; [c)] Research Centre for Natural Sciences Hungarian Academy of Sciences, Magyar tudósok körútja 2, H-1117 Budapest, Hungary ; [d)] Institute of Inorganic Chemistry, University of Vienna, Währinger Str. 42. A-1090 Vienna, Austria ;[e)] Research Cluster "Translational Cancer Therapy Research", University of Vienna and Medical University of Vienna, Vienna, Austria; email : domotor.o@chem.u-szeged.hu

Abstract

Thiosemicarbazones (TSCs), especially α-*N*-pyridyl derivatives are extensively investigated as they exhibit significant anticancer activity [1]. Triapine is the most prominent representative of this type of ligands undergoing phase I/II clinical trials acting as effective inhibitor of the ribonucleotide reductase enzyme [1].

N-terminally dimethylated derivatives are more cytotoxic among TSCs. Complex formation with transition metal ions such as Cu(II) Ni(II), Pt(II) or Pd(II) often results in compounds with even higher cytotoxicity than that of the metal-free ligands [2]. Characterizations of these TSC complexes are generally performed in solid phase, although knowledge of the actual chemical forms in the aqueous solutions strongly contributes to the structure-stability-activity relationship studies.

Herein we report the aqueous stability, isomer distribution in different solvents, fluorescence properties, lipophilicity and Cu(II) complexation of 2-formylpyridine TSC (FTSC) and its various methylated derivatives. These studies were performed using pH-potentiometry, UV-Vis spectrophotometry, spectrofluorometry, ^{1}H-NMR and EPR spectroscopy.



ligand	R_1	R_2
FTSC	H	H
PTSC	H	CH_3
AcFTSC	CH_3	H
AcPTSC	CH_3	CH_3

Acknowledgement: OTKA PD103905, GINOP-2.3.2-15-2016-00038, TÉT_15-1-2016-0024.

References

1) J. Kolesar, R.C. Brundage, M. et al., Cancer Chemother. Pharmacol. 67 (2011) 393–400.
2) A. Dobrova, S. Platzer, F. Bacher, M.N.M. Milunovic, A. Dobrov, G. Spengler, É.A. Enyedy, G. Novitchi, V.B. Arion, Dalton Trans. 45 (2016) 13427-13439.

Ruthenacarborane Complexes as Novel Highly Selective Cytotoxic Agents

Marta Gozzi,[a] Dijana Drača,[b] Danijela Maksimović-Ivanić,[b] Sanja Mijatović,[b] Evamarie Hey-Hawkins[a]

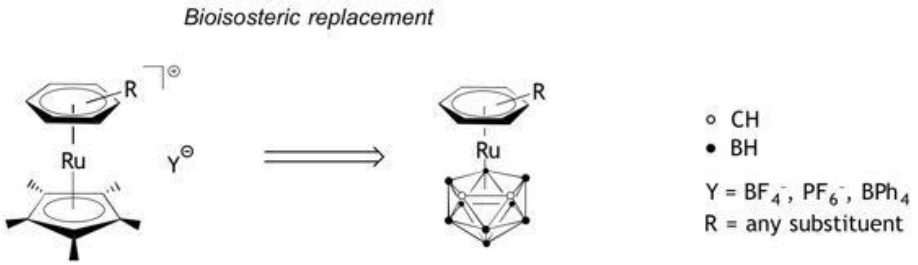
[a] Universität Leipzig, Faculty of Chemistry and Mineralogy, Institute of Inorganic Chemistry, Johannisallee 29, 04103 Leipzig, Germany; [b] University of Belgrade, Institute for Biological Research "Sinisa Stanković", Bul. despota Stefana 142, 11060 Belgrade, Serbia; email: marta.gozzi@studserv.uni-leipzig.de

Abstract

The bioisosteric replacement strategy is a widely used approach in drug design, as a possibility to overcome certain problems associated with an effective therapeutic application of the drug, e.g., metabolic instability and long-term instability in the biological medium.[1] In the medicinal chemistry of polyhedral boranes, namely dicarba-*closo*-dodecaboranes(12) and dicarba-*nido*- undecaborates(–1), the carborane cluster has been successfully used as phenyl ring or cyclopentadienyl ligand mimetic, respectively, to create novel hybrid organic-inorganic molecules, which show, in several cases, modulation of the biological activity with respect to their purely organic or organometallic counterparts.[2]

We are interested in the combination of ruthenium-arene fragments with the dicarbollide ligand $C_2B_9H_{11}^{2-}$ and its *C*- or *B*-substituted derivatives, and in the evaluation of the biological activity of such mixed-sandwich ruthenacarborane complexes.[3] Conceptually, the Cp* ligand (Cp* = pentamethylcyclopentadienyl) of ruthenium-arene complexes developed by Loughrey *et al.*[4] is replaced with the dicarbollide ligand, and the modulation of the anti-proliferative activity of the resulting complexes is investigated.

Here, we present our results from *in vitro* cytotoxicity assays, flow cytometric analysis and binding studies of the ruthenacarborane complexes to blood serum proteins.



References

1. N. A. Meanwell, *Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design*, *J. Med. Chem.* **2011**, *54*, 2529–2591.
2. a) B. Schwarze, M. Gozzi, E. Hey-Hawkins, *Half- and Mixed-Sandwich Transition Metal Dicarbollides and nido-Carboranes(–1) for Medicinal Applications,* in *Boron in Medicine*, edited by E. Hey-Hawkins and C. Vinas, John Wiley & Sons Ltd., **2017**, *accepted*; b) M. Scholz, E. Hey-Hawkins, *Carbaboranes as Pharmacophores: Properties, Synthesis, and Application Strategies*, *Chem. Rev.* **2011**, *111*, 7035–7062.
3. M. Gozzi, B. Schwarze, P. Lönnecke, D. Drača, D. Maksimović-Ivanić, S. Mijatović, E. Hey-Hawkins, *Mixed-Sandwich Ruthenacarborane Complexes as Novel Highly Selective Cytotoxic Agents*, *submitted*.
4. B. T. Loughrey, B. V. Cunning, P. C. Healy, C. L. Brown, P. G. Parsons, M. L. Williams, *Selective, Cytotoxic Organoruthenium(II) Full-Sandwich Complexes: A Structural, Computational and In Vitro Biological*

Ru(II) organometallic complexes with acylthiourea ligands: cytotoxic activity in lung cancer

Beatriz N. Cunha[a,b], Rafael G. Silveira[a,b], Legna Colina-Vegas[a], Ana M. Plutin[c], Eduardo E. Castellano[d], Marcia R. Cominetti[a], Alzir A. Batista[a]

[a] *Federal University of São Carlos- Brazil;,* [b] *Federal Institute Goiano- Brazil;* [c] *Facultad de of Chemistry, University of Habana- Cuba;* [d] *Institute of Physis of São Carlos, University of São Paulo;* beatriznc_@hotmail.com

Abstract

Half sandwich Ru(II) arene complexes have been extensively investigated for their anticancer properties [1]. In this study complexes of the type [Ru(η^6-*p*-cymene)(PPh_3)(T)Cl](PF_6) and [Ru(η^6-*p*-cymene)(PPh_3)(T)](PF_6) where PPh_3= triphenylphosphine, T= *N*-(furoyl)-*N*`-furoylthiourea **(1; 1a)**; *N*-(thiophenyl)-*N*`-thiophenylthiourea **(2);** *N*-(tiophenyl)-*N*`-furoylthiourea) **(3);** *N*-(furoyl)-*N*`-1,3-benzodioxolylthiourea) **(4)**; *N*-(thiophenyl)-*N*`-1,3-benzodioxolylthiourea) **(5)**, were synthesized, characterized and their cytotoxic properties were evaluated. Interactions studies were carried out with human serum albumin (HSA) and DNA. The cytotoxicity of the compounds was determinate in human lung cells, A549 and MRC-5, by MTT assay. The IC_{50} values of the complexes are in the range 0.25 - 0.63 µM, at 48 h, in lung cancer cells, indicating that the compounds show high cytotoxicity with values significantly lower than reference drug, cisplatin, for the same tumor cells. Complexes **1** and **1a** are able to inhibit the colony formation, to decrease the colony size and to induce morphology changes in A549 cells. These complexes induce apoptosis cell death and promote cell arrest at Sub-G1 phase with decrease in S phase portion.

References

1) C. G. Hartinger, N. Metzler-Nolte, P. Dyson, *Organometallics* **2012**, *31*, 5677.

Synthesis, solution equilibria and antitumor activity of Ru(II)(η^6-toluene) complexes of various picolinates

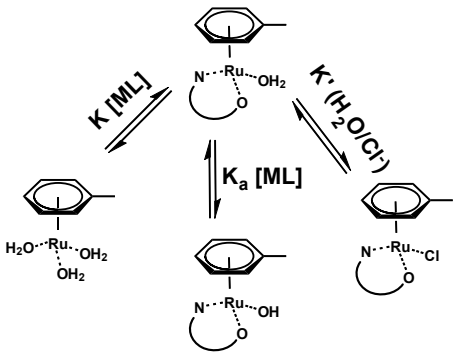
Jelena M. Poljarević,[a,b] Nóra V. May,[c] Gabriella Spengler,[d] Orsolya Dömötör,[a,e] Milica Orlović,[b] Aleksandar R. Savić,[b] Sanja Grgurić-Šipka,[b] Éva A. Enyedy[a]

[a)] Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7. H-6720 Szeged, Hungary; [b)] Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia; [c)] Research Centre for Natural Sciences Hungarian Academy of Sciences, Magyar tudósok körútja 2, H-1117 Budapest, Hungary; [d)] Department of Medical Microbiology and Immunobiology, University of Szeged, Dóm tér 10, H-6720 Szeged, Hungary; [e)] MTA-SZTE Bioinorganic Chemistry Research Group, Dóm tér 7. H-6720 Szeged, Hungary; email: enyedy@chem.u-szeged.hu

Abstract

Ruthenium complexes have been the subject of extensive drug discovery efforts yielding NKP-1339 and NAMI-A as the most promising Ru(III) compounds reaching clinical trials [1]. Half-sandwich Ru(II)(η^6-arene) complexes have tunable reactivity and numerous of them show remarkable anticancer activity such as the RAPTA or RAED compounds [2]. Despite the large number of Ru(II)(η^6-arene) based compounds limited information is available about their solution speciation. However, the knowledge on the solution stability and interactions with serum proteins strongly contributes to the understanding and controlling of the pharmacokinetic properties and mechanism of action.

Careful tuning on the physico-chemical properties of the half-sandwich organoruthenium complexes may improve the pharmacokinetic behaviour and ultimately the anticancer activity. Our aim was to investigate the effect of various substituents on the reference compound, picolinic acid on the solution chemical and biological properties of their Ru(II)(η^6-toluene) complexes.



Herein, we report on the synthesis, solution stability, chloride ion affinity, lipophilicity, X-ray crystal structures and *in vitro* cytotoxicity of Ru(II)(η^6-toluene) complexes formed with some derivatives of picolinic acid possessing (*N,O*) donor set. Equilibrium studies were performed by the combined use of pH-potentiometry, UV-Vis spectrophotometry, ^{1}H NMR spectroscopy. The *in vitro* anticancer potency of the complexes was monitored in multidrug resistant/non-resistant human cancer lines. The mechanism of cell death was assayed by flow cytometry. Interaction of the active Ru(II)(η^6-toluene)-picolinate complexes towards human serum albumin was also studied using fluorometry and ultrafiltration.

Acknowledgement: OTKA PD103905, GINOP-2.3.2-15-2016-00038, MPNTR 172035 and MPNTR postdoctoral grant (J. M. P.), J. Bolyai Research Scholarship (E. A. E.)

References

1) R. Trondl, P. Heffeter, et al., Chem. Sci. 5 (2014) 2925–2932; A. Bergamo, G. Sava, Chem. Soc. Rev. 44 (2015) 8818-8835.
2) A.A. Nazarov, C.G. Hartinger, P.J. Dyson, J. Organomet. Chem. 751 (2014) 251-260.; A.F.A. Peacock, S. Parsons, P.J. Sadler, J. Am. Chem. Soc. 129 (2007) 3348-3357.

Design of radiotrackable water soluble Ti-based complexes

Océane Florès,[a] Francisco Silva,[b] Fernanda M. Marques,[b] Franck Denat,[a] Maria Paula C. Campello,[b] Pierre Le Gendre,[a] Christine Goze,[a] Ewen Bodio[a]

[a] *ICMUB, UMR 6302 CNRS, Université de Bourgogne, 9 av. A. Savary, BP 47870, 21078 Dijon, France;*

[b] *C²TN, IST, Universidade de Lisboa, Estrada Nacional 10, 2695-066 Bobadela LRS, Portugal*

email : oceane.flores@u-bourgogne.fr

Abstract

Among the large variety of potential anticancer organometallic complexes, titanocene dichloride Cp_2TiCl_2 reached Phase 2 clinical trial. However, its poor solubility and stability in water lead to an uncertainty about the biologically active species and severely hampered its development as anticancer agents. Besides, the mechanism of action of such complexes is still unclear since the usual *in vivo* biodistribution studies and determination of metal uptake in organs by ICP-MS cannot be performed due to the natural occurrence of titanium in the human body. Scintigraphy appears then as a promising tool to visualize metal biodistribution and clarify the mechanism of action of titanocene derivatives.

To deal with the previously mentioned issues, Gansäuer and coworkers developed a synthesis of water-soluble and stable cationic titanium complexes[1] (Fig. 1). Seeking to synthesize new radiolabeled titanium based drugs, we decided to use this strategy to introduce a bifunctionnal chelating agent on the titanocene framework. The resulting titanocene-DOTA compound (Fig. 2) proved to be relatively stable in water and serum up to 48h and could handle radiolabeling-like conditions. Complexation of "cold" Lu, In, and Y has been performed successfully, as well as radiolabelling with ^{111}In. Biological assays such as determination of antiproliferative properties in cancer cells and *in vivo* biodistribution are currently under investigation.

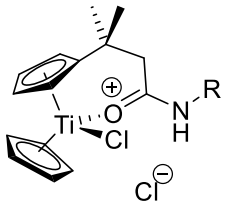


Fig. 1 : structure of cationic titanium complexes

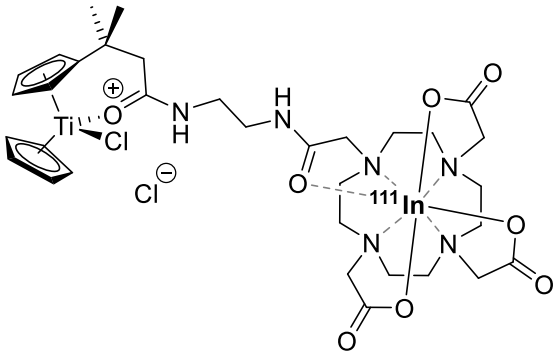


Fig. 2 : Radiolabeled titanocene derivative

References

1) Gansäuer et al., J. Am. Chem. Soc., 2005, 127, 11622

Towards applications of β-NMR in bioinorganic chemistry

D. Szunyogh[1,2], M. Stachura[3], F. H. Larsen[4], S. Pallada[1,5], P. W. Thulstrup[1], M. Kowalska[5], L. Hemmingsen[1]

[1] *University of Copenhagen, Department of Chemistry, Universitetsparken 5, 2100 Copenhagen, Denmark.*
[2] *MTA-SZTE Bioinorganic Chemistry Research Group, Dóm tér 7, 6720 Szeged, Hungary.*
[3] *TRIUMF, 4004 Wesbrook Mall, Vancouver BC, V6T 2A3, Canada.*
[4] *University of Copenhagen, Department of Food Science, Rolighedsvej 30, 1958 Copenhagen, Denmark.*
[5] *CERN, Experimental Physics Department, Geneva, Switzerland.*
email : dsz@chem.ku.dk

Abstract

β-NMR spectroscopy is an ultrasensitive NMR technique, relying on the detection of β-particles emitted from spin polarized radioactive nuclei. That is, the technique benefits from both hyperpolarization of the nuclear spins and from the high detection efficiency of radiotracer experiments, leading to a billion fold (10^9) or higher increase in sensitivity as compared to conventional NMR. The technique has been applied in nuclear and solid state physics for decades, and we aim to advance towards chemistry and biochemistry by investigation of samples in solution. Recent advances on this topic encompass measurements on Mg^{2+} in ionic liquid solution[1], and assessment if adequate resolution may be achieved[2]. Ionic liquids are attractive solvents because they exhibit low vapour pressure, and may therefore be placed in the high vacuum of beam lines at the current production facilities for the spin polarized radioisotopes. The overarching goal is to conduct experiments on biomolecules in aqueous solution, and Sugihara *et al.* progressed towards this goal in an impressive endeavour implanting ^{12}N into water[3] and recording a β-NMR spectrum.

We will present recent results on this project, including examples of β-NMR applied to metal ions in solution using ionic liquids as solvents.

References

1) A. Gottberg, M. Stachura, M. Kowalska, M. L. Bissell, V. Arcisauskaite, K. Blaum, A. Helmke, K. Johnston, K. Kreim, F. H. Larsen, R. Neugart, G. Neyens, R. F. Garcia Ruiz, D. Szunyogh, P. W. Thulstrup, D. T. Yordanov, L. Hemmingsen.
Billion-Fold Enhancement in Sensitivity of Nuclear Magnetic Resonance Spectroscopy for Magnesium Ions in Solution
ChemPhysChem 2014, 15, 3929 DOI 10.1002/cphc.201402619

2) M. Stachura, R. M. L. McFadden, A. Chatzichristos, M. H. Dehn, A. Gottberg, L. Hemmingsen, A. Jancso, V. L. Karner, R. F. Kiefl, F. H. Larsen, J. Lassen, C. D. P. Levy, R. Li, W. A. MacFarlane, G. D. Morris, S. Pallada, M. R. Pearson, D. Szunyogh, P. W. Thulstrup, A. Voss.
Towards ^{31}Mg-β-NMR resonance linewidths adequate for applications in magnesium chemistry
Hyperfine Interact. 2017, 238, 32, DOI 10.1007/s10751-017-1408-8

3) T. Sugihara, M. Mihara, J. Shimaya, K. Matsuta, M. Fukuda, J. Ohno, M. Tanaka, S. Yamaoka, K. Watanabe, S. Iwakiri, R. Yanagihara, Y. Tanaka, H. Du, K. Onishi, S. Kambayashi, T. Minamisono, D. Nishimura, T. Izumikawa, A. Ozawa, Y. Ishibashi, A. Kitagawa, S. Sato, M. Torikoshi, S. Momota.
NMR detection of short-lived β-emitter ^{12}N implanted in water
Hyperfine Interact. 2017, 238, 20, DOI 10.1007/s10751-017-1401-2

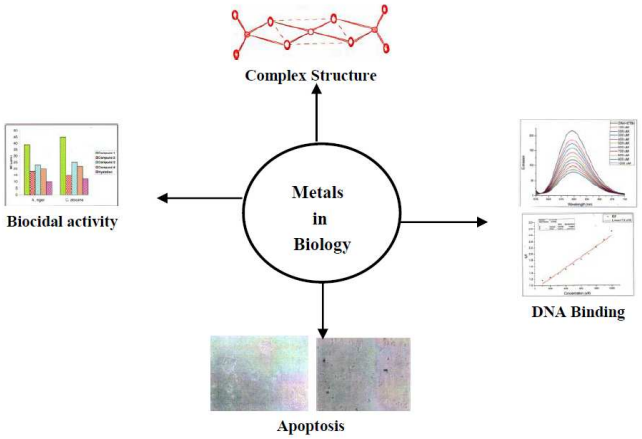
Anticancer, Biocidal and DNA Binding Studies of Bis(tetrathiotungstate) Complexes of Pt (II), Pd (II) and Ni(II) of Bioinorganic relevance

V.K. Srivastava

Chemical Research Laboratory, Faculty of Science, Department of Chemistry, D.S. College, Aligarh 202001 (U.P.) India, email: vksrivastava633@gmail.com

Abstract

The chemistry of compounds containing Transition metals bound to sulfur containing ligands has been actively studied. Interest in these compounds arises from the identification of the biological importance of Iron-sulfur containing proteins as well as the unusual behaviour of several types of synthetic metal – sulfur complexes. Bis (tetrathiotungstate) complexes of Pt (II), Pd (II) and Ni (II) of Bioinorganic relevance were investigated. The complexes [M(M'S4)2]2- were prepared with high yield and purity as salts of the variety of organic cations. The diamagnetism and spectroscopic properties of these complexes confirmed that their structures are essentially equivalent with two bidentate ligands coordinated to the central d8 metal in a square planer geometry. The electro chemical properties of the [M(M'S4)2]2- dianions in CH3CN and DMF have been determined by cyclic voltametry. The interaction of the complexes with CT-DNA was studied by using absorption, emission, spectral methods, thermal denaturation and viscometry studies. Results showed that metal complexes increase DNA's relative viscosity and quench the fluorescence intensity of EB bound to DNA. In antimicrobial activities all complexes showed good antimicrobial activity higher than ligand against gram positive, gram negative bacteria and Fungi. The antitumor properties have been tested in vitro against two tumor human cell lines, Hela (derived from cervical cancer) and MCF-7 (derived from breast cancer) using a metabolic activity tests. Results showed that the complexes are promising chemotherapeutic alternatives in the search of anticancer agents.



References

1. V. K. Srivastava, International Journal of Pharmaceutical Science and Research, **2015**, 6(4), 1000-05.
2. V. K. Srivastava, Forensic and Medical Bioinformatics **2016**, 97-104 Springer.
3. V. K. Srivastava, Review Article European Journal of Biomedical and Pharmaceutical sciences, **2016**, 3(5), 204-208.
4. V. K. Srivastava, Cancer Chemother Pharmacol, **2016** (In Press)

Acknowledgement : Author's thankful to University Grant Commission, New Delhi, India for financial support.

Importance of the iron redox state on its stable isotope composition during adsorption on cyanobacteria. Application to a thermal spring from Kamchatka, Russia.

Daniel S. Mulholland,[a,b] Franck Poitrasson, [a,b] Liudmila S. Shirokova,[b,c] Aridane G. González,[b] Oleg S. Pokrovsky,[b,d] Geraldo R. Boaventura,[a] Lucieth C. Vieira[a]

[a] Instituto de Geociências, Campus Universitário Darcy Ribeiro, Universidade de Brasília (UnB), CEP 70910-900, Brasília, DF – Brazil 1 ; [b] Laboratoire Géosciences Environnement Toulouse, Institut de Recherches pour le Développement – Centre National de la Recherche Scientifique – Université de Toulouse, 14-16, avenue Edouard Belin, 31400 Toulouse, France ; [c] Laboratory of Aquatic Ecosystems, Institute of Ecological Problems of the North, Russian Academy of Science, Arkhangelsk, Russia ; email : Franck.Poitrasson@get.omp.eu

Abstract

This study[1] aimed at testing the hypothesis that, similarly to other metal cations, Fe adsorption on bacterial phytoplankton likely cause significant isotopic fractionation with preferential adsorption of heavy isotopes on the cell surface. We measured the isotopic fractionation during the interaction of aqueous Fe with planktonic cyanobacteria (*Gloeocapsa sp., Synehococcus sp.,* and *Planthothrix sp)* in six independent experiments using two distinct Fe oxidation states (Fe(III) and Fe(II)) at pHs of 3 and 6. Isotopic analyses demonstrated that the Fe adsorption on bacterial planktonic biomass yields a clear enrichment of heavy isotopes on the cell surfaces, producing isotopically light δ^{57}Fe values in the final, remaining growth solutions. The adsorption experiments with Fe(II) in the initial solution yielded a $\Delta^{57}Fe_{cell\text{-}solution}$ ranging from 2.4 to 2.9‰, whereas the adsorption experiments with Fe(III) in the initial solution yielded $\Delta^{57}Fe_{cell–solution}$ ranging from 0.92 to 1.03‰. Because these data plot close to closed system equilibrium isotopic fractionation lines rather than Rayleigh curves, the most likely mechanism can be defined as a steady state isotopic fractionation, linked with short-term, reversible Fe adsorption on cells. The preferential enrichment of heavy Fe isotopes on the cell surfaces is attributed to the stronger covalent metal-ligand bonding (Fe-O-C/P) present in the Fe octahedrally coordinated with phosphoryl or carboxyl groups on the cell walls when compared with the Fe aquacomplexes (O-Fe-O) in solution. Our experimental results are used to interpret the iron isotope signatures measured on dissolved Fe in water and on Fe adsorbed on biofilms sampled from a thermal spring from Kamchatka, Russia. These findings suggest that Fe adsorption on cyanobacteria cell surfaces might have profound implication on Fe isotopic fractionation in continental river and lacustrine waters and oceans during phytoplankton blooms in the course of the Earth´s life evolution.

Reference

1) Mulholland, D.S., Poitrasson, F., Shirokova, L.S., Gonzales, A.G., Pokrovsky, O.S., Boaventura, G.R., Vieira, L.C., 2015. Chemical Geology, 400: 24-33.

Hg stable isotopes as tracers for dietary methyl-Hg exposure and hepatic detoxification in humans

Jeroen E. Sonke,[a] Johanna Menges,[b] Laure Laffont,[a] Laurence Maurice,[a]

[a] CNRS-IRD-University of Toulouse;[b] GFZ Potsdam, email : jeroen.sonke@get.omp.eu

Abstract

Mercury (Hg) is a natural element whose global biogeochemical cycle has been profoundly changed by human activites. In its methylmercury (MeHg) form, Hg bioaccumulates along aquatic food chains and affects human and wildlife health. MeHg is neurotoxic to the developing foetus and young children and is thought to be linked to cardiovascular disease in adults. Humans are thought to mainly absorb toxic MeHg through dietary exposure. There is however substantial variation in dose-response among different populations. A better understanding of precise dietary exposure and metabolic breakdown of MeHg would help optimizing Hg risk management and environmental policy.

Hg stable isotope signatures show large variations across Earth surface reservoirs and within biological species. These variations result from the gradual fractionation of heavy/light and even/odd Hg isotopes during the multiple physicochemical processes that shuttle Hg across the Earth's surface. Two useful Hg isotope fingerprints, δ^{202}Hg and Δ^{199}Hg, characterize its source, or code for the transformations that Hg has undergone in its biogeochemical cycle.

Recent work on Hg isotopes in human hair has given insight into dietary Hg exposure. Hair Δ^{199}Hg, which has a photochemical origin and is not affected by Hg metabolism, appears to be a robust and conservative marker for the dietary source of MeHg. Amazonion river people have low Δ^{199}Hg that are identical to that of the fresh water fish they consume; similarly Europeans have higher Δ^{199}Hg corresponding to a mixed sea food diet, while North-Americans have elevated Δ^{199}Hg corresponding to the consumption of open ocean predator fish such as tuna.

In contrast, the δ^{202}Hg signature is affected by nearly all chemical transformations of Hg. Human hair δ^{202}Hg shows a systematic 2 ‰ enrichment in the heavier isotopes compared to the fish that was consumed across all three populations. We will show that the origin of this 2 ‰ enrichment potentially lies in the metabolic demethylation of MeHg, a detoxification mechanism that lowers MeHg exposure.

Density Functional Theory: Key to transfer the entatic state concept to photochemistry

Alexander Hoffmann,[1] Julia Stanek,[1] Michael Rübhausen,[2] Wolfgang Zinth[3] and Sonja Herres-Pawlis[1]

[1] RWTH Aachen University, Institute of Inorganic Chemistry, Landoltweg 1, 52074 Aachen, Germany
2 University of Hamburg, Department of Physics, Luruper Chaussee 148, 22761 Hamburg, Germany
3 LMU Munich, Faculty of Physics, Oettingerstr. 67, 80538 Munich, Germany
Email: alexander.hoffmann@ac.rwth-aachen.de

The concept "entatic state" was coined by Vallee and Williams already in 1968.[1] Since then, the discussion about influences of a distorted coordination sphere on the function of metalloenzymes and their model complexes is alive. Especially, in the field of electron transfer proteins, the major question is if metal binding to the ligands dominates the resulting structures and electronic potentials or if the protein scaffold induces strain at the metal centers.[2]

We investigated the structures of two types of guanidine–quinoline copper complexes (being copper(I) and copper(II) complexes, see Figure 1) by single-crystal X-ray crystallography, K-edge X-ray absorption spectroscopy (XAS), resonance Raman and UV/Vis spectroscopy, cyclic voltammetry, and density functional theory (DFT). Independent of the oxidation state, the two structures, which are virtually identical for solids and complexes in solution, resemble each other strongly and are connected by a reversible electron transfer at -0.37 V versus Fc/Fc^+. By resonant excitation of the two entatic copper complexes, the transition state of the electron transfer is accessible through vibrational modes, which are coupled to metal–ligand charge transfer (MLCT) and ligand–metal charge transfer (LMCT) states.[3,4]

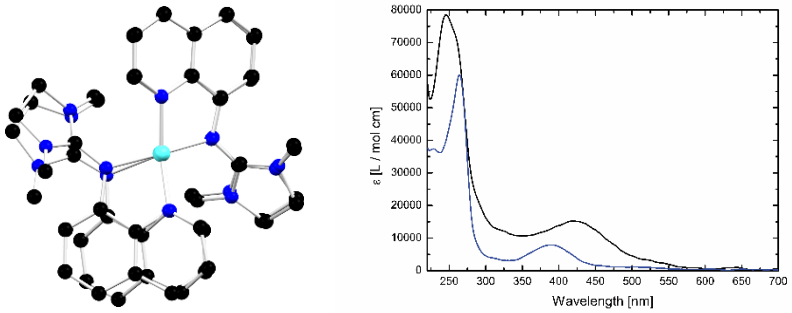


Figure 1. left: Superimposed molecular structures of $[Cu(DMEGqu)_2]^+$ and $[Cu(DMEGqu)_2]^{2+}$; right: Comparison of experimental UV/vis spectra of $[Cu(DMEGqu)_2]^+$ (black) and $[Cu(DMEGqu)_2]^{2+}$ (blue).

References

1) B. L. Vallee, R. J. P. Williams, Proc. Natl. Acad. Sci. USA 1968, 59, 498 – 505.
2) a) P. Comba, Coord. Chem. Rev. 1999, 182, 343 – 371; b) P. Comba, W. Schiek, Coord. Chem. Rev. 2003, 238 – 239, 21 – 29.
3) A. Hoffmann, J. Stanek, B. Dicke, L. Peters, B. Grimm-Lebsanft, A. Wetzel, A. Jesser, M. Bauer, M. Gnida, W. Meyer-Klaucke, M. Rübhausen, S. Herres-Pawlis, Eur. J, Inorg. Chem. 2016, 4731 – 4743.
4) A. Hoffmann, S. Binder, A. Jesser, R. Haase, U. Flörke, M. Gnida, M. Salomone Stagni, W. Meyer-Klaucke, B. Lebsanft, L. E. Grünig, S. Schneider, M. Hashemi, A. Goos, A. Wetzel, M. Rübhausen, S. Herres-Pawlis, Angew. Chem. Int. Ed. 2014, 53, 299 – 304.

Is "good enough" really enough? In search of novel chelators for Cu-64, Ga-68 and Zr-89 radiometals.

Karolina Zdyb, [a] Elzbieta Gumienna-Kontecka,[a] Oleg Varzatskii [b]

[a] *Faculty of Chemistry, University of Wroclaw, Wroclaw, Poland ;* [b] *Vernadskii Institute of General and Inorganic Chemistry NAS of Ukraine, Kiev, Ukraine; email : karolina.zdyb@chem.uni.wroc.pl*

The development of medical diagnostics over the last several decades has resulted in an increase in the radionuclide significance in areas such as cardiology, oncology and neurology, and targeted therapy.[1]

Recent advances in radiochemistry underline the importance of PET imaging, and subsequently the significance of optimal utilization of a growing number of β^+ emitting radiometal ions. Among the range of available radioisotopes, three present interesting properties and potential for PET imaging: ^{68}Ga, ^{64}Cu and ^{89}Zr.[2,3] In order to apply these isotopes, the ''free'' radiometal ions must be sequestered from aqueous solution using suitable chelating ligands to obviate transchelation and hydrolysis. A large number of radionuclides chelators described in the literature as "good enough" imaging agents may deceptively discourage the development of new compounds. However, the conscientious overview of achievements in this field shows the enormous complexity of the relationship between the metal ion and the chelator needed to satisfy the specific requirements of these radiometals in terms of donor atoms, coordination number and geometries, *in vivo* behaviour and biodistribution.[3]

The key element for the advancement of nuclear medicine is the development of novel chelators for non-invasive *in vivo* imaging agents. Moreover, this progress also requires complete structural, physico-chemical and thermodynamic characterization of ligands and their metal complexes.

Here we will present the series of compounds based on the desferrithiocin analogue structure (Fig1.) as a potential radionuclide chelators.

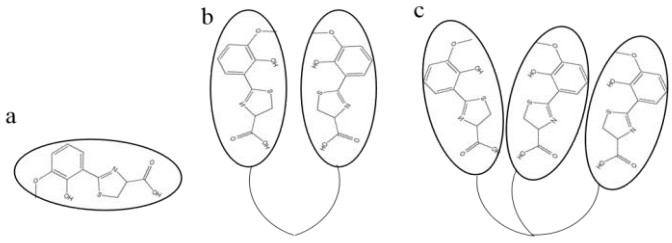


Fig.1. Structures of desferrithiocine analogue monomeric, dipodal and tripodal ligands.

References

1) Zeglis, B.M.; Houghton et.al., *Inorganic Chemistry* 2014, 53, 1880-1899.
2) Brasse, D.; Nonat, A., *Dalton Transactions* 2015, 44, 4845-4858.
3) Price, E.W.; Orvig, C., *Chemical Society Reviews* 2014, 43, 260-290.

Acknowledgement:

The financial support from the Polish National Science Centre (NCN, UMO-2015/19/B/ST5/00413 Nr 0201/1846/16) is gratefully acknowledged.

SPR Screening of Metal Chelating Peptides in a Hydrolysate for their Antioxidant Properties

Laetitia L.S. CANABADY-ROCHELLE[a], Katalin SELMECZI[b], Sabrina COLLIN[c], Tania DJEHEL[a]Andreea PASC[b], Laurence MUHR[a], and Sandrine BOSCHI-MULLER[c]

[a]*Université de Lorraine, LRGP (UMR CNRS-UL 7274), ENSIC, 1 rue Grandville, 54000 Nancy, France*
[b]*Université de Lorraine, SRSMC (UMR CNRS-UL 7565), Faculté des Sciences et Technologie,54506 Vandœuvre-lès-Nancy Cedex, France*
[c]*Université de Lorraine, IMoPA(UMR CNRS-UL 7365), Campus Biologie-Santé, Faculté de Médecine, 9, avenue de la forêt de Haye, 54505 Vandœuvre-lès-Nancy, France*
email :laetitia.canabady-rochelle@univ-lorraine.fr

Abstract

There is a growing need in the industrial sector (*i.e.* health, nutrition and cosmetic) to discover new biomolecules with various physico-chemical and bioactive properties. Various beneficial effects of peptides - notably those produced from protein hydrolysis - are reported in the literature. The antioxidant activity involves various mechanisms among them metal chelation. Since Immobilized Metal ion Affinity Chromatography is used to separate protein based on the coordination bond between metal and some aminoacid residues notably the Porath's triade (histidine, cysteine and tryptophane),in this work, we aimed to establish a link between the affinity of a peptide for a metal ion and its antioxidant activity based on metal chelation. First,method used for the determination of metal chelating ability of antioxidant peptides was set up (1). Then, an original method of screening metal chelating peptides in a hydrolysate was developed using Surface Plasmon Resonance (SPR) for their antioxidant properties. To date, the empirical approach used several cycles of hydrolysate fractionation and bioactivity evaluation until the isolation of the pure bioactive molecule and its identification. Besides, the detection of metal-chelating peptide is not sensitive enough using spectrophotometry. For the first time, metal chelating peptides were screened in hydrolysates using SPR and a correlation was established between affinity constant determined in SPR and metal chelation capacity determined from UV-visible spectrophotometry.Then peptide-metal ion interactions were further investigated in hydrolysates. Some applications of metal chelating peptides are currently under studies.

References

1) L. Canabady-Rochelle,C. Harscoat-Schiavo, V. Kessler, F. Fournier, J.-M. Girardet. Determination of reducing power and chelating ability of antioxidant peptides: Revisited Methods. *Food Chemistry*, **2015**, *183*, 129-135.
2) L.Canabady-Rochelle, K.Selmeczi, S.Collin, A.Pasc, L.Muhr, S. Boschi-Muller. SPR Screening of metal chelating peptides in a hydrolysate for their antioxidant properties, *Food Chemistry*, **2017**, submitted.

Aggregation-Induced Emission in Benzoxazole-based Rhenium Complexes

Jin-Hui Wang, [a] Romain Eychenne, [a] Eric Benoist, [a] Laure Gibot, [b] Suzanne Fery-Forgues [a]

[a] *SPCMIB, UMR5068, CNRS-Université Paul Sabatier-Toulouse III, 118 route de Narbonne, 31062 Toulouse cedex 9, France*

[b] *Equipe de Biophysique Cellulaire, IPBS-CNRS UMR 5089, 205 route de Narbonne, BP 64182 F31077 Toulouse Cedex, France*

E-mail: wangjh@chimie.ups-tlse.fr

Fluorogenic molecules that are non-emissive when molecularly dissolved but highly emissive when aggregated have attracted intense research interest for applications in optics, optoelectronics and bioimaging. [1] This aggregation-induced emission (AIE) phenomenon has initially proposed as a cause of restriction of intramolecular rotation (RIR) and is generally observed in molecules with rotating units such as phenyl rings. Since their discovery in 2001, organic AIE-fluorogens have been rapidly developed and the study has been subsequently extended to a number of organometallic compounds. [2] However, some types of complexes like tricarbonyl Rhenium(I) complexes have hardly been investigated.

Our group has been working on tricarbonyl rhenium(I) complexes for years and used them as potential imaging agents for luminescence in solution.[3] The $[Re(CO)_3]^+$ core possesses three facial positions available for substitution by various organic ligands, which allow the spectroscopic and biological properties to be widely varied. These Re(I) complexes usually are air- and water stable, and biocompatible. Consequently, it seemed interesting to associate the AIE effect with the Re(I) complexes to generate a new series of compounds for applications such as light-responsive materials, sensing and imaging.

In the present work, we describe two isomeric tricarbonyl Re(I) complexes, ReL_1 and ReL_2, that possess respectively a 3-(pyridine-2-yl)-1,2,4-triazole and a 3-(pyridine-2-yl)-1,2,3-triazole fragment. They both bear the same 2-phenylbenzoxazole moiety that has been introduced on the triazole group to enhance the emission properties. The synthesis and crystallographic data of these compounds are reported, together with the spectroscopic and photophysical studies, as well as preliminary imaging tests. We show that these compounds display clear AIE-behavior, with unexpected variations due to modifications of the organic ligand. This study paves the way for a new generation of AIE-active Re complexes.

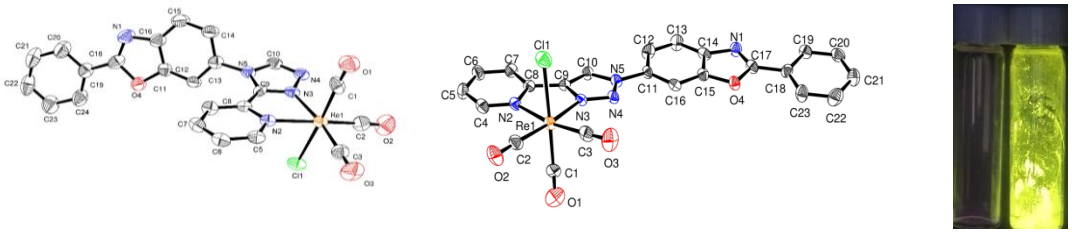


Figure1. From left to right: Molecular view of ReL_1 and ReL_2; Picture of ReL_2 in solvent (DCM) and in the solid state under UV light (365 nm)

References

[1]] J. Mei, N. L. C. Leung, R. T. K. Kwok, J. W. Y. Lam, B. Z. Tang. *Chem. Rev.,* **2015**, *115*, 11718-11940.
[2] V. Sathish, A. Ramdass, P. Thanasekaran, K.-L. Lu, S. Rajagopal. *J. Photochem. Photobiol. C,* **2015**, 23, 25-44.
[3] A. François, C. Auzanneau, V. L. Morvan, C. Galaup, H. S. Godfrey, L. Marty, A. Boulay, M. Artigau, B. Mestre-Voegtlé, N. Leygue, C. Picard, Y. Coulais, J. Robertc, E. Benoist. *Dalton Trans.*, **2014**, 43, 439-450.

Searching for PARACEST effect in DOTA-like complexes with free pendant amine/amide

Peter Urbanovsky,[a] Tereza Krchova,[a] Jan Kotek,[a] Petr Hermann[a]

[a] *Dept. of Inorg. Chem., Faculty of Sciences, Charles University, Prague, CZ ; email: urbanop@natur.cuni.cz*

Abstract

Magnetic resonance imaging (MRI) is frequently used non-invasive diagnostic technique. It possesses a quite high potential to localize abnormalities in patient body thanks to the proton relaxation properties in magnetic field. However, pathological tissue is not always clearly evident in the MRI scan. To improve that, contrast agent (CA) which alters surrounding proton relaxation rates is administered during scanning. Up-to-date, the CAs based on Gd^{3+} are clinically used because of the suitable physical properties of this ion. Gd^{3+} is injected in the form of thermodynamically stable and kinetically inert complex due to the severe toxicity of the free ion (LD_{50}(mouse, *i.v.*) = 0.35 mmol/kg)[1]. The CAs based on DOTA ligands (**Figure**) meet these conditions. To utilize more possibilities, ligands of CAs can be modified in a specific way so that CAs have unique properties such as specific biodistribution, slower excretion rate, bifunctionality etc.

Another type of MRI is utilizing PARACEST effect (paramagnetic chemical exchange saturation transfer). The main goal of enhanced imaging is to alter relaxation rates of water protons and this can be done by simple chemical exchange. Selectively irradiated protons from protonable group (e.g. amine) of CA could be exchanged with water protons and this phenomenon leads to reduced observable water signal. For this reason, lanthanide complexes (*e.g.* Eu^{3+}, Yb^{3+}) are used.

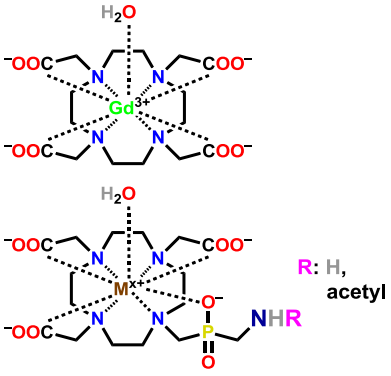


Figure: The drawings of the CA based on the ligand DOTA (up) and the investigated CAs (down).

We have focused on the type of CAs based on $DO3AP^R$ moiety with the free pendant amine and its acetyl derivative (**Figure**). The universal nature of the amino group is due to its strong pH-dependency, *e.g.* its protonation results in the different specie with the new properties. Moreover, amine could be easily synthetically modified and properties of CAs could be tuned, for instance coupling with vector or biomolecule. The presented work summarizes some of the findings and describing PARACEST effect in the CAs.

Acknowledgements

This work was supported by Grant Agency of Charles University (no. 1170317).

References

1) Bousquet, J. C.; Gd-DOTA: characterization of a new paramagnetic complex, Radiol., 1988, 166, 3, 693–698.

Mn(II) complexes of open-chain ligands as possible magnetic resonance imaging (MRI) contrast agents (CAs)

G. Tircsó[a], Z. Garda[a], F. K. Kálmán[a], K. Póta[a], E. Molnár[a], B. Váradi[a], R. Botár[a], E. Tóth[b] and I. Tóth[a]

[a]*Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010, Debrecen, Egyetem tér 1, Hungary*

[b]*Centre de Biophysique Moléculaire, CNRS, Rue Charles-Sadron, 45071 Orleans Cedex 2, France*

Presenting author: gyula.tircso@science.unideb.hu

Abstract

Over the past decades, research and development of novel Magnetic Resonance Imaging (MRI) contrast agents produces structurally diverse ligands and complexes with a variety of metals. Although the majority of approved MRI contrast agents are still based on gadolinium complexes, manganese has gained certain attention due to its ability to form high spin complexes with five unpaired electrons. An appropriate ligand would form a redox- and thermodynamically stabile, kinetically inert Mn^{2+} complex with high relaxivity. All of these, often contradictory, requirements are needed for in vivo applications. Mn is an essential trace element in the human body with likely less toxic effect compared to the highly toxic Gd, however the knowledge collected for gadolinium based CAs can not be directly translated to Mn^{2+}. Our attempt to find a suitable ligand for Mn^{2+} complexation started from equilibrium, kinetic and relaxometric characterization of Mn^{2+} complexes with known open-chain (OCh)[1] and macrocyclic (MC)[2] amino-polycarboxylates in order to collect data for structure – function relations, than we synthesized some new ligands. One of the key finding is, that the kinetic inertness of the Mn^{2+} complexes plays very important role. Suitable structural changes to increase the inertness involve the rigidifying of the ligand backbone, or the pendant arms, changing the protonation properties and charge of the ligands etc., exemplified on Figure 1.

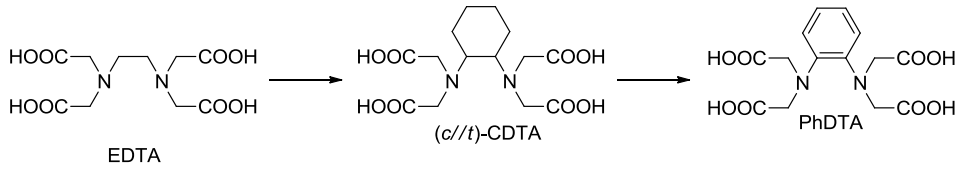


Figure 1. EDTA-derivatives studied and compared for complexation of Mn^{2+}

References

1) F. K. Kálmán, G. Tircsó, Inorg. Chem., 2012, 51, 10065.
2) Z. Garda, A. Forgács, Q. N. Do, F. K. Kálmán, S. Timári, I. Tóth, Zs. Baranyai, L. Tei, Z. Kovács and Gy. Tircsó, J. Inorg. Biochem., 2016, 163, 206.

Acknowledgements

The authors thank the Hungarian Scientific Research Fund (NKFIH K-120224 project), Le Studium, Loire Valley Institute for Advanced Studies (Gy.T. and F.K.K.) and the János Bolyai Research Scholarship (Gy.T. and F.K.K.) of the Hungarian Academy of Sciences. E. T. acknowledges support of the Ligue contre le Cancer (France). The research was also supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008.

Penetration study by multimodal imaging of labelled hyaluronic acids of different molecular weights into the skin

Lucas Henry,[a] Cécile Laugel,[b] Nicolas Delsuc,[a] François Lambert,[a] Hélène Bertrand, [a] Sarah Hostachy, [a] Christophe Sandt,[c] Arlette Baillet-Guffroy,[b] Clotilde Policar.[a]

[a] *École Normale Supérieure-PSL Research University, Département de Chimie, Sorbonne Universités – UPMC Univ Paris 06, UMR 7203 CNRS-ENS-UPMC LBM, 24, rue Lhomond, 75005 Paris, France*
[b] *Laboratory of Analytical Chemistry, Analytical Chemistry Group of Paris-Sud (GCAPS-EA 4041), Faculty of Pharmacy, University Paris-Sud, 5 Rue Jean-Baptiste Clément, 92296 Chatenay-Malabry, France*
[c] *SMIS beamline, Synchrotron SOLEIL Saint-Aubin, Gif-sur-Yvette Cedex, 91192, France*

Abstract

Recently, we demonstrated that Single Core Multimodal Probe for Imaging (SCoMPI) could be used for the labelling and the study of the penetration into skin biopsies by fluorescence and infrared imaging of a cell penetrating peptide (CPP), which is used to deliver biological active cargoes into cells.[1]

The aim of this study is to use the same correlative approach to study the penetration of a widely used cosmetic agent: hyaluronic acid (HA). HA is a carbohydrate naturally present in the human body that is commonly used as a wrinkle filler in cosmetic applications.[2] Although HA is often injected into the dermis, it can be applied at the surface of the skin. In order to estimate the penetration after topical application, hyaluronic acids of different molecular weights were labelled with a SCoMPI and their penetration was studied into human skin using both IR microscopy and fluorescence imaging. To label HA, a Ugi reaction was chosen because of its efficiency.[3]

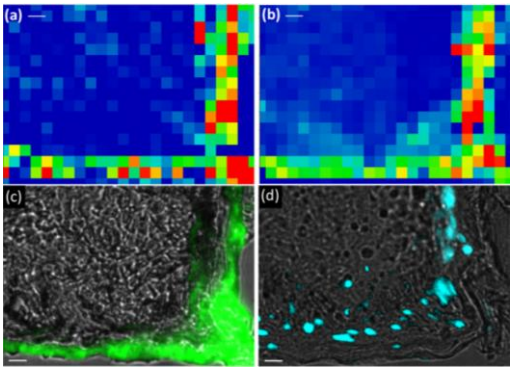


Figure 1. Map of a 10 µm-thick skin section after a 24-hour exposure with a 10 kDa labelled hyaluronic acid, mounted on a CaF_2 window (a-b) SR-FTIR-SM images based on the integration of specific absorption bands: (a) A_1-band (2005-2055 cm^{-1}) (b) CH_2 (2838-2868 cm^{-1}), using a false color code, from blue (low) to red (high intensity), (c) Bright field image merged with the luminescence signal of the SCoMPI (d) Bright field image merged with nuclei staining (Hoechst, blue), scale bar = 10 µm

After a 7-hour exposure, the low molecular weight HA was localized at some hotspots in the *stratum corneum* whereas the high molecular weight HA was not detected at the surface of the skin. After a 24-hour exposure, the low molecular weight HA was homogeneously distributed in the *stratum corneum* but was not detected in deeper layers of the skin (figure 1) whereas the high molecular weight HA was only located at some spots.

This study showed that after a topical application, the effect of HA is limited to the upper layer of the skin, the *stratum corneum*, as no penetration was observed in deeper layers.

References

1) Clede, S.; Delsuc, N.; Laugel, C.; Lambert, F.; Sandt, C.; Baillet-Guffroy, A.; Policar, C., An easy-to-detect nona-arginine peptide for epidermal targeting. *Chem Commun* **2015,** *51* (13), 2687-9.
2) Kogan, G.; Šoltés, L.; Stern, R.; Gemeiner, P., Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnology Letters* **2007,** *29* (1), 17-25.
3) Fudala, R.; Mummert, M. E.; Gryczynski, Z.; Gryczynski, I., Fluorescence detection of hyaluronidase. *Journal of Photochemistry and Photobiology B: Biology* **2011,** *104* (3), 473-477.

Ratiometric or Turn-on Responses of Pyrone-Cored Donor-Acceptor-Donor Fluorophores to Amyloid-β Peptides

Sonam Kim,[a] Hyuck Jin Lee,[b] Eunju Nam,[b] Mi Hee Lim,*[b] and Youngmin You*[a]

[a]Division of Chemical Engineering and Materials Science, Ewha Womans University, Seoul 03760, Republic of Korea; [b]Department of Chemistry, Ulsan National Institute of Science and Technology (UNIST), Ulsan 44919, Republic of Korea ; email : odds2@ewha.ac.kr

Abstract

Alzheimer's disease (AD) is the most common form of dementia. The accumulation of amyloid-β (Aβ) plaques in the cortical brain is recognized as a hallmark of AD.[1] However, the early detection of Aβ aggregate formation remains a significant challenge. Fluorescence techniques are advantages for detecting Aβ species, because they carry capacity for rapid detection, non-invasiveness, and low limits of detection.[2] In this study, we rationally designed a series of pyrone-cored donor–acceptor–donor (D–A–D) triads (SN series), and evaluated their ability for fluorescence detection for Aβ aggregation (from monomers to fibrils). SN molecules spontaneously formed nanoparticles in aqueous solutions, producing *J*-aggregate fluorescence emission with strong intramolecular charge-transfer character. Among the tested SN compounds, SN2 nanoparticles exhibited strong red fluorescence emission at 615 nm which shifted hypsochromically with $\Delta\lambda_{ems}$ = 747 cm^{-1} upon interaction with Aβ species. Control experiments with the use of a cetrimonium bromide (CTAB) surfactant produced fluorescence changes similar to Aβ cases. This result suggests that the ratiometric, fluorescent responses of SN2 could be ascribed to hydrophobic interactions with Aβ species. The fluorescence emission of SN nanoparticles was very sensitive to the identity and morphology of Aβ peptides, enabling fluorescence monitoring of the progress of Aβ aggregation. In addition, the fluorescence signaling was selective to Aβ peptides, as demonstrated by the absence of fluorescence responses to non-amyloidogenic proteins, such as ubiquitin.

References

1) S. J. C. Lee et al., *Chem. Soc. Rev.* **2017**, *46*, 310-323.
2) M. Ono, *Chem. Commun.* **2015**, *51*, 17124-17127.

Visible Light-Driven Photogeneration of Hydrogen Sulfide (H_2S)

Seung Yeon Yi, Yu Kyung Moon, and Youngmin You*

Division of Chemical Engineering and Materials Science, Ewha Womans University, Seoul 03760, Republic of Korea; email : odds2@ewha.ac.kr

Abstract

Hydrogen sulfide (H_2S) has emerged as an important gasotransmitter because it performs a variety of functions in our body, including vasodilation and cytoprotection.[1] For therapeutic purposes, a range of small-molecule H_2S donors have been developed based on H_2S-release strategies, such as hydrolysis and activation by biothiols.[2] It is notable that there have been few researches of the development of photo-induced H_2S donors,[3] although the donors carry enormous advantages with respect to spatiotemporal resolution for H_2S delivery.

In this research, we have developed and evaluated a photo-induced, singlet oxygen ($^1O_2$)-mediated H_2S donor. The donor system was based on polymer nanoparticles of Pluronic F-127 which incorporated a $^1O_2$ photosensitizer $[Ir(ppyOMe)_2(phen)]^+$ and a $^1O_2$-responsive H_2S donor 1,7-diphenylisobenzothiophene (DPBS). A fluorescent H_2S probe SF4 was also included to detect photogenerated H_2S. We hypothesized that, upon photoirradiation of the nanoparticles, $[Ir(ppyOMe)_2(phen)]^+$ converts $^3O_2$ to $^1O_2$ which undergoes a Diels–Alder reaction with DPBS to produce endoperoxide. The endoperoxide is very unstable, and readily hydrolyzed into diketone with producing H_2S. To examine this hypothesis, we prepared polymer nanoparticles of a ternary mixture of 10 μM $[Ir(ppyOMe)_2(phen)]^+$, 500 μM DPBS, and 20 μM SF4. Photoirradiation at 380 nm of an aqueous solution (buffered to pH 7.4; 10 mM PBS, 1.0 mM CTAB) of the nanoparticles produced strong fluorescence turn-on of SF4 due to H_2S generation, in proportion with the duration of photoirradiation time. A photochemical quantum yield as high as 0.2% was determined, using the standard ferrioxalate actinometry. The H_2S generation was ascribed to photosensitization of $^1O_2$, because other reactive oxygen species did not produce H_2S. Finally, the validity of our approach has been successfully demonstrated by employing PtOEP (platinum(II) octaethylporphyrin) which can initiate production of H_2S under green light (500 nm) photoirradiation.

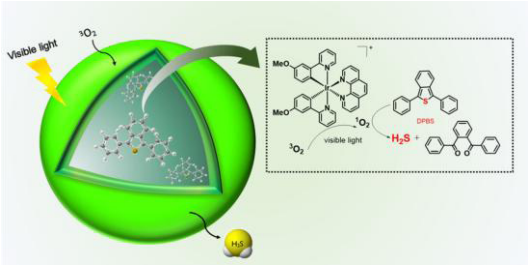


Figure. Schematic diagram showing the mechanism of photogeneration of H_2S

References

1) C. Szabó, *Nat. Rev. Drug Discovery* **2007**, *6*, 917.
2) M.D. Pluth, *Chem. Soc. Rev*. **2016**, *45*, 6108.
3) For examples, M. Xian, *Org. Lett.* **2013**, *15*, 2786; Y. Liu, *Chem. Commun.* **2015**, *51*, 9193; J. C. Scaiano, *J. Am. Chem. Soc*. **2005**, *127*, 7698; H. Nakagawa, *Chem. Commun*. **2014**, *50*, 587; H. Nakagawa, *Bioorg. Med. Chem. Lett*. **2015**, *25*, 175.

Rhenium Carbonyl Complexes for the Labeling and Multimodal Imaging of Biomolecules

S. Hostachy,[a] C. Sandt,[b] A. Somogyi,[b] I. Hamachi,[c] N. Delsuc,[a] C.Policar[a]

[a] Laboratoire des Biomolécules, Département de chimie, Ecole normale supérieure, PSL Research University, Sorbonne Universités, UPMC Univ. Paris 06, CNRS, 24 rue Lhomond, 75005 Paris, France; [b] Synchrotron SOLEIL Saint-Aubin, F-91192, Gif-sur-Yvette Cedex, France; [c] Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, Japan.

email : nicolas.delsuc@upmc.fr

Abstract

Fluorescence microscopy has been widely used over the past decades to study the function, localization and interactions of proteins in their native environment. Small-size probes have therefore been developed for the imaging of proteins not only by fluorescence microscopy but also by interesting alternative methods of imaging.[1,2] For instance, IR imaging, although less sensitive than fluorescence methods, does not induce photo-bleaching or photo-damaging of cells due to the lower energies involved. The most widely used IR-probes are probably those based on metal-carbonyl moiety, as they show attractive properties for bio-imaging[3,4] (e.g. stability in biological environment and strong C=O absorption at 1800-2200 cm^{-1} in the transparency window of the cell). Interestingly, when bound to specific ancillary ligand with low π* orbitals, metal-carbonyl complexes may also be luminescent.[5] Recently, our group has thus developed bimodal probes (IR and luminescent) based on rhenium-carbonyl complexes and used them to label various biomolecules and image them by both IR and fluorescence microscopies.[6] In particular, the spectroscopic properties of two luminescent Re(I) tricarbonyl complexes conjugated with two cell-penetrating peptides have been examined in details. Fluorescence experiments and IR quantification in membrane models and in cells showed unexpectedly strong luminescence enhancement for one of the complexes in lipid environment.[7] We aim now at labeling proteins, using various covalent labeling methods. We labeled *in vitro Engrailed* homeodomain, a protein that can then be internalized into cells and we used the so-called traceless affinity labeling strategy developed in particular by Hamachi et al[2,8] to label human carbonic anhydrase IX, a membrane protein overexpressed in some cancers. The labeling strategies and the imaging of these proteins will be presented.

References

[1] H. M. O'Hare, K. Johnsson, A. Gautier, *Curr. Opin. Struct. Biol.* **2007**, *17*, 488–494.
[2] T. Hayashi, I. Hamachi, *Acc. Chem. Res.* **2012**, *45*, 1460–1469.
[3] K. V. Kong, W. Chew, L. H. K. Lim, W. Y. Fan, W. K. Leong, *Bioconj. Chem.* **2007**, *18*, 1370–1374.
[4] C. Policar, J. B. Waern, M.-A. Plamont, S. Clède, C. Mayet, R. Prazeres, J.-M. Ortega, A. Vessières, A. Dazzi, *Angew. Chem. Int. Ed.* **2011**, *50*, 860.
[5] A. J. Amoroso, M. P. Coogan, J. E. Dunne, V. Fern?ndez-Moreira, J. B. Hess, A. J. Hayes, D. Lloyd, C. Millet, S. J. A. Pope, C. Williams, *Chem. Commun.* **2007**, 3066.
[6] S. Clède, F. Lambert, C. Sandt, S. Kascakova, M. Unger, E. Harté, M.-A. Plamont, R. Saint-Fort, A. Deniset-Besseau, Z. Gueroui, et al., *Analyst* **2013**, *138*, 5627.
[7] S. Hostachy, J.-M. Swiecicki, C. Sandt, N. Delsuc, C. Policar, *Dalton Trans.*, **2016**, *45*, 2791.
[8] K. Matsuo, Y. Kioi, R. Yasui, Y. Takaoka, T. Miki, S. Fujishima, I. Hamachi, *Chem. Sci.* **2013**, *4*, 2573.

Design of *de novo* coiled coils as ligands for catalysis

A. I. O. Almeida, [a] A. F. A. Peacock [a] and P. W. Davies [a]

[a] *University of Birmingham, School of Chemistry; email aia543@bham.ac.uk*

Abstract

Transitions metals are capable of catalysing some of the most versatile chemical transformations. Studies have been made regarding the inclusion of these metals in peptide scaffolds to obtain the highly selective, reactive and sustainable catalytic system.[1] One can expect that the peptide moiety will provide a chemo- and stereoselective environment causing the system to behave in an enzyme-like manner. Known for their high selectivity and specificity, enzymes are highly complex systems. Inclusion of transition metals in proteins with well-defined folding has been achieved, this could be improved by designing a simpler more tuneable *de novo* system.

Early attempts to develop metal-peptide hybrid catalysts for enantioselective organic transformations have been reported. To achieve this goal two different approaches were used. A non-covalent approach provided a chiral and highly functionalized environment for organometallic gold complexes, using a well-structured peptide as an additive in aqueous catalysis. This has been demonstrated by observing the influence of a trimeric coiled coil present in solution with gold(I) complexes, in the catalysis of the hydroxylation of alkynes.[2]

The synthesis of unnatural amino acids has been explored with emphasis upon metal-coordinating species such as phosphites, phosphines and NHC's. Phosphite amino acids were designed based on the reaction of a variety of phosphochloridites and different amino acids with a hydroxyl group side chain.

Despite being in its early stages, preliminary results suggests great potential in achieving a library of well-folded peptide ligands enabling enzyme-like catalysis.

References

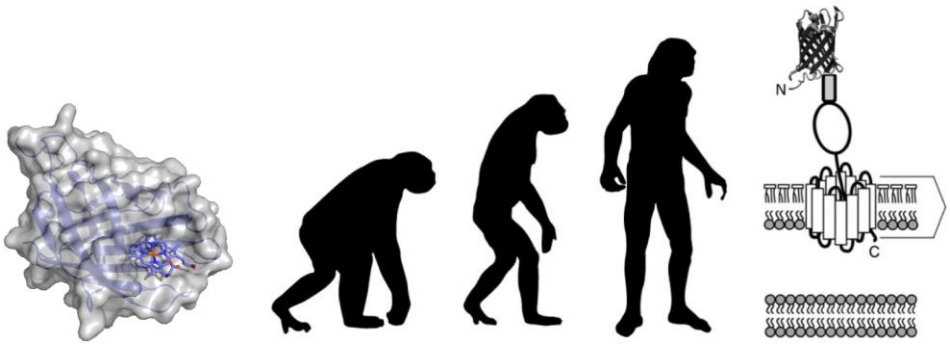
1) i) M. Creus *et al.*, *Org. Biomol. Chem*, 2007, 5, 1835. ii) J. C. Lewis *et al.*, *ACS Catalysis*, 2013, 3, 2954.
2) i) M. Berwick *et al.*, *J. Am. Chem Soc.*, 2014,136,1166. ii) M. Berwick *et al.*, *Chem. Sci.*, 2016, 7, 2207.

Nitrobindin: Evolution of a Protein-Host for Biohybrid Catalysts

Daniel F. Sauer[1], Alexander R. Grimm[2], Akira Onoda[3], Takashi Hayashi[3], Ulrich Schwaneberg[2], Jun Okuda[1]

[1] *Institute of Inorganic Chemistry, RWTH Aachen University, Landoltweg 1, 52074 Aachen, Germany*
[2] *Institute of Biotechnology, RWTH Aachen University, Worringerweg 3, 52074 Aachen, Germany.*
[3] *Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*
daniel.sauer@rwth-aachen.de

Abstract



The heme-containing protein nitrobindin was discovered in 2010.[1] Two years later, the heme-moiety was reconstituted with artificial metal cofactors to perform polymerization of phenylacetylene[2,3] and to evolve hydrogen.[4] In the polymerization reaction, the *trans*/*cis*-selectivity was nearly inverted by the protein sphere from 82/18 to 22/78 and with the hydrogenase model, the activity could be increased in comparison to the protein free catalyst. Furthermore, incorporation of a Grubbs-Hoveyda type complex to construct an artificial metathease resulted in an unexpected highly active catalyst for the ring-opening metathesis polymerization (ROMP).[5] This activity shows realtion to the hydrophobic cavity structure, that is provided by the protein nitrobindin.

In the recent work, we applied new strategies on the protein scaffold to generate a versatile platform for biohybrid catalyst design, either in solution or as diffusion-barrier free whole-cell systems.

References

1) Bianchetti, C. M.; Blouin, G. C.; Bitto, E.; Olson, J. S.; Phillips, G. N., The structure and NO binding properties of the nitrophorin-like heme-binding protein from Arabidopsis thaliana gene locus At1g79260.1, Proteins: Struct., Funct., Bioinf., 2010, 78, 917.
2) Onoda, A.; Fukumoto, K.; Arlt, M.; Bocola, M.; Schwaneberg, U.; Hayashi, T., A rhodium complex-linked β-barrel protein as a hybrid biocatalyst for phenylacetylene polymerization, Chem. Commun., 2012, 48, 9756.
3) Fukumoto, K.; Onoda, A.; Mizohata, E.; Bocola, M.; Inoue, T.; Schwaneberg, U.; Hayashi, T., Rhodium-complex-linked hybrid biocatalyst: Stereo-controlled phenylacetylene polymerization within an engineered protein cavity, ChemCatChem., 2014, 6, 1229.
4) Onoda, A.; Kihara, Y.; Fukumoto, K.; Sano, Y.; Hayashi, T., Photoinduced Hydrogen Evolution Catalyzed by a Synthetic Diiron Dithiolate Complex Embedded within a Protein Matrix, ACS Catal., 2014, 4, 2645.
5) Sauer, D. F.; Himiyama, T.; Tachikawa, K.; Fukumoto, K.; Onoda, A.; Mizohata, E.; Inoue, T.; Bocola, M.; Schwaneberg, U.; Hayashi, T.; Okuda, J., A Highly Active Biohybrid Catalyst for Olefin Metathesis in Water: Impact of a Hydrophobic Cavity in a β-Barrel Protein, ACS Catal., 2015, 5, 7519.

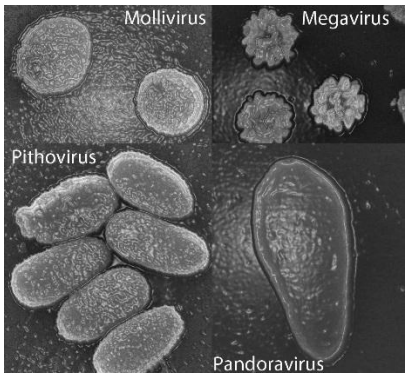
Structural characterization of a novel iron-sulfur proteins family in giant viruses

Burlat, B. [a]; Lartigue, A.[b] ; Couillaud, J.[a] ; Byrne, D.[c] ; Chaspoul, F.[d] ; Guigliarelli, B. [a] ; and Abergel, C.[b].

CNRS & Aix-Marseille University ; [a]Bioénergétique et Ingénierie des Protéines, UMR7281, 13402 Marseille ; [b]Information Génomique et Structurale, CNRS-AMU UMR 7256, 13288 Marseille ; [c]Plateforme d'Expression des Protéines, Institut de Microbiologie de la Méditerranée, FR3479, 13402 Marseille ; [d]Institut Méditerranéen de la Biodiversité et d'Ecologie marine et continentale, UMR 7263, 13288 Marseille, France ;
email : burlat@imm.cnrs.fr

Abstract

Giant viruses were discovered about more than a decade ago (1). They display unexpected features for viruses, namely the size of the viral particle higher than 0.5 µm and complex genomes containing from 500 to 2500 genes, depending on the virus. Most of their genes encode proteins never encountered before in a virus. Four families are identified to date, Mimiviridae, Pandoraviridae, Pithoviridae and Molliviridae (see SEM pictures above, ©IGS), and it is believed that these viruses are widespread and abundant in the environment (2).



Transcriptional analysis of Mimivirus infecting *Acanthamoeba* hosts revealed transcripts corresponding to unpredicted genes (3). Among them, the most transcripts encode a small protein of 6kDa featuring a sequence mainly made of glycine and cysteine. This protein is among the most abundant proteins in the viruses and is conserved among the Mimiviridae. While its function remains unknown, it is believed that this protein is essential in the infection process of Mimiviridae.

In the work herein, we present a structural characterization of this new proteins family by combining UV-Visible and EPR spectroscopies to elementary analysis (Fe, acid-labile sulfide) and *in vitro* Fe-S reconstitutions. Our first results suggest that this viral protein, named GG-FeS, houses a new type of iron-sulfur (FeS) cluster, different from those found so far in the cellular world (rubredoxin-like, [2Fe-2S], [3Fe-4S], [4Fe-4S]) (4) and which could be the signature of the ancestral proto-cell and of specific function not selected by the cellular world.

References

1. La Scola, B., Audic, S., Robert, C., Jungang, L., de Lamballerie, X., Drancourt, M., Birtles, R., Claverie, J. M.; Raoult, D. *Science* 2003, *299*, 2033.
2. Abergel, C., Legendre, M. & Claverie, J.-M. *FEMS Microbiol. Rev.* 2015, *39*(6), 766.
3. Legendre, M., Audic, S., Poirot, O., Hingamp, P., Seltzer, V., Byrne, D., Lartigue, A., Lescot, M., Bernadac, A., Poulain, J., Abergel, C., Claverie, J. M. *Genome Res* 2010, *20*, 664.
4. Beinert, H. *Science* 1997, *277*, 653.

.

Characterization of the CODHs from Thermococcus AM4

Martino Benvenuti[a], Vincent Fourmond[a], Christophe Léger[a], Sebastien Dementin[a]

Laboratoire de Bioénergétique et Ingénierie des Protéines – UMR 7281 CNRS,

e-mail : mbenvenuti@imm.cnrs.fr

Abstract

Carbon monoxide dehydrogenases (CODHs) are metallo-enzymes that catalyze the reversible interconversion of CO and CO_2 according to the reaction

$$CO + H_2O \leftrightarrow CO_2 + 2H^+ + 2e^-$$

These homodimeric enzymes harbor five clusters (D, B and C clusters) of three different types[1],[2]. The B and D clusters are classical FeS clusters. The C-cluster is the active site where the chemical reaction occurs. Different models of the structure of the C-cluster have been proposed but it is now accepted that its chemical composition is a [Ni-4Fe-4S].

The folding and the metallocluster assembly of CODHs are key steps for the production of the active enzyme. Several accessory genes, CooC, CooJ CooT, seem to be involved in the assembly of the C-cluster[3]. In the CODH operons of *Desulfovibrio vulgaris* and *Carboxydothermus hydrogenoformans* some of the accessory genes are not present[4],[5], which raises the question of their functionality in the C-cluster assembly.

Thermococcus AM4 produces two uncharacterized CODHs (CooS-582 and CooS-1067) whose physiological functions and catalytic properties are unknown. The operons of these two CODHs contain CooC but neither CooJ nor CooT and we observed that in the operon of CooS-528 there are several genes, with unknown function, that can be important for the maturation of the protein.

The aim of my study is the biochemical and electrochemical characterization of these two novel CODHs, which I have produced and purified for the very first time, and to assess the function of CooC in the assembly of the C-cluster.

References

(1) Drennan, C. L. *et al Proc. Natl. Acad. Sci.* **2001**, *98* (21), 11973–11978.

(2) Jeoung, J.-H.; Dobbek, H. *Science.* **2007**, *318* (5855), 1461–1464.

(3) Kerby, R.L *et al*. *J Bacteriol*, **1997**, 197(7), 2259-66

(4) Hadj-Said, J.*et al*. *Biochim. Biophys. Acta - Bioenerg.* **2015**, *1847* (12), 1574–1583.

(5) Wu M, Ren Q, *et al PLoS Genet 1***2005** (5): e65.

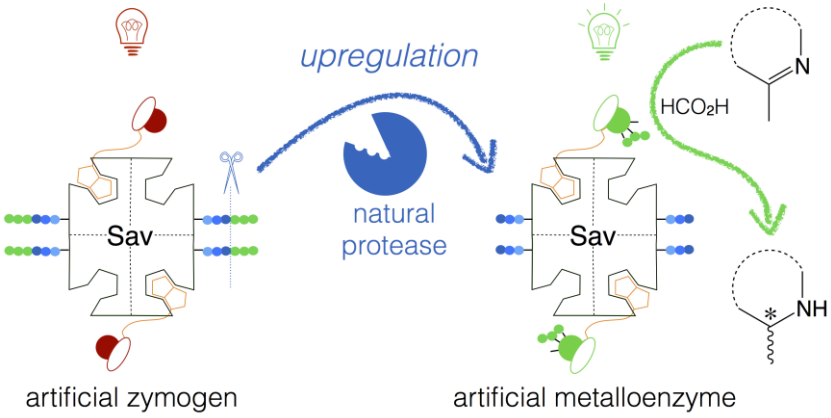
Development of an artificial zymogen

Zhe Liu,[a] Vincent Lebrun,[a] Taku Kitanosono,[b] Hendrik Mallin,[a] Valentin Köhler,[a] Daniel Häussinger,[a] Donald Hilvert,[c] Shu Kobayashi[b] and Thomas R. Ward[a]

[a]*Department of Chemistry, University of Basel, 4056 Basel, Switzerland*
vincent.lebrun@unibas.ch ; [b]*Department of Chemistry, School of Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan* ; [c]*Laboratory of Organic Chemistry, ETH Zürich, 8093 Zürich, Switzerland.*

Abstract

Complex biochemical cascades, which are essential to sustain life, rely on tightly cross-regulated enzymatic processes. Several complementary mechanisms are used by the cell to achieve such exquisite regulation including (i) the control of the expression, (ii) chemical modification, (iii) allosteric regulation and (iv) selective proteolysis by protease.[1] The latter strategy consists in expressing an enzyme in an inactive state, also called zymogen, and activating it via partial proteolysis by a cognate protease. In recent years, artificial metalloenzymes, resulting from anchoring an organometallic catalyst within a macromolecular scaffold, have emerged as an attractive alternative to organometallic catalysts.[2,3] With the aim of developing a biocompatible strategy for the upregulation of an artificial metalloenzyme, we report the development and the genetic optimization of an artificial zymogen requiring the action of a natural protease to unleash its latent asymmetric transfer hydrogenase activity.[4]



References

1) J. M. Berg, J. L. Tymoczko, L. Stryer, *Biochemistry, 7th Edition*, W H Freeman, New York, **2012**.
2) O. Pàmies, M. Diéguez, J.-E. Bäckvall, *Adv. Synth. Catal.* **2015**, *357*, 1567–1586.
3) T. Heinisch, T. R. Ward, *Eur. J. Inorg. Chem.* **2015**, 3406–3418.
4) Z. Liu, V. Lebrun, T. Kitanosono, H. Mallin, V. Köhler, D. Häussinger, D. Hilvert, S. Kobayashi, T. R. Ward, *Angew. Chem. Int. Ed.* **2016**, *55*, 11587–11590.

Exploring bioinspired copper peptides as oxidation catalysts

Alexandre Hautier, Thierry Tron, Olga Iranzo,
Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, Marseille, France
olga.iranzo@univ-amu.fr

With the increasing concerns about the environmental protection, the development of sustainable chemical processes has become one of the most challenging topic in chemistry. To serve this interest, catalysis has gained a lot of interest since it allows efficient transformations at the same time that reduces or eliminates the use and the generation of hazardous substance.

In this regard, Nature has been a source of inspiration for chemists for its capability to perform challenging chemical transformations with high rates and high selectivity under mild conditions, in water. For example, copper dependent proteins can react with O_2 and H_2O (or H_2O_2) to perform oxidative and oxygenation reactions toward a broad range of substrates,[1,2] whose products are of great interest for pharmaceutical and chemical industries.

With the idea of combining the best of homogeneous catalysis and bio-catalysis, numerous artificial metalloenzymes have been developed.[3,4] However, it is quite often hard to predict their reactivity and their stability. In recent years, small metallopeptides have emerged as a new interesting platform to evolve new catalysts because they are easier to synthesize, handle and fine-tune.

Our group have been developing a family of His-containing peptides whose backbones have different degrees of conformational constrains.[5–7] Their copper(II) coordination properties were studied using pH potentiometry and different spectroscopic methods (UV-Vis, CD, EPR and NMR). The results indicate the formation of a similar major copper(II) species at close to neutral pH value where copper(II) is coordinated to the His. Nonetheless, due to the distinct flexible nature of their scaffolds and the presence of an Asp residue different stability constants as well as copper(II) exchange rates and redox potentials (Cu(II)/Cu(I)) were observed.

In this poster, we will present our recent studies where we are investigating their catalytic activities for the oxidation of different substrates. Our preliminary data suggest that all the complexes are able to catalyze the oxidation of sulfides in aqueous solution and at room temperature. Interestingly, although all the copper(II) complexes are chemoselective (sulfoxide product), their catalytic efficiencies are different reflecting somehow their intrinsic properties.

References

[1] J. P. Klinman, *Chem. Rev.* **1996**, *96*, 2541–2562.
[2] E. I. Solomon, D. E. Heppner, E. M. Johnston, J. W. Ginsbach, J. Cirera, M. Qayyum, M. T. Kieber-Emmons, C. H. Kjaergaard, R. G. Hadt, L. Tian, *Chem. Rev.* **2014**, *114*, 3659–3853.
[3] J. C. Lewis, *ACS Catal.* **2013**, *3*, 2954–2975.
[4] F. Yu, V. M. Cangelosi, M. L. Zastrow, M. Tegoni, J. S. Plegaria, A. G. Tebo, C. S. Mocny, L. Ruckthong, H. Qayyum, V. L. Pecoraro, *Chem. Rev.* **2014**, *114*, 3495–3578.
[5] A. Fragoso, R. Delgado, O. Iranzo, *Dalton Trans.* **2013**, *42*, 6182.
[6] A. Fragoso, P. Lamosa, R. Delgado, O. Iranzo, *Chem. - Eur. J.* **2013**, *19*, 2076–2088.
[7] A. Fragoso, T. Carvalho, P. Rousselot-Pailley, M. M. Correia dos Santos, R. Delgado, O. Iranzo, *Chem. - Eur. J.* **2015**, *21*, 13100–13111.

Selectivity in FhuA ΔCVF^{tev} biohybrid catalyst

M. A. Stephanie Mertens,[1] Marcus Arlt,[1] Daniel Sauer,[2] Andreas Thiel,[2] Hassan Osseili,[2] Takashi Hayashi,[3] Jun Okuda,[2] Ulrich Schwaneberg[1]

[1] *Institute of Biotechnology, RWTH Aachen University, Worringerweg 3, 52074 Aachen, Germany.*
[2] *Institute of Inorganic Chemistry, RWTH Aachen University, Landoltweg 1, 52074 Aachen, Germany*
[3] *Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*
u.schwaneberg@biotec.rwth-aachen.de

Abstract

The use of biohybrid catalysts enables highly active and selective reactions like the ring-opening metathesis polymerization[1,2], demonstrating the effect of a protein scaffold surrounding a transition metal catalyst. The outer membrane protein FhuA of *Escherichia coli* provides a suitable second ligand sphere for such catalysts, as it shows high stability due to its β-barrel structure and a wide inner channel diameter to host large catalysts and substrates.[2]

FhuA ΔCVF^{tev} based biohybrid catalysts allow the combination of reactivity in chemical catalysis and selectivity in biocatalysis. Structural characteristics of this biohybrid catalysts are a single cysteine at position 545 for covalent coupling of a catalyst through a maleimide moiety.[2] TEV-protease cleavage sites included in two loops enable analysis of covalently bound catalyst by MALDI-TOF-MS.

FhuA ΔCVF^{tev} based biohybrid catalysts constructed in previous work resulted in the artificial metathease FhuA-Grubbs-Hoveyda[1,2], active in various metathesis reactions and the artificial Diels-Alderase FhuA-Cu(II)-terpy[3].

References

1. Sauer, D. F. *et al., Chem. Asian J.* **10,** 177–182 (2015)
2. Philippart, F. *et al., Chem. Eur. J.* **19,** 13865–13871 (2013)
3. Osseili, H. *et al., Bellstein J. Org. Chem*. **12,** 1314–1321 (2016)

Financial support by DFG and SeleCa is gratefully acknowledged.

Synthesis and reactivity of dinuclear copper complexes inspired by tyrosinase active site

Eliana Lo Presti ,[a] Enrico Monzani,[a] Luigi Casella[a]

[a] *University of Pavia, Viale Taramelli 12, 27100, Pavia ; email : eliana.lopresti01@gmail.com*

Abstract

Metal containing enzymes inspired several bioinorganic chemists who wanted to replicate thermodynamically unfavourable selective reactions in mild conditions. This research work is centred on mimicking the structure and activity of one of those enzymes, tyrosinase. The active site of tyrosinase is basically represented by a dinuclear copper complex in which each copper ion is coordinated by three histidine residues, to give a final hexa-coordinated chiral nitrogen environment. Several attempts of mimicking the structure and the activity of this enzyme were done in the past few decades, but only few models able to catalyze stereoselective copper-mediated oxidations were presented. To replicate the features of this active site, dinucleating ligands, able to host two copper ions, were synthesized. The dinuclear copper complexes of these ligands (Figure 1) were tested in their capability to promote typical tyrosinase activity, catecholase and monophenolase activity. With regard to catecholase activity, a set of enantiomeric couples of chiral catechols of biological interest were chosen as substrates (L/D-dopa, L/D-dopa methyl esters, R/S-norepinephrine) and the best discrimination was found, in almost all cases, with the methyl esters of dopa. Monophenolase activity was studied with small functionalized phenols, but in the majority of cases a radical pathway was preferred rather than a genuine tyrosinase-like hydroxylation.

Tyrosinase was more recently found to catalyze enantioselective oxidation of aryl-alkyl sulphides to sulfoxides, in presence of L-dopa as sacrificial reducing agent, with an enantiomeric excess up to 90 % [1]. The synthesized model complexes were employed in an analogue reactivity, with hydroxylamine hydrochloride as co-reducing agent. In the majority of cases (Figure 1- a); b); c)), poor reaction yields, accompanied by low enantiomeric excesses were detected. An increase of enantiomeric excess (up to 40 %) was detected with $[Cu_2(mXPhI)](ClO_4)_4$ as catalyst and thioanisole as substrate. 18-O incorporation experiment confirmed that the inserted oxygen atom derived from the atmospheric oxygen. This is, so, the first example of copper-mediated asymmetric sulfoxidation, promoted by molecular dioxygen as oxidant.

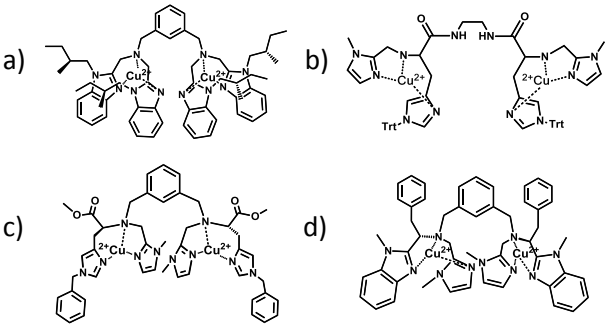


Figure 1. Synthesized dicopper complexes. a) $[Cu_2(L55Bu_4*)]^{4+}$ [2]; b) $[Cu_2(EHI)]^{4+}$ [3]; c) $[Cu_2(mXHI)]^{4+}$; d) $[Cu_2(mXPhI)]^{4+}$

References

1) R. Pievo, M. Gullotti, E. Monzani, L. Casella, *Biochemistry*, **2008**, 47, 3493–3498;

2) M. Perrone, E. Lo Presti, S. Dell'Acqua, E. Monzani, L. Santagostini, L. Casella, *EurJIC*, **2015**, 21, 3493–3500;

3) M. Perrone, E. Salvadeo, E. Lo Presti, L. Pasotti, E. Monzani, L. Santagostini L., L. Casella, *Dalton Transaction,* **2017,** 46, 4018-4029.

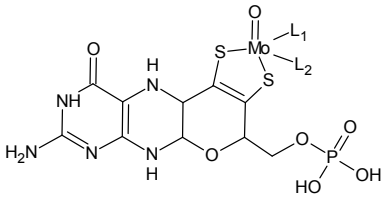
Pyrazine-pyran-dithiolene complexes of molybdenum mimicing specific features of the molybdenum cofactor (MoCo)

Claudia Schindler,[a] Carola Schulzke[a]

[a] *Universität Greifswald, Felix-Hausdorff-Straße 4, 17487 Greifswald, email: claudia.schindler@uni-greifswald.de*

Abstract

Molybdopterin plays an essential role in nature because it forms the molybdenum cofactor, which serves as a catalytic center for various enzymes. While the biological synthesis of MoCo is well understood, a chemical synthetic pathway is very challenging and is still being studied. The aim of the project is the synthesis of model compounds for the molybdopterin depending cofactor, mimicing specific features of MoCo. The focus of our research is the development of ligand precursors that address the dithiolene function, the pyran ring and the adjacent pyrazine ring and their coordination with especially molybdenum, but also the development of novel, alternative model compounds which are able to catalyse oxygen atom transfer and which can be incorporated into the apoenzyme.

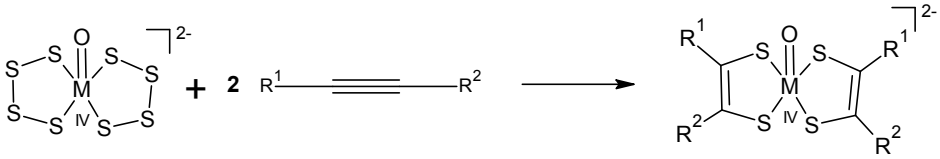


molybdenum cofactor

L_1, L_2 = O, OR, SR

For complex formation with suitable ligands, two different approaches were carried out:

1. the formation of complexes by reaction of a triple bond with $(Et4N)_2[MoO(S4)_2]$: The cycloaddition of alkynes with the M-S4 moiety of $(Et4N)_2[MoO(S4)_2]$ precursors results in the formation of a dithiolene complex ion of the structure $[MoO\ (S_2C_2\ (R_1R_2))_2]^{2-}$, where the metal does not change its oxidation state.



M = Mo, W

2. The reaction with the precursor complex $K_3Na[MoO_2(CN)_4]\bullet 6H_2O$:

By using $K_3Na[MoO_2(CN)_4]\bullet 6H_2O$ precursor in complex formation water-soluble model complexes are formed with properties being essential for applications in biological studies.

Financial support of the European Research Council is gratefully acknowledged.

References

1) C. Schindler, C. Schulzke, Chemistry of Heterocyclic Compounds, 2015.

2) D. Coucouvanis, A. Iladjikyriacou, A. Toupadakis, Sang-Man Koo, O. Ileperuma, M. Draganjac, A. Salifoglou, *Inorg. Chem.*, **1991**, *30*,154-161.

3) Davies, E. S.; Beddoes, R. L.; Collison, D.; Dinsmore, A.; Docrat, A.; Joule, J. A.; Wilson, C. R.; Garner, C. D., J. Chem. Soc., Dalton Trans. 1997

4) Davies, E. S.; Aston, G. M.; Beddoes, R. L.; Collison, D.; Dinsmore, A.; Docrat, A.; Joule, J. A.; Wilson, C. R.; Garner, C. D., J. Chem. Soc., Dalton Trans. 1998

Determination of Thermodynamic Parameters of Cyclophane-Cu^{2+} Complex and Its Open Chain Analogous By Isothermal Titration Calorimetry (ITC)

Rocio Sugich-Miranda[a], Hisila Santacruz[b], Enrique Velázquez[a,b], Fernando Rocha[a]; Melissa Beltrán-Torres[a]

[a] Departamento de Cs. Químico Biológicas. Universidad de Sonora. Hermosillo, Sonora, México, CP 83000 ; [b] Departamento de Investigación en Polímeros y Materiales. Universidad de Sonora. Hermosillo, Sonora, México, CP 83000; email : rsugich@polimeros.uson.mx

Abstract

The isothermal titration calorimetry (ITC) is a widely used biophysical technique to study the formation or dissociation of molecular complexes (Falconer, 2016) through which a complete thermodynamic characterization of the ligand-substrate interaction (?H, Ka, N, ?G and ?S). On the other hand, it is well known that in general, the complexes with macrocyclic ligants may be more thermodynamically stable than their open chain analogous. Therefore, the objective of the present work was to measure the thermodynamic parameters of the formation in aqueous medium of the Cu2+ complexes with two EDTA-derived ligants, one with a macrocyclic structure (edtaOD) and the other with an open chain (EdtaBZ). The relevance of studying the behavior of the formation of these complexes is that both have SOD activity, and their analogous compounds (previously reported) are non-toxic on peripheral blood mononuclear cells. The measurements were carried out in a MicroCal VP-ITC microcalorimeter with 0.1mM binder solutions and 0.6mM CuCl2 solutions at pH=5.0, 7.4 and 9.0. The behavior of the thermograms showed comparable stabilities but significant differences between the studied species, in some cases attributable to the coordination geometry (pH effect).

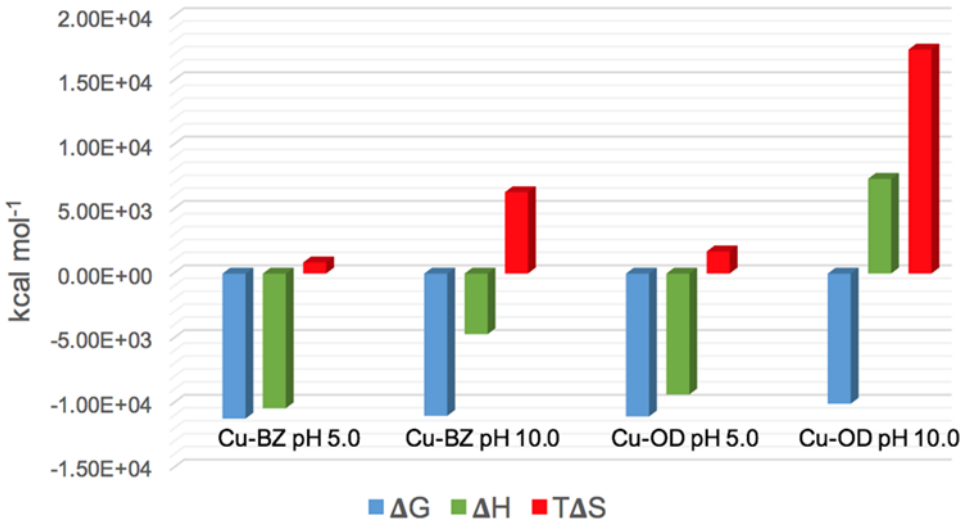


Figure 1. Thermodynamic parameters of cyclophane-Cu2+ complexes and its open chain analogous.

References

1) Salazar-Medina AJ, *Molecules*, 2013, 29; 18(2):1762-74. DOI: 10.3390/molecules18021762.
2) Sugich-Miranda, *J Biochem Mol Toxicol.*, 2010, 24(6):379-83. DOI: 10.1002/jbt.20350

Synthesis of Isoquinoline Derivatives from Oxime and Alkyne Catalyzed by a RhCp*-Linked β-Barrel Protein

Shunsuke Kato, [a] Kengo Tachikawa, [a] Akira Onoda, [a] Takashi Hayashi, [a]

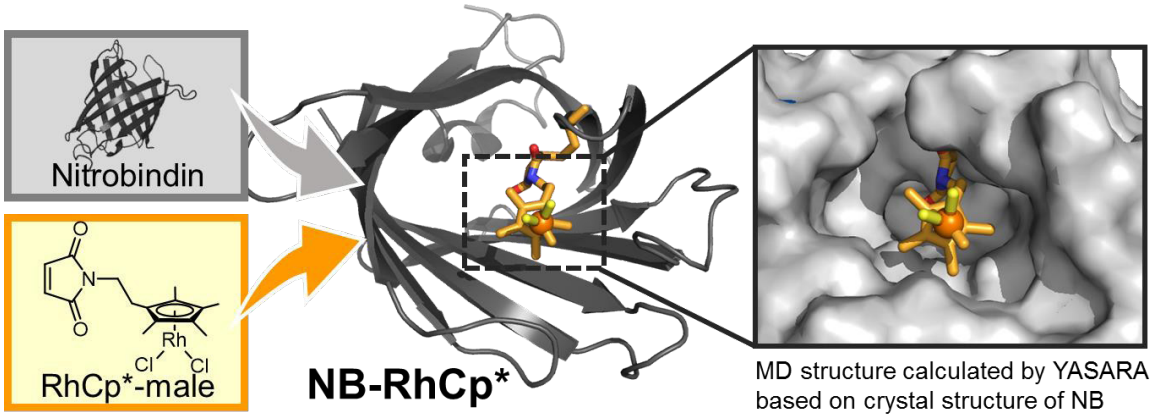
[a] *Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, 565-0871, Japan*

email : s_kato@chem.eng.osaka-u.ac.jp

Abstract

Enzymes as a biocatalyst that efficiently promote a series of vital reactions with high substrate specificity and/or high regio- and stereo-selectivities under mild conditions. The attractive features of natural enzymes are derived from the evolutionally-optimized first and second coordination spheres of the active site. To extend the versatility of the enzymes, incorporation of artificial transition metals as an active site into a protein scaffold has been attracted over decades. Combining non-native transition metal with a protein scaffold leads to the construction of a tailored enzyme, biohybrid catalyst, which exhibits non-natural reactivity and enzyme-like selectivity using engineered second coordination sphere.[1]

Along this concept,[2] we have recently constructed a new biohybrid catalyst, in which a RhCp* complex with a maleimide group (RhCp*-male) was covalently linked to the hydrophobic cavity of nitrobindin (NB) with β-barrel structure. The precise conjugation of RhCp*-male with NB yielding the NB-RhCp* conjugate was confirmed by MALDI-TOF MS and UV-vis. We then performed the cycloaddition of oximes and alkynes to synthesize isoquinoline derivatives using the NB-RhCp* conjugate. The reactions using the biohybrid catalyst NB-RhCp* toward the synthesis of isoquinoline derivatives will be presented.



References

(1) T. R. Ward *et al.* Recent achievments in the design and engineering of artificial metalloenzymes. *Curr. Opin. Chem. Biol.* **2014**, *19*, 99.

(2) T. Hayashi *et al*. A rhodium complex-linked β-barrel protein as a hybrid biocatalyst for phenylacetylene polymerization, *Chem. Commun.* **2012**, *48*, 9756.

(3) S. Chiba *et al*. Rhodium(III)-Catalyzed Synthesis of Isoquinolines from Aryl Ketone *O*-Acyloxime Derivatives and Internal Alkynes. *Org. Lett*. **2010**, *12*, 5688.

Electronic Structure of Metal (II) Methylthiophenoxyl Radical Complexes

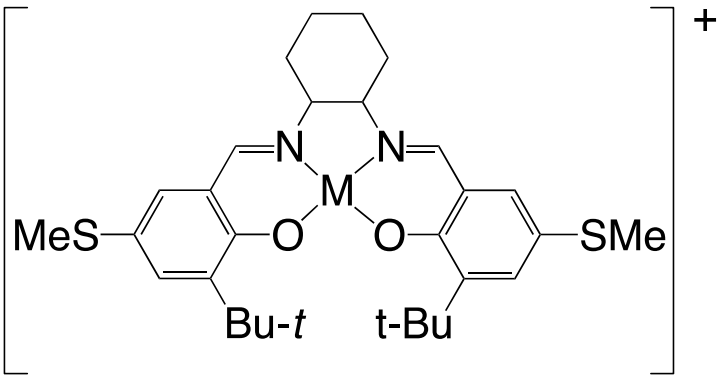
Yuichi Shimazaki

College of Science, Ibaraki University ; email : yshima@mx.ibaraki.ac.jp

Abstract

The Cu(II)–phenoxyl radical formed during the catalytic cycle of galactose oxidase (GO) attracted much attention, and the structures and properties of a number of metal–phenoxyl radical complexes have been studied.[1] Some of functional model system of GO have been reported previously that the Cu complexes showed the oxidation of primary alcohols to aldehydes, and formation of the Cu(II)-phenoxyl radical species was revealed in the catalytic cycle. The phenoxyl radical in GO has carbon-sulfur covalent bond at *ortho*-position of the phenol ring. However, properties and detailed electronic structures of the methylthiophenoxyl radical coordinated metal complexes have been reported only a few.

As an extension of the studies on one-electron oxidized Cu(II)–phenolate species and a clarification of role of the methylthio group of phenoxyl radical in GO, we synthesized one-electron oxidized square-planar Cu(II) and group 10 metal(II) complexes of di(*p*-methylthiophenolate) Schiff base ligands and characterized their geometric and electronic structures and reactivity. The geometric and electronic structures difference dependent with the central metal ion in di(*p*-methylthiophenolate) Schiff base ligands will be mainly discussed in this presentation.



M = Cu, Ni, Pd, Pt

References

1) Y. Shimazaki, "Phenoxyl radical metal complexes", in *The Chemistry of Metal Phenolates*; Zabicky, J., Ed.; Wiley: Chichester, U.K., **2014**; pp 593.

Electrochemical studies of FeFe hydrogenase from *Chlamydomonas reinhardtii*: kinetics of gas binding and release

M. del Barrio, C. Baffert, V. Fourmond, C. Leger

Laboratoire de Bioénergétique et Ingénierie des Protéines, Aix-Marseille Université/CNRS UMR 7281
Marseille, FRANCE
mdelbarrio@imm.cnrs.fr

FeFe hydrogenases are the most efficient biological catalysts of hydrogen production. Their active site, the H-cluster, consists of a $[Fe_2(CO)_3(CN)_2$(dithiomethylamine)] subsite covalently bound to a cubane [4Fe4S] subcluster. It is known that the inhibitors O_2 and CO bind the vacant site on the Fe that is remote to the cubane, but to completely uncover their mechanisms of inactivation, we have to keep in mind that they also involve the diffusion of these gases from the solvent and the subsequent reactions at the active site.[1]

We used protein film voltammetry (whereby the enzyme is adsorbed onto an electrode and electron transfer is direct) to characterize the kinetics of inhibition of the FeFe hydrogenase from the photosynthetic organism *Chlamydomonas reinhardtii* (*Cr*) and various mutants, obtained by substitution of the V296 (an amino acid present in the vicinity of the H-cluster). The binding and release rate constants of the competitive inhibitor CO were determined. This provides information about the diffusion of O_2, since both gases, at least for NiFe hydrogenases, use the same pathway to access the active site. [2] Their Michaelis constants for H_2 have been calculated and the inhibition by O_2 has also been studied. The differences in the kinetic properties found between the enzymes tested (such as those shown in the figure for CO inhibition) evidence the impact of the amino acid side chains on the diffusion rates to the active site.

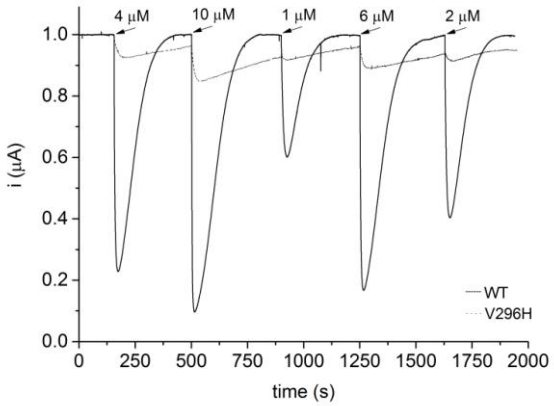


Figure: Effect of V296H substitution on the inhibition by CO of *Cr* FeFe hydrogenase (WT, thick line; V296H, dashed line). Catalytic activity, recorded as a current of H_2 oxidation, changes with the exposure to inhibitor (amounts injected are indicated on the graph).

References

1) Kubas, A. *et al*. Mechanism of O_2 diffusion and reduction in FeFe hydrogenases. Nat Chem. 9, 88–95 (2017)
2) Liebgott, P.-P. *et al*. Relating diffusion along the substrate tunnel and oxygen sensitivity in hydrogenase. Nat. Chem. Biol. 6, 63–70 (2010)

SOD activity and O_2 activation by Mn-Schiff-base complexes. "All has not been said!"

Palopoli, Claudia[a]; Bernatene Ezequiel[a]; Disetti, María Eugenia[a]; Amandine Conte-Daban[b], Christelle Hureau[b], Elodie Anxolabéhère Mallart[c], Sandra Signorella[a]

[a] *Instituto de Química Rosario IQUIR-CONICET/ Fac. de Cs. Bioq. y Farm. UNR. Suipacha 531.S2002LRK Rosario, Argentina*

[b] *Laboratoire de Chimie de Coordination CNRS Toulouse. 205 Route de Narbonne 31077 Toulouse cedex 4-France*

[c)] *Laboratoire d'Electrochimie Moléculaire UMR 7591 Univ Paris Diderot, Sorbonne Paris Cité, 15 rue Jean-Antoine de Baïf, Paris, France*

email : palopoli@iquir-conicet.gov.ar

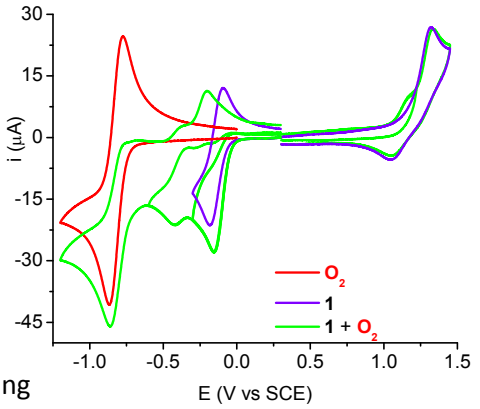
Abstract

A number of mononuclear Mn complexes of Schiff base ligands have been proven to be good structural mimics of MnSOD enzymes and some of them have been tested as ROS scavengers.[1,2] However, little is known about the mechanism of the redox processes.

In this work we obtained four Mn^{III} complexes with the tetradentate N_2O_2 ligands 1,3-bis(3,5-X_2salicylidenamino)propane (3,5-X_2salpn), where X = H, Cl, F and NO_2, evaluated their SOD activity and studied the reaction of O_2 with the electrochemically generated Mn(II) complexes in order to provide new insights on the key intermediates involved in the redox reactions.

The studied complexes have the formulas $[Mn(salpn)(H_2O)_2]ClO_4{\cdot}H_2O$ (**1**), $[Mn(3,5\text{-}F_2salpn)(H_2O)_2]PF_6$ (**2**), $[Mn(3,5\text{-}Cl_2salpn)(H_2O)_2]ClO_4$ (**3**) and $[Mn(3,5\text{-}(NO_2)_2salpn)(H_2O)_2]$ (**4**), and were obtained by monodeprotonation of the Schiff base in MeOH/EtOH with 1 equiv. of aqueous NaOH, followed by addition of 1 equiv. of $Mn(ClO_4)_2$. Complexes **1** - **3** contain Mn(III), while **4** is a Mn(II) complex.

Complexes **1** - **4** react with superoxide and their SOD activity was determined by measuring inhibition of the NBT photoreduction. For these complexes, IC_{50} values were in the 2.9 – 4.9 range, indicating that SOD activity is only slightly affected by the substituent.



The electrochemically reduced forms of complexes **1** - **3** showed ability to react with O_2. These complexes are reversibly reduced to the Mn(II) form, in deoxygenated DMF, at -153, 31 and 145 mV vs SCE, respectively. Cyclic voltammetry in the presence of O_2 exhibited new redox processes together with the loss of reversibility of the one electron reduction wave of the starting complex, indicating reaction of the reduced complex with O_2. The intensity of the reduction peak of the Mn(II)-O_2 adduct increased with $[H^+]$. EPR and electronic spectroscopy of complex + O_2 mixtures at different controlled potentials were used to characterize species formed in the redox processes.

References

1) O. Iranzo, Bioorg. Chem., 2011, 39, 73–87.

2) C. Palopoli, G. Gomez, A. Foi, F. Doctorovich, S. Mallet-Ladeira, C. Hureau, S. Signorella, J. Inorg. Biochem., 2017, 167, 49-59.

The hidden effect in the stability diversification of classical zinc fingers

Justyna Adamczyk and Artur Krężel

Department of Chemical Biology, Faculty of Biotechnology, University of Wroclaw, Joliot-Curie 14, 50-383, Wroclaw, Poland;

E-mail : justyna.adamczyk@uwr.edu.pl; artur.krezel@uwr.edu.pl

Abstract

Bioinformatic analysis of human proteome shows that the most common ββα zinc fingers (ZFs) group differs in the number of residues present between binding cysteines $CX_{1-7}C$. Extensive research has shown that Zn^{2+} ion affinities to ZF proteins (K_d: 10^{-8}-10^{-15} M) influence complex stability and might be essential for the controlling of cellular free Zn^{2+} concentration that varies from 10^{-9} to 10^{-11} M.[1] In this work structural analysis of distorted classical ZFs and their comparison with canonical motifs have been studied. We examined which factor may influence on Zn^{2+} affinity to ββα zinc fingers and thus affects diversification. We believe that the formation of β-loop in ZF is a crucial part in the initiation of metal ion binding process and entire domain stabilization. Reorganization in β-loop has particular impact on both NH···O and NH···S the hydrogen bond network. Therefore, our research are focused on the localization and amount of hydrogen bonds in zinc fingers. To map hydrogen bonds network we used two complementary methods: HDX-MS and NMR. Our study shows that ZFs with various number of residues localized between two binding cysteines (CX_nC) are very dynamic in terms of Zn^{2+} coordination and demonstrate wide range of affinities (K_d: 10^{-11}-10^{-14} M).[2] We believe that this is very important for cellular activation of ZFs under cellular fluxes of free Zn^{2+}. The variation of Zn^{2+} affinity within the same, highly conserved class of zinc fingers shows that the stabilization effects are hidden both in the sequence (β-linker length) and structural rearrangement (hydrogen bonds, electrostatic and hydrophobic interactions) of particular amino acid residues.[3] We believe that our findings are critical factors that adjust Zn^{2+} affinity of ZFs to cellular fluctuations of free Zn^{2+} and protein compartmentalization.

This work was supported by the National Science Centre (2012/07/E/NZ1/01894).

References

1) A. Krężel, W. Maret, Zinc-buffering capacity of eukaryotic cell at physiological pZn. J. Biol. Inorg. Chem., 2006, 11, 1049-1062.
2) A. Miłoch, A. Krężel, Metal binding properties of zinc finger metallome – insights into variations in stability. Metallomics, 2014, 6, 2015-2024.
3) T. Kochańczyk, A. Drozd, A. Krężel, Relationship between the architecture of zinc coordination and zinc binding affinity in proteins – insights into zinc regulation, Metallomics, 2015, 7, 244-257.

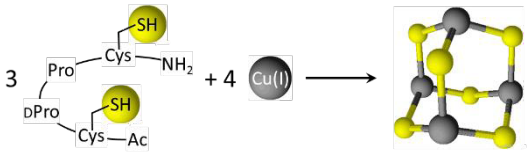
An Efficient Peptidic Copper(I) Chelator with Two Cysteines Linked by a Strong Turn

Edit Mesterhazy,[a,b] Colette Lebrun,[a] Attila Jancso,[b] Pascale Delangle[a]

[a] *INAC/SyMMES UMR 5819 CEA, CNRS, Université Grenoble Alpes, 17 rue des Martyrs 38 054 Grenoble, France;*
[b] *University of Szeged, Department of Inorganic and Analytical Chemistry, 7. Dóm tér, 6720 Szeged, Hungary;*
email : medit90@chem.u-szeged.hu; edit.mestherhazy@cea.fr;

Abstract

One of the most important micro nutrient is copper. Thanks to its ability to alternate between Cu(I) and Cu(II) redox states it is a usual cofactor in redox enzymes, such as Cu, Zn superoxide dismutase or ceruloplasmin. Available copper in cells is in Cu(I) form due to the overall intracellular reducing environment. Its concentration is under strict control to avoid the damages caused by reactive oxygen species produced in Fenton like reactions of excess Cu(I). Therefore the removal of excess Cu(I) is really important in Wilson's disease when the copper trafficking malfunctions and copper accumulates in patient's liver cells.



In this communication we present an efficient Cu(I) binding peptide where two cysteines are linked by the DPro-Pro motif. This short peptide's high affinity for Hg(II), another metal ion with high thiophilicity had been already demonstarted.[1] The interaction between the peptide and Cu(I) was investigated by direct titration with Cu(I) followed by UV-Vis and CD spectroscopy. In both case, the evolution of the characteristic LMCT[2] band was monitored and a sharp breakpoint occurred in the previous linear increase at the addition of 1.3 equiv. Cu(I). Already from this, a $\{Cu_4S_6\}$ coordination mode could be inferred, which was confirmed by ESI-MS, where the most abundant species was $Cu_4L_3^{2-}$ and by DOSY NMR spectroscopy, where the $M(Cu_xL_y)$ = 1645 g/mol molar mass calculated from the measured diffusion coefficient was in a good correlation with the $M(Cu_4L_3)$ = 1631 g/mol molar mass calculated from the molecular formula of the species. The affinity between thiolates and Cu(I) is too high to determine it directly, but it could be obtained in the presence of a well characterised Cu(I) ligand, namely bathocuproine disulfonate.[3] The apparent stability constant happened to be $\log\beta$ = 17.4 at physiological pH.

The DPro-Pro motif turned out to be strong enough to rigidify the peptide structure and to promote the formation of the water soluble, well-defined Cu_4L_3 cluster with high stability.

We also would like to present how the modification of the turn affect the chelating properties.

Acknowledgement

Edit Mesterhazy kindly acknowledge the financial support of the French Government (Campus France) and the Institute of Metals in Biology of Grenoble (IMBG).

References

1) S. Pires et al., Inorganic Chemistry 2012, 51, 11339.
2) P. Faller et al., Biochemistry 1997, 36, 13341.
3) Z. Xiao et al., Journal of the American Chemical Society 2004, 126, 3081.

Specific Sterilization of *Pseudomonas aeruginosa* Using Its Heme Acquisition Protein HasA

Yuma Shisaka,[a] Shiho Yamada,[a] Yusuke Iwai,[a] Hiroshi Sugimoto,[b] Yoshitsugu Shiro,[c] Osami Shoji,[a] Yoshihito Watanabe[d]

[a] *Department of Chemistry, Graduate School of Science, Nagoya University ;* [b] *RIKEN SPring-8 Center, Harima Institute ;* [c] *Graduate School of Life Science, University of Hyogo ;* [d] *Research Center for Materials Science, Nagoya University ; email : shisaka.yuuma@g.mbox.nagoya-u.ac.jp*

Abstract

Most of pathogens, such as *Pseudomonas aeruginosa, Staphylococcus aureus, and Serratia marcescens,* have heme acquisition system to pillage iron from their hosts. In the case of *P. aeruginosa*, the heme acquisition protein A (apo-HasA) was secreted toward extracellular to acquire heme. The heme captured HasA (holo-HasA; Fig. 1a) was interacted with outer membrane receptor HasR, and the heme was transported from HasA to HasR (Fig. 1b).[1)] We have previously reported that HasA can capture several synthetic metal complexes. Especially, we discovered that iron phthalocyanine bound HasA (FePc-HasA; Fig. 2a) can inhibit growth of *P. aeruginosa* due to the inhibition of heme uptake from holo-HasA through HasR (Fig. 2b). [2)]

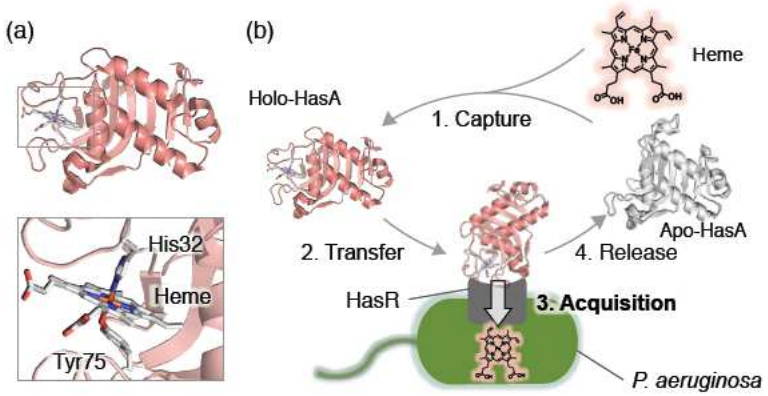


Fig. 2 (a) Crystal structure of holo-HasA (PDB ID: 3ELL), and (b) Heme Acquisition System (Has) in *Pseudomonas aeruginosa*

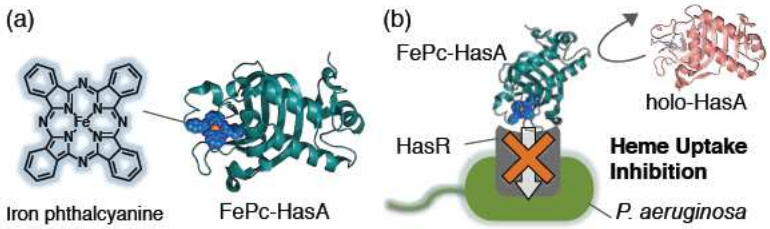


Fig. 1 (a) Crystal structure of Iron phthalocyainine bound HasA (PDB ID: 3W8O), and (b) Expected Mechanism of Growth Inhibition Using FePc-HasA

To further understand this growth inhibition, we decided to examine the interaction between FePc-HasA and HasR *in vitro*. Here we report expression and purification of HasR. HasR function was confirmed by the complex formation with holo-HasA. We also examined whether FePc-HasA could interact with HasR in the same manner as holo-HasA. Furthermore, we will report a novel sterilization system using HasA having other synthetic metal complexes.

References

1) N. Izadi-Pruneyre, et al., *J. Biol. Chem.*, 2006, 281, pp.25541-25550
2) C. Shirataki, et al., *Angew. Chem. Int. Ed.*, 2014, 53, pp.2862-2866

7-deazapurine ligands: towards hydrogen-mediated Watson-Crick base pairs in double-stranded DNA

Méndez-Arriaga J. M., [a] Galindo M. A.,[a*]

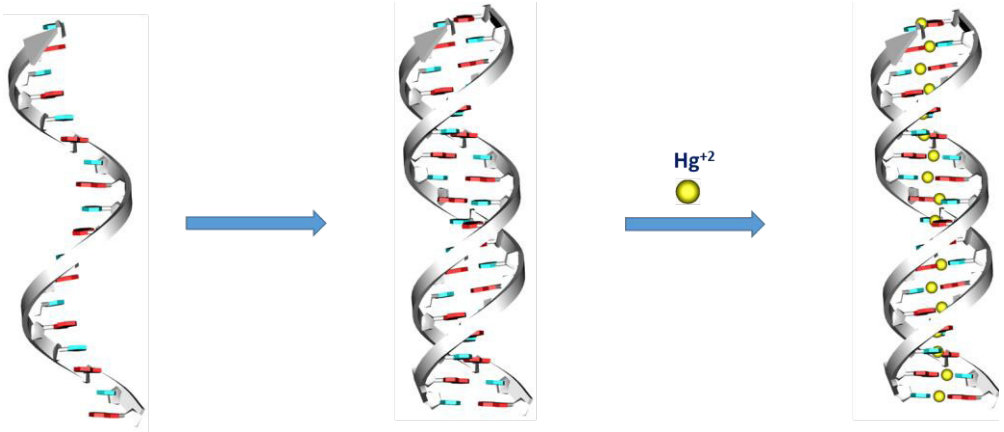
[a] *Dpto Químico Inorgánica, Facultad de Ciencias, Universidad de Granada, avda Fuentenueva s/n 18071 GRANADA (Spain) ; *email : magalindo@ugr.es*

Abstract

Non-canonical 7-deazaguanine (^{7C}G) and 7-deazaadenine (^{7C}A) and canonical cytosine (C) and thymine (T) nucleobases are capable of forming Watson-Crick base pairs via hydrogen bonds as well as forming Ag(I)-mediated base pairs by coordination to central Ag(I) ions. [1]

However, other metal ions such as Hg(II) also provide linear coordination that could substitute linear hydrogen bonds.[2] With this aim, we decided to explore the reactivity between Hg(II) metal ions and base pairs comprising 7-deazapurines nucleobases. Different duplexes, consisting in palindromic and non-palindromic sequences, holding ^{7C}G-C or ^{7C}A-T base pairs, have been synthesized and characterized. The incorporation of Hg(II) ions into these duplexes leads to the formation of Hg(II)-mediated base pairs, with a concomitant increase in the duplexes thermal stability and leading to noticeable conformations changes observed by circular dichroism.

Temperature-dependent UV-spectroscopy, circular dichroism and DFT calculations have been employed to confirm and the formation of sequential Hg(II)-mediated base pairs and to determine their arrangement. This finding launches a powerful methodology to generate customized Hg-DNA nanostructures with multiple potential applications.



References

1) Santamaría-Díaz N., Méndez-Arriaga J. M., Salas J. M., Galindo M. A. "Highly stable double-stranded DNA containing sequential silver(I)-mediated 7-deazaadenine/thymine watson-crick base pairs", Angew. Chem. Int. Ed. 2016, *55*, 6170–6174.
2) Kondo J., Yamada T., Hirose C., Okamoto I., Tanaka Y., Ono A., "Crystal Structure of Metallo DNA Duplex Containing Consecutive Watson–Crick-like T–Hg(II)–T Base Pairs", Angew. Chem. Int. Ed. 2014, 53, 2385 –2388.

A study on the secondary structure of the metalloregulatory protein CueR: effect of pH, metal ions and DNA

Ria K. Balogh,[a] Attila Jancsó,[a] Béla Gyurcsik,[a] Eszter Németh,[a]

[a] *Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, H-6720 Szeged, Hungary; email : baloghr@chem.u-szeged.hu*

Abstract

CueR is a fascinating example of proteins that regulates the intracellular level of a certain group of metal ions in several strains of bacteria. CueR provides a sensitive and selective transcriptional response to single-charged (Cu^{I}, Au^{I}, Ag^{I}), but not to double-charged (Zn^{II}, Hg^{II}) transition metal ions.[1)] Understanding the details of the metal ion response mechanisms operated by such bacterial proteins may forward the design of molecules for selective metal ion binding, accumulation or detection.

The function of CueR is based on the conformational change of the protein upon metal ion coordination, influencing the structure of the protein-bound DNA. Metal ion coordination occurs in a metal binding loop of the protein close to the C-terminus, where the sidechains of two cysteine residues provide linear coordination geometry around the effector metal ion. Based on a study of CueR model peptides, the participation of a protonated Cys residue in metal ion binding and the operation of a protonation switch have been proposed as a possible element of the regulatory mechanism.[2)]

To obtain further insights into the selective metal ion binding and regulatory function of the CueR protein, we investigated the effect of pH, the presence of mono and divalent metal ions, the promoter DNA sequence and combination of these on the relative proportion of secondary structure elements. The fractions of the secondary structure elements were calculated from the circular dichroism spectra of the protein, with the BeStSel[3)] and CDNN[4)] software, respectively. The CD spectrum of the protein in the presence of Ag^{I} ions was different from the spectra recorded with Zn^{II}, Hg^{II} and Cd^{II} ions and suggested that the structure of the Ag^{I}-bound form of CueR was richer in α-helices. Furthermore, decreasing the pH to 6.0 induced the transformation of the helix-rich structure into a β-sheet rich form. This secondary structure switch could be slowed down by adding Ag^{I} ions to the protein and fully prevented in the presence of the promoter DNA. In order to reveal the effect of pH on the structure of CueR, we performed Molecular Dynamic simulations, as well.

References

1) A. Changela, K. Chen, Y. Xue, J. Holschen, C.E. Outten, T.V. O'Halloran, A. Mondragon, Molecular Basis of Metal-Ion selectivity and Zeptomolar Sensitivity by CueR, 2003, Science, 301, 1383-1387
2) D. Szunyogh, H. Szokolai, P.W. Thulstrup, F.H. Larsen, B. Gyurcsik, N.J. Christensen, M. Stachura, L. Hemmingsen, A. Jancsó, Specificity of the Metalloregulator CueR for Monovalent Metal Ions: Possible Functional Role of a Coordinated Thiol?, 2015, Angew. Chem. Int. Ed. 54, 15756–15761
3) A. Micsonai, F. Wien, L. Kernya, Y.H. Lee, Y. Goto, M. Réfrégiers, J. Kardos, Accurate secondary structure prediction and fold recognition for circular dichroism spectroscopy, 2015, PNAS, 112, E3095-E3103
4) G. Böhm, R. Muhr, R. Jaenicke, Quantitative analysis of protein far UV circular dichroism spectra by neural networks, 1992, Protein Engineering, 5, 191-195

Bacterial Holdase Evolution Towards the Inhibition of Alzherimer's Amyloid Beta Peptide Aggregation

Sara Ayala[a,c], Ambre Sala[a,b], Christelle Hureau[c], Peter Faller[d], Pierre Genevaux[a]

[a)] LMGM (Laboratoire de Microbiologie et Génétique Moléculaires) 118 route de Narbonne Bât IBCG 31062 Toulouse Cedex 9, E-mail : pierre.genevaux@ibcg.biotoul.fr [b)] Morimoto Laboratory, Northwestern University, Evanston, IL [c)]LCC (Laboratoire de Chimie de Coordination) 205 route de Narbonne, BP 44099, 31077 Toulouse Cedex 4, E-mail: christelle.hureau@lcc-toulouse.fr [d)] Institute de Chimie (UMR 7177), 4 rue B. Pascal, F-67081 Strasbourg, E-mail: pfaller@unistra.fr

Abstract

Alzheimer's disease is the most frequent type of dementia and to this date there is no early diagnosis technic nor curative treatment available (1). Alzheimer along with more than 50 other diseases are directly linked to protein aggregation (2,3). Although, the cellular proteostasis network balances protein synthesis, folding and degradation, its weakening due to aging or deleterious mutations can challenge this delicate equilibrium giving place to disease. Molecular chaperones are key players of the proteostasis network and are able to modulate the aggregation of several proteins implicated in disease (4). In this work we look upon a small bacterial export chaperone, with an unusual robust antifolding activity, as a promising modulator of Alzheimer's related Amyloid Beta (AB) peptide aggregation. Our data shows that this chaperone is indeed a robust inhibitor of AB aggregation. Furthermore, we demonstrate that this chaperone malleable binding area could be modified to efficiently accommodate specific clients, including AB peptide (5,6).

References

1) World Alzheimer Report 2016
2) Knowles et.al, 2014, Nat Rev Mol Cell Biol 15:384-96
3) Stefani, 2004, BBA 1739:5-25
4) Morimoto and Labbadia, 2015, Annu Rev Biochem 84:435-64
5) Bordes et.al, 2016, Nat Commun 7:13339
6) Sala et.al, submitted

The Influence of Water Molecule Coordination to a Metal Ion on Water-Nucleic Base Hydrogen Bonds

J. Andrić, [a] I. Stanković, [b] S. Zarić [c,d]

[a] Innovation center, Department of Chemistry, University of Belgrade, Serbia ; [b] ICTM, University of Belgrade, Serbia ; [c] Department of Chemistry, University of Belgrade, Serbia ; [d] Department of Chemistry, Texas A&M University at Qatar, Doha, Qatar ; e-mail : ivana_stankovic@chem.bg.ac.rs

Abstract

Hydrogen bonds of coordinated water are much stronger than those between noncoordinated water molecules [1,2]. The nucleic base – coordinated water interactions are applied in heavy metal ion detection [3] as artificial short single-stranded DNA or RNA sequences can fold into specific secondary and tertiary structures on binding to certain heavy metal targets with extremely high specificity. Here, the hydrogen bond interactions of nucleic bases with noncoordinated and coordinated water molecule were studied by analysing geometry of 2403 Protein Data Bank [4] crystallographic structures and by MP2/def2-QZVP quantum chemical calculations implemented in the ORCA software [5].

Both PDB search and calculations revealed shorter hydrogen bonds with coordinated water, with the acceptor-donor distance in the range of 2.6–2.8 Å independently of metal type, in contrast to the noncoordinated water in the range of 2.8–3.0 Å. A number of interactions found in PDB are of type nucleic base - hydrated metal – protein, Figure 1, which points out that hydrated metal ions can intermediate protein-DNA contacts.

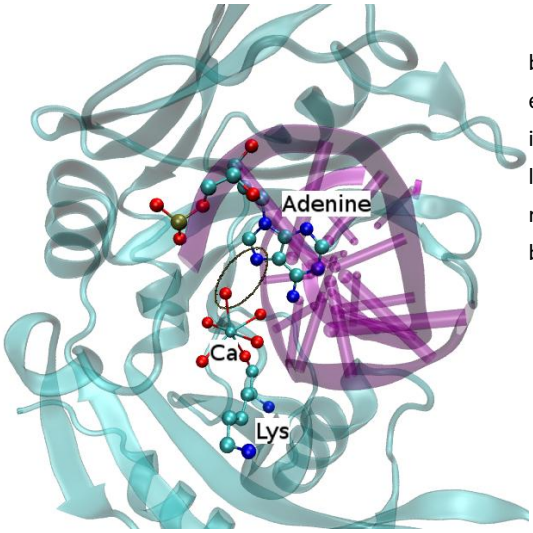


Figure 1: An example of the hydrogen bond between a nucleic base and a coordinated water, encircled in black, d(O/N)= 2.83 Å. The hydrated calcium ion intermediates protein-DNA contact as one of the Ca ligands is the protein's amino acid lysine. DNA represented in purple, protein in cyan, nitrogen atoms in blue balls and oxygen atoms in red balls. PDB id: 2OAA.

The results of the calculations are in agreement with the PDB data and show that the hydrogen bond interactions for doubly charged $[Mg(H_2O)_6]^{2+}$ complex (-12.94 to -49.96 kcal/mol) and for singly charged $[Na(H_2O)_6]^{+}$ complex (-6.66 kcal/mol to -19.63 kcal/mol) are stronger than for noncoordinated water (from -4.63 to -8.93 kcal/mol).

Ratiometric Zinc Luminescent Probe based on Zinc Finger Peptide Conjugated to Lanthanides Complexes

Manon Isaac[a], Svetlana Eliseeva[b], Colette Lebrun[c], Stephane Petoud[b], Olivier Sénèque[a]

[a] *Chemistry of Metal in Biology Lab CNRS/CEA/Grenoble Alpes University;*

[b] *Center for Molecular Biophysics Orleans CNRS;*

[c] *Institut for Nanosciences and Cryogenics CEA/Grenoble Alpes University*

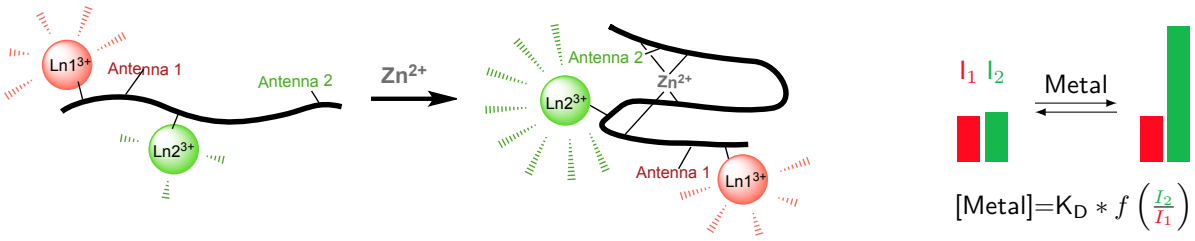
email : manon.isaac@ens-lyon.org

Abstract

Zinc dynamic fluxes plays an important role in signaling pathways in the nervous system, pancreas signaling, and in the reproductive system. Fluorescent probes able to sense the labile pool of zinc, are commonly used to image these dynamic processes. However, these intensity-based probe do not allow direct quantification of local zinc concentration. On contrary to intensiometric probes, ratiometric probes display two distinct emissions, which can be altered independently upon metal binding. The ratio of their intensities is proportional to the complexation level, allowing easy quantification of zinc concentration.

We have designed ratiometric probes for Zn^{2+} based on a classical ββα zinc finger peptide decorated with two different lanthanide complexes (Tb^{3+} and Eu^{3+}), as emissive moieties. f-f transitions are *Laporte* forbidden, so direct excitation of lanthanides is not efficient, but they can be sensitized by energy transfer from an organic chromophore, called antenna. As antennas for Tb^{3+} and Eu^{3+}, we have chosen tryptophan and naphthylalanine, respectively.

We will describe the principle of ratiometric Zn^{2+} detection with these systems and compare the behavior of four zinc finger-based probes, in which the position of the antenna and lanthanide chelates are different.



Hydrolytic properties and stability of sequentially diverse ββα zinc fingers

Katarzyna Kluska, [a] Artur Krężel, [a]

[a] *Department of Chemical Biology, Faculty of Biotechnology, University of Wroclaw, Joliot-Curie 14a, 50-383 Wrocław, Poland, email: artur.krezel@uwr.edu.pl*

Abstract

It has been established that zinc finger motifs are consisted of cysteine and histidine residues coordinated to Zn^{2+} ion tetrahedral geometry. These specific metal binding motifs occurred in proteins are able to facilitate biological processes like transcription, DNA repair, apoptosis and gene expression. Commonly known Cys_2His_2 zinc finger motif called classical or ββα possess strictly conserved sequence with amino acids responsible for Zn^{2+} binding and hydrophobic core formation. Extensive research in this area have shown that elimination of the conserved amino acid has impact on the Zn^{2+} binding and in consequence influence proteins biological function. Our bioinformatics analysis showed that sequence alteration within zinc finger proteins appears naturally. Therefore in this study, using eight diverse zinc fingers, Co^{2+} substitution and circular dichroism measurement were used to investigate metal ion geometry as well as changes in domain folding that occurred during metal binding. Furthermore, we examine stability constants using competition assay with metal chelators like 4-(2-pyridylazo)resorcinol and zincon. The obtained data proved that sequence alteration within conserved amino acids has impact on the zinc finger complex structure and stability. Thus, further investigation is essential to examine if this specific modifications can transform zinc finger domain from structural to catalytic one. In order to explore if structurally diverse zinc fingers are able to hydrolyse ester or phosphoester bonds the hydrolytic study with two substrates *p*-nitrophenyl acetate and *p*-nitrophenyl phosphate were performed, respectively.

Financial support by 2012/07/E/NZ1/01894 (National Science Centre) is gratefully acknowledged.

References

1) T. Kochańczyk, et al., Relationship between the architecture of zinc coordination and zinc binding affinity in proteins - insights into zinc regulation,*Metallomics*, 2015, 7, 244.

2) A. Miłoch, A. Krężel, Metal binding properties of zinc finger metallome – insights into variations in stability, *Metallomics*, 2014, **6**, 2015.

3) J. H. Laithy, et al., Zinc finger proteins: new insights into structural and functional diversity, *Curr Opin Struct Biol*., 2001, 11, 39.

Fine tuning of the thermodynamic and structural properties of transition metal peptide complexes

Gizella Csire,[a] Judit Erdei, Judit Király, Júlia Pinkóczy, Judit Kocsi, Katalin Várnagy[a]

[a]*Department of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary.*
email: csire.gizella@science.unideb.hu

Abstract

The systematic studies of the protected and non-protected multihistidine peptides with sequences mimicking metal binding sites Cu,Zn SOD on the one hand gave information about the metal ion and protein interaction and on the other hand made it possible to conclude general trends in the coordination of metal complexes of multihistidine peptides. The results clearly show that the stability of the metal complexes significantly depend on the metal ion and the number and position of histidines in the peptide [1-2]. The metal binding ability of the peptide is, however, affected by the amino acids which are present in the neighbourhood of the histidine amino acids, also. These conclusions strongly suggest that the thermodynamic and structural properties of the peptide complexes could be finely tuned by the change of quality and sequence of amino acids around the side chain donor atoms of coordinating the metal ions. This means, that the systematic planning of the sequence of peptides could increase the metal binding selectivity of peptides as well.

Aims of the continuation of our metallopeptide chemistry research work were the synthesis, equilibrium, and structural studies of complexes of such series of multihistidine peptides, in which the systematic change of the amino acid sequence is carried out. The presence of positively or negatively charged and polar or bulky side chains of other amino acids in the neighbourhood of the metal binding sites, can significantly contribute to the stability of these complexes and selectivity of the peptides. To understand the specific effects of these side chains lysine, aspartic acid or phenylalanine were systematically inserted into the sequence of the multihistidine peptides: Ac-HDAH-NH_2, Ac-HADH-NH_2, Ac-HXHZH-NH_2 (X, Z = Ala, Phe, Asp, Lys) and Ac-HXHAHXH-NH_2 (X = Asp or Phe). The results in studies of copper(II), nickel(II) complexes of these peptides have shown that the aspartic acid and phenylalanine residue in the molecule change the stability of metal complexes and the preference of the metal binding site, while the lysine residue does not influence the complex formation processes.

References

1) Cs. Kállay, K. Várnagy, G. Malandrinos, N. Hadjiliadis, D. Sanna, I. Sóvágó, Dalton Trans., 2006, 4545-4552.
2) S. Timári, Cs. Kállay, K. Ősz, I. Sóvágó, K. Várnagy, Dalton Trans., 2009, 1962-1971.

Acknowledgements

This work was supported by the Hungarian Scientific Research Fund (OTKA K 115480) and the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008.

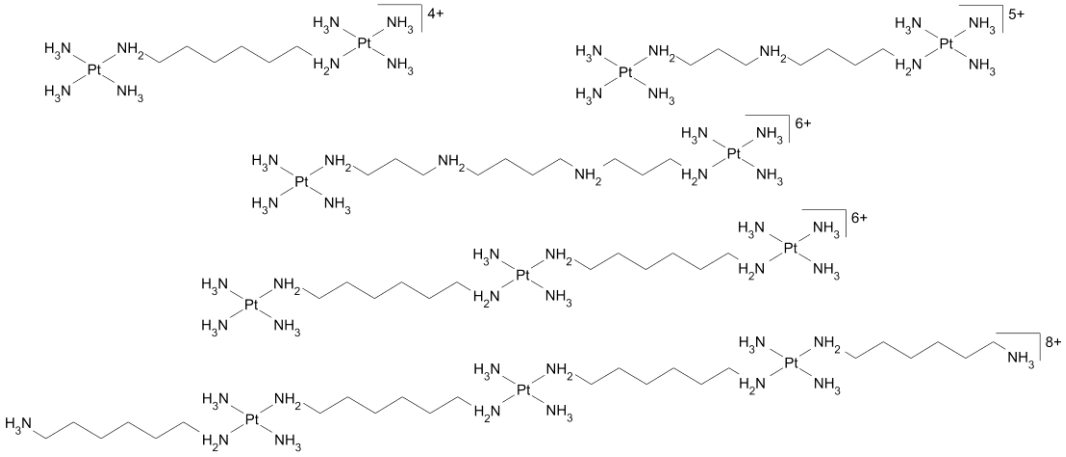
Stabilization of DNA and RNA triplexes by substitution-inert polynuclear platinum complexes

Jaroslav Malina,[a] Nicholas P. Farrell,[b] and Viktor Brabec[a]

[a] Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Královopolská 135, CZ-61265 Brno, Czech Republic; [b] Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia 23284-2006, United States; e-mail: malina@ibp.cz

Abstract

The substitution-inert polynuclear platinum complexes (PPCs) represent a unique class of antitumor agents that bind with high affinity to DNA and RNA [1]. Their binding to nucleic acids is mediated exclusively by noncovalent (hydrogen bonding, electrostatic) interactions. PPCs bind to DNA through use of 'phosphate clamp' - a discrete mode of DNA-ligand binding distinct from canonical intercalation and minor-groove binding [2]. Substitution-inert PPCs display interesting biological activities including *in vitro* cytotoxicity in a wide range of cell lines, antitumor activity that is independent of p53 status and high cellular accumulation. The mechanism of their antitumor and cytotoxic activity is not fully understood, however it has been suggested [1, 3] that it may be associated with the high potency of these compounds to condense nucleic acids along with their sequence-specific DNA binding and also on the competition with naturally occurring polyamines such as spermine for intracellular binding sites, but with altered function.



Here we present our results on a group of substitution-inert PPCs and their ability to promote formation of various DNA and RNA triplexes. The results are compared to those obtained with spermine, which is regarded as an excellent triplex stabilizer. In addition, we examined the potency of substitution-inert PPCs to inhibit DNA synthesis by DNA polymerase and reverse transcriptase in sequences that are prone to the formation of triplexes. Our data show that substitution-inert PPCs are capable of promoting triplexes at concentrations significantly lower than spermine.

Acknowledgements

Financial support by the Czech Science Foundation (Grant 16-03517S) is gratefully acknowledged.

References

1) N.P. Farrell, *Chem. Soc. Rev.* **2015**, 44, 8773-8785.
2) S. Komeda, Y. Qu, J.B. Mangrum. A. Hegmans, L.D. Williams, N.P. Farrell, *Inorg. Chim. Acta* **2016**, 452, 22-33.
3) J. Malina, N.P. Farrell, V. Brabec, *Angew. Chem. Int. Ed.* **2014**, 53, 12812-12816; J. Malina, N.P. Farrell, V. Brabec, *Inorg. Chem.* **2014**, 53, 1662-1671.

Thermodynamic study of metal ion coordination by Neclu_MT1

Monica Perinelli, [a] Matteo Tegoni,[b] Eva Freisinger [a]

[a)] *Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland;*

[b)] *Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Italy;*

email : monica.perinelli@chem.uzh.ch

Abstract

In a German polluted spring, the aquatic fungus *Heliscus lugdunensis* was found to be able to survive in the presence of high metal ion concentrations. This fungus produces a number of different thiolate-containing molecules, among them the metallothionein Neclu_MT1[1].

MTs are a large super-family of cysteine-rich metalloproteins; besides their small size (<10 kDa), the significant number of thiolate residues (in vertebrates up to 33% of the amino acids are Cys residues) confers them with a high binding capacity towards transition metal ions with d^{10} electron configuration. Neclu_MT1 is with only 24 amino acids one of the smallest MTs but features with 8 Cys residues the same high Cys content as the vertebrate forms. In addition, Neclu_MT1 contains a single C-terminal His residue, which is one of its most interesting features since it was shown to be a Cd(II)-specific MT, even on the gene transcription level.[2]

In order to better understand the role of the His residue, an arginine mutant was produced (Neclu_H24RMT1). The binding capacity of Neclu_H24RMT1 is comparable to the wild-type: both are able to complex three Cd(II) or Zn(II) ions. With potentiometric measurements we were able to calculate the pK_a values of all titratable residues of wild-type and mutant Neclu_MT1. Furthermore, complexation studies are being carried out on the two systems with both Cd(II) and Zn(II).

The obtained data is intended to provide information about the coordinating residues and the specific role of the His residue. UV-visible and circular dichroism spectroscopy as well as mass spectrometry experiments complement the potentiometric data.

Project funding by the Swiss National Science Foundation (SNSF) to EF is gratefully acknowledged.

References

1) Miersch, J. & Grancharov, K. Cadmium and heat response of the fungus Heliscus lugdunensis isolated from highly polluted and unpolluted areas. Amino Acids 34, 271–277 (2008).

2) Loebus, B. Leitenmaier, D. Meissner, B. Braha, G. Krauss, D. Dobritzsch, E. Freisinger. The major function of a metallothionein from the aquatic fungus Heliscus lugdunensis is cadmium detoxification. J. Inorg. Biochem. 127, 253-260 (2013).

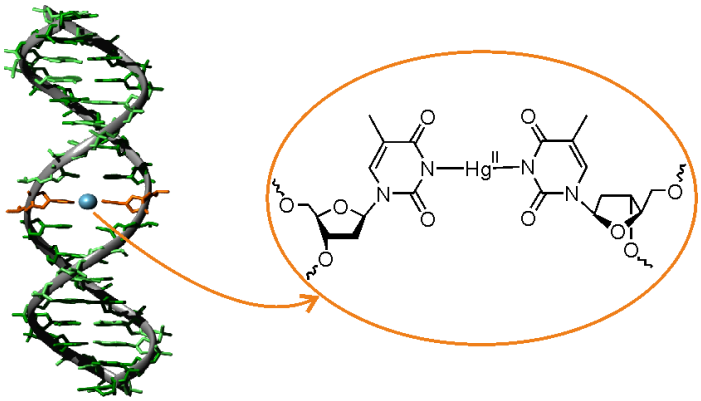
Studies of Charge Transfer through DNA with a Metal-Mediated Base Pair

Susanne Hensel, Kevin Eckey, Uwe Karst, Jens Müller*

Westfälische Wilhelms-Universität Münster, Institut für Anorganische und Analytische Chemie, Corrensstraße 28/30, 48149 Münster, Germany

susanne@muellerlab.org

Charge transfer in DNA and its potential of applications are not only a subject in chemistry but also in biology and physics.[1] Its applicability in sensors, nanoelectronics as well as its proposed relevance in DNA damage and skin cancer makes it an interesting research area.[2] The underlying mechanisms are well understood both for hole transfer and excess electron transfer.[3,4]



To investigate the effects of excess electron transfer, we describe here a simple method for the synthesis of a diaminonaphthalene-based electron donor that can be easily incorporated into DNA oligonucleotides. Based on earlier work by *Rokita et al.*,[5] these oligonucleotides can be paired with bromouracil-containing electron acceptor oligonucleotides. Upon excitation of the electron donor via irradiation, an electron is injected into the base pair stack. After electron transfer through the DNA duplex, bromouracil is reduced and cleaves off a bromide anion. We have synthesized different oligonucleotide strands containing natural base pairs in-between electron donor and acceptor as well as thymine-thymine mismatches. Metal-mediated thymine-Hg(II)-thymine base pairs are easily formed from these mismatches and the effect of the metal ions inside the DNA duplex on the electron transfer properties can be investigated. The charge transfer was evaluated after enzymatic digestion of the irradiated oligonucleotides by quantifying the amount of the unreacted bromouracil via LC-ICP/MS detection of Br.

References

1) H.-A. Wagenknecht: *Charge Transfer in DNA. From Mechanism to Application*. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, **2005.**
2) P. A. Sontz, N. B. Muren, J. K. Barton, *Acc. Chem. Res*. **2012**, *45*, 1792.
3) N. P. Campbell, S. E. Rokita, *Org. Biomol. Chem.* **2014**, *12,* 1143.
4) M. Fujitsuka, T. Majima, *Phys. Chem. Chem. Phys.* **2012**, *14,* 11234.
5) T. Ito, S. E. Rokita, *J. Am. Chem. Soc.* **2003**, *125*, 11480.

Targeting G-quadruplexes with two Zn(II) complexes with terpyridine derivatives. Nuclear uptake and cytotoxicity.

Natalia Busto,[a] José M. Leal,[a] M. Carmen Carrión,[b] Félix A. Jalón,[b] Blanca R. Manzano,[b] and Begoña García[a]

[a]*Chemistry Department, University of Burgos, Pza. Misael Bañuelos s/n, 09001, Burgos, Spain ;* [b] *Departamento de Química Inorgánica, Orgánica y Bioquímica, Universidad de Castilla-La Mancha, Avda. Camilo J. Cela 10, 13071 Ciudad Real, Spain; e-mail: nataliabyv@msn.com*

Abstract

G-quadruplexes are non-canonical secondary nucleic acids structures formed by guanine rich stretches. These highly polymorphic structures have become more than a mere structural curiosity due to the discovery of thousands of potential G4-forming sequences in biologically functional regions of many sequenced genomes. Over-represented in telomeres and promoters, these structures seem to be involved in genome stability and in a large number of biological processes[1] and they are related to some pathologies such as cancer, neurodegenerative and infectious diseases (viral replication).[2]

Therefore, the search of molecules able to interact and stabilize these structures with therapeutic purposes has attracted growing attention in recent years.[3] Among them, terpyridine derivatives constitute an important family of potential G4 ligands.[4] Nevertheless, more efforts have to be made in order to search more selective G4 ligands. In this work, we show two newly synthesized Zn(II) complexes with terpyridine derivatives, Zn(II) mono- and bis- terpyridine, able to target G-quadruplexes. FRET melting assays reveal that both Zn(II) complexes thermally stabilize G-quadruplex structures (Figure 1) but with different selectivity.

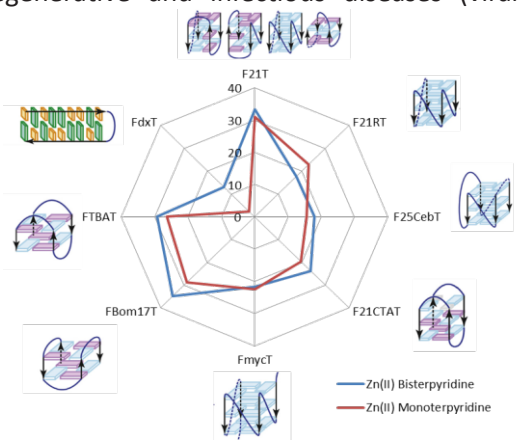


Figure 1. ΔTm Spider Plot of several oligonucleotides in the presence of 10 μM of Zn(II) complexes.

These Zn(II) complexes are fluorescent molecules, hence their cellular uptake was studied by means of fluorescence microscopy. Both terpyridine derivatives are successfully internalized in the cells at 50 μM within 1h being localized mainly in the nucleus (Figure 2). In addition, their cytotoxicity towards SW480 (colon tumor cells) was also evaluated, being the Zn(II) bis-terpyridine derivative more cytotoxic than the Zn(II) mono-terpyridine derivative.

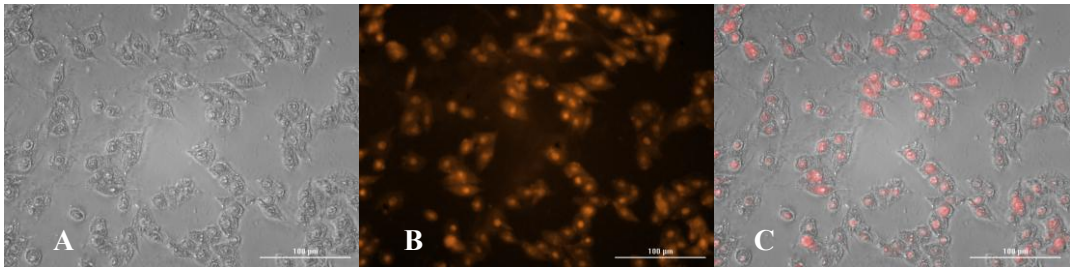


Figure 2: Images of SW480 cells treated with 50 μM of Zn(II) mono-terpyridine derivative after 1h of incubation: (A) Phase Contrast, (B) Orange Fluorescence Emission and (C) Overlay.

Artificial protein assemblies of chemically engineered hexameric hemoprotein with functional molecules

Shota Hirayama,[a] Koji Oohora,[a,b] Takashi Hayashi[a]

[a] *Department of Applied Chemistry, Graduate School of Engineering, Osaka University;* [b] *PRESTO, JST*

email: s_hirayama@chem.eng.osaka-u.ac.jp

Abstract

In nature, many proteins form various assemblies with unique functions derived from the sophisticated structures. Recently, artificial protein assemblies inspired by the natural protein assemblies have attracted attentions due to the significant potentials toward new soft materials.[1]

In order to construct an artificial hemoprotein assembly, we focus on the hexameric tyrosine-coordinated heme protein (HTHP) as a building block.[2] HTHP has a ring-shaped homohexameric structure with C_6-symmetry and each monomer possesses one heme molecule as a cofactor. For construction of the assembly, the chemical modification of HTHP was carried out (**Fig. 1**). To modify HTHP with maleimide–thiol coupling reaction, Cys-introduced HTHP mutant was designed and prepared, because native HTHP contains no Cys-residue and the site-selective modification at the Cys residue can be promoted in this mutant. Pyrene or poly(*N*-isopropylacrylamide) (PNIPAAm) as a functional molecule was introduced on the surface of the HTHP mutant. Pyrene is a hydrophobic fluorescent molecule and the pyrene-linked HTHP (pyr-HTHP) spontaneously forms the dimer of the hexamer structure, $(pyr\text{-}HTHP)_2$, via pyrene–pyrene interaction. The dimer $(pyr\text{-}HTHP)_2$ was characterized by size exclusion chromatography and dynamic light scattering. Another functional molecule, PNIPAAm is a thermo-responsive polymer. PNIPAAm-linked HTHP (PNIPAAm-HTHP) shows the reversible thermo-responsive assembling behavior. PNIPAAm-HTHP exhibits a monomeric structure under low temperature conditions, whereas the large structure is observed under high temperature conditions. The diameter is estimated to be 40 nm by the dynamic light scattering.

The chemical modification of HTHP mutant provides the formation of the artificial protein assemblies, and these shapes significantly depend on the artificially introduced molecule onto the protein surface. This diversity of the assembling behavior will contribute to the development of novel soft materials.

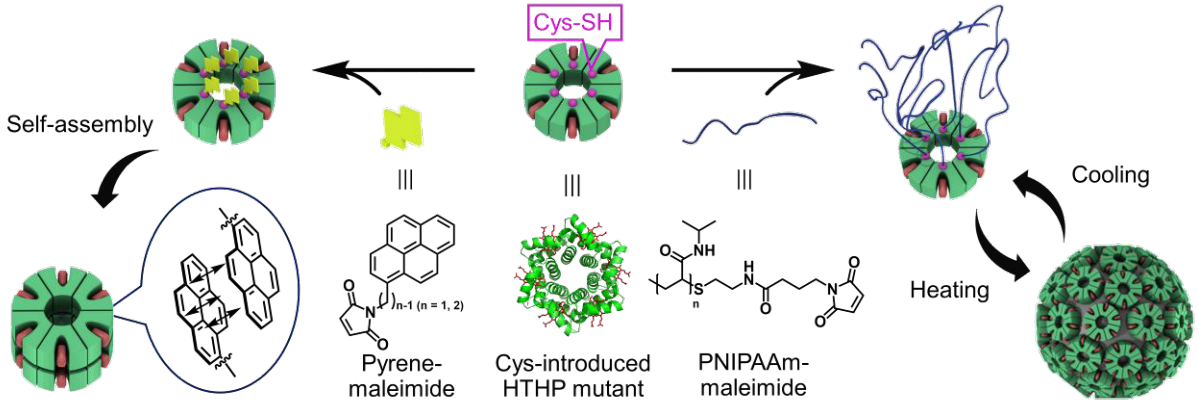


Figure 1. Construction of protein assemblies of HTHP by chemical modifications

References

1) C. Wagner *et al.*, *Chem. Rev*. **2010**, *110*, 3315–3336.
2) H. Dobbek *et al.*, *J. Mol. Biol.* **2007**, *368*, 1122–1131.

Ru-Complex Introduction to PNA for Highly Efficient Invasion

Masaki Hibino,[a] Yuichiro Aiba,[a] Yoshihito Watanabe,[b]

[a] *Department of Chemistry, Graduate School of Science, Nagoya University ;* [b] *Research Center for Materials Science, Nagoya University ; email : hibino.masaki@b.mbox.nagoya-u.ac.jp*

Abstract

Peptide Nucleic Acid (PNA, Figure 1) is a synthetic nucleic acid analogue, possessing an electrostatically neutral *N*-(2-aminoethyl)glycine backbone instead of the negatively-charged sugar-phosphate backbone of DNA[1]. Due to the lack of electrostatic repulsion, PNA/DNA duplexes are more stable than DNA/DNA duplexes. This high DNA affinity makes it possible for two PNA strands to invade double-stranded DNA (dsDNA) and form a so-called double-duplex invasion complex (Figure 2) through Watson-Crick base pairing[2]. PNAs can directly recognize specific dsDNA sequences, and this invasion does not require prior denaturing of the dsDNA. Thus, PNA is a potential candidate as a biochemical or molecular-biological tool.

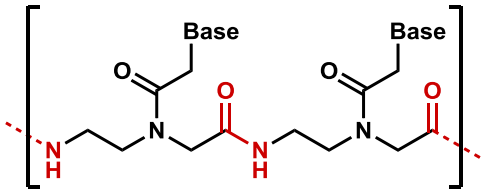


Figure 1 PNA structure

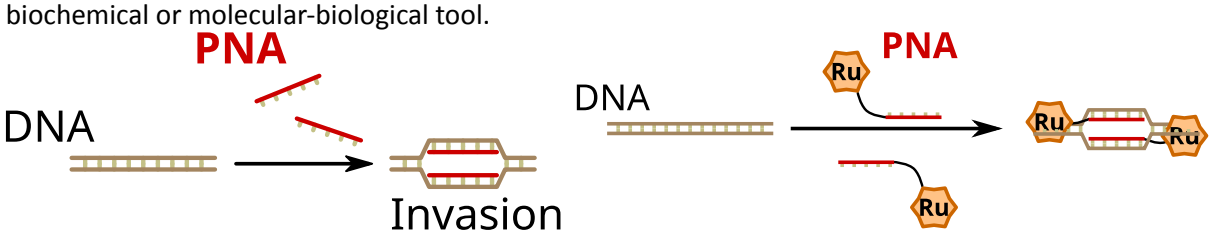


Figure 2 Invasion complex formation by PNA (left) and Ru-complex-PNA conjugate (right).

In this study, PNA was conjugated with a Ru-complex to improve its invasion efficiency. It is reported that Ru-complexes coordinated with bipyridine-derived ligands can interact with DNA via electrostatic and/or hydrophobic interactions[3]. The Ru-complex was prepared with phenanthroline ligands and introduced into the PNA through n-terminal amino group (Figure 3). This PNA is tightly anchored with the metal complex to DNA, and a more stable invasion complex can be formed.

The invasion efficiency of the synthesized PNAs was evaluated by electrophoresis mobility shift assay, and the design of the modified PNA was optimized by changing the positions of the metal complex and the length of the linker between the Ru-complex and the PNA. The optimized Ru-complex-PNA conjugate shows the higher invasion efficiency than the corresponding PNA without the Ru-complex.

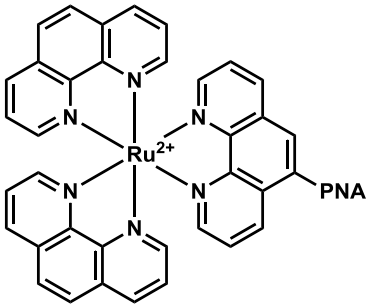


Figure 3 Ru-complex used for the PNA modification.

References

1) Nielsen, P. E. *et al. Science* **1991**, *254*, 1497.
2) Lohse, J. *et al. Proc. Natl. Acad. Sci.* **1999**, *96*, 11804.
3) Metcalfe, C.; Thomas, J. a. *Chem. Soc. Rev.* **2003**, *32*, 215.

Interaction of hystidine and histamine with complex of Yb^{3+}

Rosa Elena Navarro,[a] Octavio Serna Medina,[a] Yedith Soberanes,[b] Enrique F. Velazquez,[a] Hisila Santacruz [a]

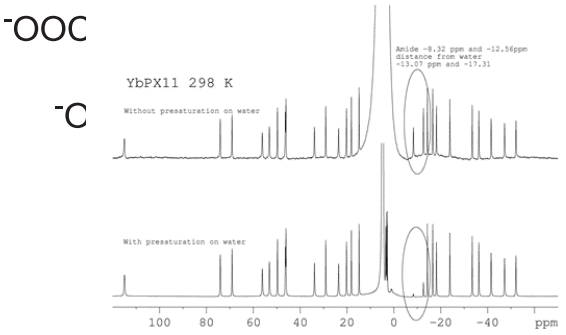
[a] Universidad de Sonora, Blvd Luis Encinas y Calle Rosales. Hermosillo, Sonora, México, CP 83000 ; [b)] Centro de Investigación en Alimentos, CIAD Hermosillo, Sonora
email : rnavarro@guaymas.uson.mx

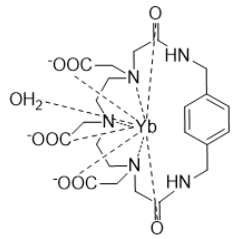
Abstract

The importance of histidine and histamine interactions with metal ions is of great importance because of their biological function as the primary component of the active centers of many metalloproteins.

We form the complex Yb[Cy1a] from the cyclic isomer Cy1a, obtained from diethylenetriaminepentaacetic (or DTPA) with the amine p-xylenediamine. This complex is soluble in water and is very stable at different pH values, mainly at physiological pH.

In this work, the ability to form adducts between the Yb [Cy1a] complex in the presence of histidine and histamine was assessed by ^{1}H NMR and UV-Vis titrations at pH 7.2, keeping the concentration of the complex constant and varying the concentration of histidine and histamine . For both histidine and histamine a second complexation was observed to occur. For both histidine and histamine a second complexation was observed to occur. In the NMR spectra there is a greater shift in the signals of the host's aromatic protons, which indicates that the imidazole ring is a type of interaction with the comple





NMR ^{1}H spectra of the complex Yb(cy1a)H_3 at 25 °C, pH 7, 18 mM in 10/90% D_2O/H_2O. Top without pulse of presaturation in the water, lower part with pulse of presaturation in the water.

References

1. Sheyla D-Yañez, Olivia Jatomea, Enrique F. Velázquez, Hisila Santacruz**, Rosa Elena Navarro*** and Motomichi Inoue.Ion-pairing of anionic DTPA-based cyclophanes with diaminoalkanes and methylated amino acids, lysine and arginine, in their dicationic form *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 80 (*2014) 295-302.
2. **Rosa Elena Navarro***, Yedith Soberanes, Sheyla D-Yañez, Olivia Jatomea, José Zeferino Ramírez and Motomichi Inoue. "Isomeric DTPA-amide macrocycles of *p*-xylenediamine and their complexation with Gd^{3+}" Polyhedron 92 (2015) 105-110. 28 de mayo 2015

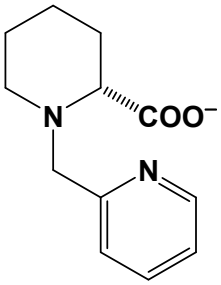
Optical resolution of *N*-acylated amino acids via complexation with (*R*)-*N*-(2-pyridylmethyl)pipecolatocopper(II)

Tatsuo Yajima, Takeo Fukushima, Atsuya Yamada, Tadashi Shiraiwa

Faculty of Chemistyr, Materials and Bioengineering, Kansai University ; email : t.yajima@kansai-u.ac.jp

Abstract

Amino acids are among the most important materials for our life. Many substances for food additives, medicines, and so on are produced from amino acids. Most of optically active non-proteogenic amino acids, for instance D-amino acids, are obtained from racemic amino acids through some chemical or biochemical methods, such as hydrolysis of protected amino acids by enzymes or catalysts and optical resolutions. Optical resolution is a traditional but still a common method for obtaining optically active amino acids. Some methods for optical resolutions and asymmetric syntheses using metal complexes have been reported.

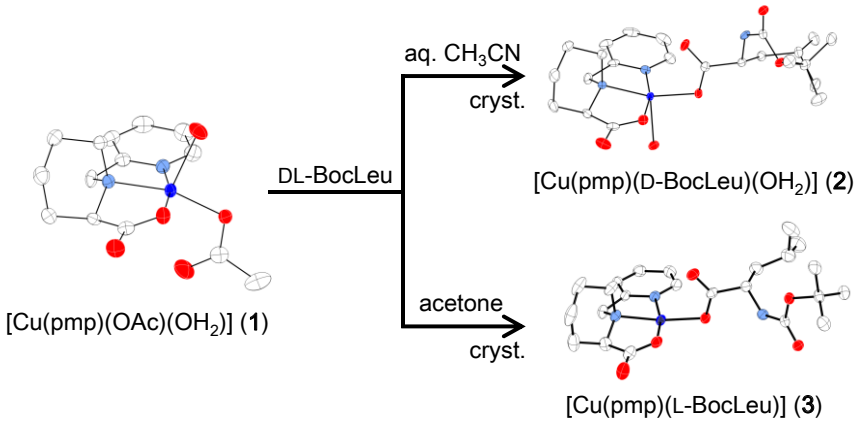


pmp

(*R*)-*N*-(2-Pyridylmethyl)pipecolic acid (Hpmp), prepared from D-pipecolic acid and 2-chloromethylpyridine, was treated with copper(II) acetate to give $[Cu(pmp)(OCOCH_3)(OH_2)]\cdot H_2O$ (**1**$\cdot H_2O$) as blue crystals, which was revealed to have a five-coordinated structure with a pmp as a tridentate ligand and an acetate and a water molecule as monodentate ligands by an X-ray crystal structure analysis. A mixed-ligand complex obtained from *N*-*t*-butoxycarbony-DL-leucine (DL-BocLeu) and **1** gave $[Cu(pmp)(D\text{-}BocLeu)(OH_2)]\cdot H_2O$ (**2**$\cdot H_2O$) as a soluble-less compound by recrystallization in aqueous acetonitrile (Scheme 1). X-Ray structure analyses showed that **2**$\cdot H_2O$ has hydrogen-bond networks involving the coordinated and the crystallization water molecules. Recrystallization of the ternary complex of DL-BocLeu in acetone gave [Cu(pmp)(L-BocLeu)] (**3**) as a soluble-less compound, which forms a dimer connected by a coordination and a hydrogen bond between ligands instead of the hydrogen-bond networks of coordinated and crystal waters in **2**$\cdot H_2O$.

The differences in intermolecular interactions between the crystals of **2**$\cdot H_2O$ and **3** influence their solubilities. In aqueous acetonitrile **2** was about 3 times less soluble than **3**. This is due to the hydrogen bond network consisting of the coordinated and crystal waters in **2**$\cdot H_2O$. On the other hands, **2** was about 20 times more soluble than **3** in acetone. The difference is probably caused by lack of crystal waters forming the hydrogen bond network in **3**, which is crystallized more favorably by promotion of dimerization by interligand hydrogen bonds in non-aqueous solvents.

Complexes **2** and **3** provided optically-pure D-BocLeu and 97 %*ee* of L-BocLeu, respectively, by treatment with diluted acetic acid.



Scheme 1. Mixed-ligand complexes crystallized in aqueous CH_3CN and acetone

Structural Design and Synthesis of De Novo Proteins for Light Harvesting Applications

Oliver J. Daubney,[a] Graham A. Worth,[b] Scott A. White,[c] Anna F. A. Peacock,[a]

[a)] University of Birmingham, School of Chemistry; [b)] University College London, School of Chemistry; [c)] University of Birmingham, School of Biosciences; email : ojd583@bham.ac.uk

Abstract

Solar power is an exceptionally abundant and reliable energy source, which is made use of extensively by natural systems. This work is inspired by natural proteins and uses biology to inform the design of novel materials. Utilising proteins as chemical scaffolds we have designed, with the aid of computational modelling, and analysed a series of synthetic systems. Focusing on the use of super-helical assemblies and previous work within the group,[1, 2] we have constructed a family of peptides that self-assemble around a lanthanide tri-cation. These peptides have been studied by circular dichroism (CD) and x-ray diffraction (XRD) experiments to ascertain structural features both to understand and improve the design.

The photo-physics of energy transfer from tryptophan sensors to a Tb(III) or Eu(III) bound ion has been studied to understand the ability to trap energy within the system. Interestingly, the lack of waters around the lanthanide should allow long lifetimes with low quenching coefficients. However, tryptophan is a poor sensor for lanthanides so we have carried out modelling to allow rapid prediction of emission spectra from the molecular structure of small aromatic and conjugated chromophores. This tool has allowed us to start optimising the design of unnatural amino acids to impart enhanced absorption and energy transfer properties upon the overall structure.

The synthesis of unnatural amino acids has been explored, making use of Negishi cross coupling and natural amino acid feedstocks to impart enantiopurity.[3, 4] This work demonstrates early attempts to mimic the complex architecture of a natural light harvesting complex. The use of solid-phase peptide synthesis has allowed the structure to be altered and the effect of residue mutation studied. We hope to use this tailorable architecture to create new devices to harvest light energy for applications from electricity generation to sensor design and light activated catalysis.

References

1) M. R. Berwick *et al.*, *J. Am. Chem. Soc.*, 2014, **136**, 1166-1169.
2) M. R. Berwick *et al.*, *Chem. Sci.*, 2016, **7**, 2207-2216.
3) H. J. C. Deboves *et al.*, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1876-1884.
4) S. Tabanella *et al.*, *Org. Biomol. Chem.*, 2003, **1**, 4254-4261.

List of participants


INP
ENSIACET


La Région
Occitanie
Pyrénées - Méditerranée


cnrs


IMRG


Hôpitaux de Toulouse


INP

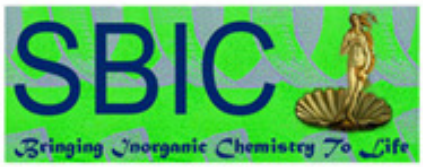

BMG LABTECH
The Microplate Reader Company


UNIVERSITÉ
TOULOUSE III
PAUL SABATIER
Université
de Toulouse


JCC
2016


SCF


COORDINATION
CHEMISTRY REVIEWS


SBIC
Bringing Inorganic Chemistry To Life


FrenchBIC


BACHEM


VEOLIA


DOSTAL
Scientific Engineering Solutions


vacuubrand
Technologie du vide


MDPI


FONDATION
ONCOPOLE
TOULOUSE CANCER SANTÉ