毎月1回1日発行　124巻増刊号　平成16年6月1日発行　昭和25年1月19日　第3種郵便物認可　ISSN 0031-6903

薬學雑誌 YAKUGAKU ZASSHI
Journal of the Pharmaceutical Society of Japan
Vol.124 Suppl. 1

The 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04

第14回 金属の関与する生体関連反応シンポジウム

Program and Abstracts
プログラム・アブストラクト集

Thursday, June 10 - Friday, June 11, 2004
GranShip (Shizuoka Convention & Art Center)
Shizuoka, Japan
2004年6月10日（木）～11日（金）
グランシップ・静岡

Organized by : The Pharmaceutical Society of Japan
Cooperated by : Japan Society for Biomedical Research on Trace Elements, The Chemical Society of Japan, The Japan Society for Analytical Chemistry, The Japanese Society for Hygiene, Japan Society for Occupational Health, The Japanese Society of Immunotoxicology, A Strongpoint for driving 21 Century COE Program, Japan Health Nutrition Food Association

主催：日本薬学会
共催：日本微量元素学会、日本化学会、日本分析化学会、日本衛生学会、日本産業衛生学会、日本免疫毒性学会、21世紀COEプログラム推進拠点、（財）日本健康・栄養食品協会

毎月1回1日発行　124巻増刊号　平成16年6月1日発行　昭和25年1月19日　第3種郵便物認可　ISSN 0031-6903

藥學雜誌 YAKUGAKU ZASSHI
Journal of the Pharmaceutical Society of Japan
Vol.124 Suppl. 1

The 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04

第14回 金属の関与する生体関連反応シンポジウム

Program and Abstracts
プログラム・アブストラクト集

Chairman of the Organizing Committee:
Yasuaki ARAKAWA (Professor, The University of Shizuoka)
Date: Thursday, June 10 - Friday, June 11, 2004
Place: GranShip (Shizuoka Convention & Art Center)
Shizuoka, Japan

実行委員長：　荒川　泰昭　（静岡県立大学教授）
会期：2004年6月10日（木）～ 11日（金）
会場：　グランシップ・静岡

Organized by : The Pharmaceutical Society of Japan
Cooperated by : Japan Society for Biomedical Research on Trace Elements, The Chemical Society of Japan, The Japan Society for Analytical Chemistry, The Japanese Society for Hygiene, Japan Society for Occupational Health, The Japanese Society of Immunotoxicology, A Strongpoint for driving 21 Century COE Program, Japan Health Nutrition Food Association

主催：日本薬学会
共催：日本微量元素学会、日本化学会、日本分析化学会、日本衛生学会、日本産業衛生学会、日本免疫毒性学会、21世紀COEプログラム推進拠点、（財）日本健康・栄養食品協会



Welcome Message

Yasuaki ARAKAWA
Chairman, the Organizing Committee
(Professor, The University of Shizuoka)

It is a great pleasure for me to open the 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04 (SRM2004) at Shizuoka and to welcome all of you to this symposium. This symposium is an interdisciplinary scientific meeting dealing with the relation between metals and organism, including the fields of an interaction between metals and organism, biological activity and function of metals, structure and property of metal and metal-complexes, analysis of metals, design of functional metal molecule and metal drug, etc.

This symposium is designed to integrate the recent advances made by the various biological, medical, chemical, analytical, environmental and nutritional disciplines in the rapidly expanding field of metals. By bringing together investigators ranging in their interests from chemistry to medicine, we have attempted to present a fairly comprehensive coverage. Particularly in this symposium, we are going to focus our attention on the four main theme; 1) Isogenies of Drugs and Food, and Metals, 2) Essentiality and Toxicity of Metals, 3) Metal Complexes and Organism, 4) Metal Allergy.

Research on metals is entering a period of mushrooming growth. Therefore, it is highly desirable that interested scientists gather to report new advances, to review processes, to discuss conflicting findings and divergent interpretations regarding metals, and to make more meaningful plans for the future phases and directions of research.
We hope that your participation in this meeting will be rewarding to you and that sharing ideas with colleagues will help deepen understanding and friendship.

Shizuoka is located in the central part of the main island of Japan. The surrounding area provides excellent opportunities for participants to enjoy visits to scenic and interesting spots such as Mt. Fuji or the hot springs of the Izu Peninsula. I wish all participants of the 14th SRM a pleasant stay in Shizuoka and an enriching experience, both personal and professional.

Finally, I wish to express my appreciation to the members of the council who assisted in making this symposium, to the moderators, speakers, and other participants, and to the dedicated staffs of the Secretariat for their valuable contributions. I would like to thank many companies for the financial support and cooperation we received in organizing and conducting this symposium.

History of Symposium

シンポジウムの歴史

Time 回	Year 開催年	Organizing Chairman 実行委員長	Place 開催地
1	1982年(S57)	Yasuhiro YAMANE (Chiba University) 山根　靖弘　(千葉大)	Tokyo 東京
2	1984年(S59)	Hisashi TANAKA (Kyoto University) 田中　久　(京都大)	Kyoto 京都
3	1986年(S61)	Yoshinori KIDANI (Nagoya City University) 喜谷　喜徳　(名古屋市立大)	Nagoya 名古屋
4	1989年(H1)	Yoshikazu MATSUSHIMA (Kyoritsu College of Pharmacy) 松島　美一　(共立薬科大)	Tokyo 東京
5	1991年(H3)	Motoichi MIYAZAKI (Kanazawa University) 宮崎　元一　(金沢大)	Kanazawa 金沢
6	1993年(H5)	Akira YOKOYAMA (Kyoto University) 横山　陽　(京都大)	Kyoto 京都
7	1995年(H7)	Akira HANAKI (Shizuoka University) 花木　昭　(静岡大)	Hamamatsu 浜松
8	1996年(H8)	Eiichi KIMURA (Hiroshima University) 木村　栄一　(広島大)	Hiroshima 広島
9	1998年(H10)	Masafumi GOTO (Kumamoto University) 後藤　正文　(熊本大)	Kumamoto 熊本
10	2000年(H12)	Tetsuo Nagano (The University of Tokyo) 長野　哲雄　(東京大)	Tokyo 東京
11	2001年(H13)	Yutaka SAITO (Okayama University) 斉藤　寛　(岡山大)	Okayama 岡山
12	2002年(H14)	Hiromu SAKURAI (Kyoto Pharmaceutical University) 桜井　弘　(京都薬科大)	Kyoto 京都

13	2003年(H15)	Kazuo SUZUKI (Chiba University) 鈴木　和夫　(千葉大)	Chiba 千葉
14	2004年(H16)	Yasuaki ARAKAWA (The University of Shizuoka) 荒川　泰昭　(静岡県立大)	Shizuoka 静岡
15	Next	Masahiko CHIKUMA (Osaka University of Pharmaceutical Sciences) 千熊　正彦　(大阪薬科大)	Osaka 大阪

ORGANIZATION

Organized by : The Pharmaceutical Society of Japan

Cooperated by :

Japan Society for Biomedical Research on Trace Elements,
The Chemical Society of Japan, The Japan Society for Analytical Chemistry,
The Japanese Society for Hygiene, Japan Society for Occupational Health,
The Japanese Society of Immunotoxicology, A Strongpoint for driving 21 Century COE Program, Japan Health Nutrition Food Association

主催：日本薬学会
共催：日本微量元素学会、日本化学会、日本分析化学会、日本衛生学会、日本産業衛生学会、日本免疫毒性学会、21 世紀 COE プログラム推進拠点、(財) 日本健康・栄養食品協会

Organizing Committee

実行委員会

Chairman 実行委員長	Yasuaki ARAKAWA 荒川　泰昭	The University of Shizuoka 静岡県立大学
Members 委員	Masayoshi YAMAGUCHI 山口　正義	The University of Shizuoka 静岡県立大学
	Atsushi TAKEDA 武田　厚司	The University of Shizuoka 静岡県立大学
	Hiroyuki YANAGISAWA 柳沢　裕之	Saitama Medical University 埼玉医科大学
	Masahiro KAWAHARA 川原　正博	Kyushu University of Health and Welfare 九州保健福祉大学
	Masashi TSUNODA 角田　正史	Kitazato University 北里大学
	Teruaki SAKURAI 櫻井　照明	Tokyo University of Pharmacy and Life Science 東京薬科大学
Secretary 事務局	Shuji TAKAKU 高久　修司	The University of Shizuoka 静岡県立大学

The 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04
Thursday, June 10 – Friday, June 11, 2004

Program

SRM2004 Program at a glance

Thursday June 10, 2004; the first day

	GranShip Medium Hall: Earth (1,2,3 F)	GranShip Conference Room 1001-1 (10F) Exhibition Gallery-1 (6F)	
8:30—	Doors Open		
9:10—	Opening Address Organizing Chairman: Yasuaki Arakawa		— 9:10
9:20—	Session Ⅰ (Lecture 1—3) Chair: Hideo Saji		
10:20—	Session Ⅱ (Lecture 4—6) Chair: Osamu Yamauchi	Poster Setting Exhibition Gallery-1 (6F)	
11:20—	Special Lecture Ⅰ Yoshinori Itokawa Chair: Yasuaki Arakawa		
12:00—	Lunch	Council Conference room1001-1(10F)	—12:00
		Move/Break	—13:00
13:10—	Topic Atsuko Adachi Chair: Hiroyuki Yanagisawa		—13:10
13:50—	Special Lecture Ⅱ Osamu Wada Chair: Hiromu Sakurai		
14:30—	Break		
14:40—	Session Ⅲ (Lecture 7—8) Chair: Hiroyuki Nakazawa	Poster On Display Exhibition Gallery-1 (6F)	
15:20—	Session Ⅳ (Lecture 9—11) Chair: Seiichiro Himeno		
16:20—	Session Ⅴ (Lecture 12—14) Chair: Saburo Neya		
17:20—	Souvenir Photographing		
17:50—	Move		
18:30—	Social Gathering Hotel Associa Shizuoka Terminal Sunpu (3F)		
20:30—			

SRM2004 Program at a glance

Friday June 11, 2004; the second day

	GranShip Medium Hall: Earth (1,2,3 F)	GranShip Exhibition Gallery-1 (6F)	
8:30—	Doors Open		
9:00—	Session Ⅵ (Lecture 15—16) Chair: Masayoshi Yamaguchi		— 9:00
9:40—	Session Ⅶ (Lecture 17—19) Chair: Masahiro Kawahara		
10:40—	Session Ⅷ (Lecture 20—21) Chair: Atsushi Takeda		
11:20—	Session Ⅸ (Lecture 22—23) Chair: Shuichi Enomoto	Poster On Display	
12:00—	Lunch		
13:00—		Poster Session	—13:00
		Move/Break	—14:30
14:40—	Special Lecture Ⅲ Eiichi Kimura Chair: Tetsuo Nagano		—14:40
15:20—	Session Ⅹ (Lecture 24—25) Chair: Toshihiko Ozawa		
16:00—	Session Ⅺ (Lecture 26—27) Chair: Hideki Masuda	Poster Removal	
16:40—	Session Ⅻ (Lecture 28—29) Chair: Masahiko Chikuma		
17:20—	Closing Address Organizing Chairman: Yasuaki Arakawa		
17:30—			

Special Lectures

Thursday June 10, 2004 ; the first day
GranShip · Medium Hall · Earth (1,2,3 F)

Special Lecture I 11:20—12:00

Isogenies of drugs and food, and metals

Yoshinori Itokawa (Professor Emeritus, Kyoto University · Medicine)

Chairperson : Yasuaki Arakawa (Professor, The university of Shizuoka)

Special Lecture II 13:50—14. 30

Essentiality and Toxicity of Metals -Fundamental and Practical considerations-

Osamu Wada (Professor Emeritus, The University of Tokyo · Medicine)

Chairperson : Hiromu Sakurai (Professor, Kyoto Pharmaceutical University)

Friday June 11, 2004 ; the second day
GranShip · Medium Hall · Earth (1,2,3 F)

Special Lecture III 14. 40—15:20

Elucidation and Application of Intrinsic Properties of Molecular Recognition by Zinc Ions in Biology

Eiichi Kimura (ex-President, The Pharmaceutical Society of Japan)

Chairperson : Tetsuo Nagano (Professor, The University of Tokyo)

Topic Lecture

Thursday June 10, 2004 ; the first day
GranShip · Medium Hall · Earth (1,2,3 F)

Topic Lecture 13:10—13:50

Isogenies of drugs and food, and metals

Atsuko Adachi (Head, Hyogo Prefectural Kakogawa Hospital, Devmatology)

Chairperson : Hiroyuki Yanagisawa (Professor, Saitama Medical University)

Lectures

Thursday June 10, 2004 ; First day

GranShip・Medium Hall・Earth (1, 2, 3 F)

Session I Development of New Metal Drug (9:20—10:20)

Chairperson : **Hideo SAJI** (University of Kyoto)

Lecture 1

A ^{99m}Tc-labeled hydrazino nicotinamide derivatized bisphosphonate for bone scintigraphy

○Kazuma OGAWA[1,2], Takahiro MUKAI[1,3], Yasuyuki INOUE[1], Hideo SAJI[1]

1) Department of Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan 2) Central Institute of Radioisotopes Science, Division of Tracer Kinetics, Advanced Science Research Center, Kanazawa University, Kanazawa, Japan 3) Department of Biomolecular Recognition Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

Lecture 2

A new potent insulin-mimetic vanadyl(IV)-allixin complex

○Yusuke ADACHI[1], Jiro YOSHIDA[2], Yukihiro KODERA[2], Akira KATOH[3], Hiromu SAKURAI[1]

1) Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan 2) Healthcare Institute, Wakunaga Pharmaceutical Co., Ltd., Hiroshima, Japan 3) Department of Applied Chemistry, Faculty of Engineering, Seikei University, Tokyo, Japan

Lecture 3

Non-covalent interactions of an antitumor dinuclear platinum(II) complex with DNA

○Wada RYUHEI[1], Sachie WADA[1], Takaji SATO[1], Katsuhiko MINOURA[1], Keiko NOGUCHI[1], Keiko FUJII[1], Seiji KOMEDA[1], Yoshihiro SAITO[1], Masahiko CHIKUMA[1]

1) Osaka University of Pharmaceutical Sciences

Session II Design of Functional Metal Molecule (10:20—11:20)

Chairperson : **Osamu YAMAUCHI** (Kansai University)

Lecture 4

Structure-based design of the irreversible inhibitors to metallo-β-lactamase (IMP-1)

○Yoshihiro YAMAGUCHI[1], Kimitaka SOGA[1], Toshihiro HIGASHI[1], Shogo MISUMI[2], Hiromasa KUROSAKI[1], Yuriko YAMAGATA[3], Yoshichika ARAKAWA[4], Masafumi GOTO[1]

1)Department of Structure-Function Physical Chemistry, Graduate School of Pharmaceutical Sciences, Kumamoto University 2)Department of Biochemistry, Graduate School of Pharmaceutical Sciences, Kumamoto University 3)Department of Structural Biology, Graduate School of Pharmaceutical Sciences, Kumamoto University 4)Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases

Lecture 5

Co(III) complexes of an N2S3-type ligand designed as an active site model of nitrile hydratase: Influences of the oxygenated sulfurs on the central metal ion

○Tomohiro OZAWA[1], Tomohiro IKEDA[1], Yasuhiro FUNAHASHI[1], Koichiro JITSUKAWA[1], Hideki MASUDA[1]

1)Graduate School of Engineering, Materials Science & Engineering, Nagoya Institute of Technology, Nagoya, Japan

Lecture 6

Syntheses and Functional Analyses of Artificial Zinc Finger Proteins Linked with Metal Complex Moiety

○Akira ONDOA[1], Nozomi ARAI[1], Shigenori KOBAYASHI[1], Kenji KANEKO[1], Takeshi YAMAMURA[1]

1)Department of Chemistry, Faculty of Science, Tokyo University of Science, Tokyo, Japan

Session Ⅲ Biological Activity of Organometal in an Ecosystem — Marine Life

(14:40—15:20)

Chairperson : **Hiroyuki NAKAZAWA** (Hoshi University)

Lecture 7

Effect of tributyltin on reproduction of fish and shellfish

○Yuji OSHIMA[1], Shimasaki YOHEI[1], Suguru INOUE[2], Kei NAKAYAMA[2], Tsuneo HONJO[1]

1)Faculty of Agriculture, Kyushu University, Fukuoka, Japan 2)Center for Marine Environmental Studies, Ehime University, Ehime, Japan

Lecture 8

Imposex induced by organotin compounds in gastropods: from molecular mechanism to population-level effects

○Toshihiro HORIGUCHI[1], Hiroaki SHIRAISHI[1], CHO HYEON-SEO[2], Yasuhiko OHTA[3], Taisen IGUCHI[4], Tomohiro NISHIKAWA[1], Fujio SHIRAISHI[1], Masatoshi MORITA[1], Jun-ichi NISHIKAWA[5]

1)Endocrine Disruptors & Dioxin Research Project, National Institute for Environmental Studies, Tsukuba, Japan 2)Yosu National University, Yosu, Korea 3)Faculty of Agriculture, Tottori University, Tottori, Japan 4)Center for Integrative Bioscience, Okazaki National

Research Institutes, Okazaki, Japan 5)Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

Session Ⅳ Biological Activity of Organometal in an Ecosystem — Land Life

(15:20—16:20)

Chairperson : **Seiichiro HIMENO** (Tokushima Bunri University)

Lecture 9

Triphenyltin (TPT) chloride increased production of interleukin-1β (IL-1β) by murine macrophages after subacute oral exposure

○Masashi TSUNODA[1], Ken NAKANO[2], Nobuhiro KONNO[3]

1) Department of Preventive Medicine and Public Health, Kitasato University School of Medicine, Kanagawa, Japan 2)Fukushima Institute of Public Health, Fukushima, Japan 3)Koriyama Women's University & College, Koriyama, Japan

Lecture 10

Metabolism of triphenyltin

○Shuji OHHIRA[1], Mitsunori ENOMOTO[1], Yoshikazu MIURA[1], Hisao MATSUI[1]

1)Department of Hygiene, Dokkyo University School of Medicine, Tochigi, Japan

Lecture 11

Effects of organoltin compounds on endocrine functions of human placental cells

○Tsuyoshi NAKANISHI[1], Jun-ichi NISHIKAWA[2], Keiichi TANAKA[1]

1)Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan 2)Laboratory of Environmental Biochemistry, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

Session Ⅴ Development of New Metal-Complex・Metal-Probe (16:20—17:20)

Chairperson : **Saburo NEYA** (Chiba University)

Lecture 12

Radiometal Labeled Probes for in vivo Redox Imaging

○Takako FURUKAWA[1], Takeshi TANAKA[2], Yasuhisa FUJIBAYASHI[1]

1)Biomedical Imaging Research Center, University of Fukui, Fukui, Japan 2)Department of Otorhinolaryngology

Lecture 13

Design and Synthesis of a Novel Lanthanide-based Zinc Ions-Sensitive Chemosensors for Magnetic Resonance Imaging (MRI) and/or Luminescence Measurement

○Kenjiro HANAOKA[1], Kazuya KIKUCHI[1,2], Tetsuo NAGANO[1]

1)Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

2)Presto, JST Corporation, Kawaguchi, Saitama, Japan

Lecture 14

A Versatile Strategy for the Synthesis of Crown Ether Bearing Heterocycles: Discovery of Calcium Selective Fluorescent Probe

Yuko AOKI[1], Naoki UMEZAWA[1], Yuko ASANO[1], Kei-ichiro HATANO[1], Shin-ichi NAGAI[1], ○Tsunehiko HIGUCHI[1]

1)Graduate School of Pharmaceutical Sciences, Nagoya City University

Friday June 11, 2004 ; Second day

GranShip・Medium Hall・Earth (1,2,3 F)

Session Ⅵ Metal and Biological Function−1 (9:00−9.40)

Chairperson : **Masayoshi YAMAGUCHI** (University of Shizuoka)

Lecture 15

Immune disrupting effect of arsenic; Inorganic arsenite induces HLA-DR strong positive dendritic macrophage generation from human peripheral blood monocytes

○Teruaki SAKURAI[1], Takami OHTA[1], Kitao FUJIWARA[1]

1)Laboratory of Environmental Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Tokyo, Japan

Lecture 16

Molecular mechanism of cytolethality of dimethylated trivalent arsenic compound

○Chikara KOJIMA[1], Teruaki SAKURAI[1], Kitao FUJIWARA[1]

1)Laboratory of Environmental Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Tokyo, Japan

Session Ⅶ Metal and Biological Function−2 (9.40−10.40)

Chairperson : **Masahiro KAWAHARA** (Kyushu University of Health and Welfare)

Lecture 17

Role of zinc in the regulation of protein tyrosine phosphatase activity in osteoblastic MC3T3-E1 cells

Masayo FUKAGAWA[1], ○Satoshi UCHIYAMA[1], Masayoshi YAMAGUCHI[1]

1)Graduate School of Nutritional Sciences, University of Shizuoka

Lecture 18

Inhibitory zinc action against excitation of hippocampal glutamatergic neurons

○Akira MINAMI[1], Atsushi TAKEDA[1], Jun-ichi KOSUGE[2], Noboru FUKASAKU[2], Tetsuo NAGANO[3],

Naoto OKU[1]

1)Department of Medical Biochemistry, School of Phrmaceutical Sciences, University of Shizuoka, Shizuoka, Japan 2)Daiichi Pure Chemicals Co., LTD., Ibaraki, Japan 3)Laboratory of Bioorganic and Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

Lecture 19

Studies on the valid utilization of abundant zinc contained in Oyster (*Crassostrea gigas*)

○Yoshikazu MATSUDA[1], Natsumi SUMIDA[2], Sachiko KITANI[2], Shoko KITANI[2], Mamiko ABE[1], Nami KOMURA[1], Tomoe KAWASHIMA[1], Kenji FUKUNAGA[2], Munehiro YOSHIDA[2]

1)Central Research Institute, Japan Clinic Co., Ltd., Kyoto, Japan 2)Department of Biotechnology, Faculty of Engineering, Kansai University, Osaka, Japan

Session Ⅷ Metal and Biological Function−3 (10.40−11.20)

Chairperson : **Atsushi TAKEDA** (University of Shizuoka)

Lecture 20

Activation of Iron Regulatory Protein 1 through Heme Oxygenase 1 Signaling Pathway after Oxidative Stressful Stimuli

○Satoru OSHIRO[1], Koji NOZAWA[2], CHUN ZHANG[3], Toshio SONE[4], Ken-ichi KAWAMURA[5]

1)Biochemical Genetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan 2)Showa College of Pharmaceutical Sciences, Tokyo, JAPAN 3)Van Andel Research Institute, Michigan, USA 4)Allergy and Immunology, Saitama Medical School, Saitama, JAPAN 5)TK Craft Co., LTD, Gunma, Japan

Lecture 21

Implications of calcium homeostasis and neurotrophic factors in neurotoxicity of aluminum on primary cultured neurons

Keiko KONOHA[1], Yutaka SADAKANE[1], Lisa IMAMURA[2], Masaaki TSUDA[2], ○Masahiro KAWAHARA[1]

1)Department of Analytical Chemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, Miyazaki, Japan 2)Department of Biological Chemistry, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama, Japan

Session Ⅸ Analytical Method of Metals (11:20−12:00)

Chairperson : **Shuichi ENOMOTO** (RIKEN)

Lecture 22

A robust speciation method for arsenic in biological matrices by ion-exclusion

chromatography-ICPMS using reaction cell and hydride generation techniques

○Tetsuya NAKAZATO[1], Hiroaki TAO[1]

1) Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan

Lecture 23

Analysis of inorganic antimicrobial agents used in antimicrobial products

○Harunobu NAKASHIMA[1], Ooshima TOMOKO[2]

1) Osaka Prefectural Institute of Public Health, Osaka, Japan 2) Osaka City Institute of Public Health and Environmental Sciences, Osaka, Japan

Session X Metal-Associated Bio-Chemical Reaction－1 (15.20－16.00)

Chairperson : **Toshihiko OZAWA** (National Institute of Radiological Sciences)

Lecture 24

Reaction Intermediates Detected during the Four-Electron Reduction Process of Dioxygen by Bilirubin Oxidase Mutants

○Rieko KITAGAWA[1], Megumi INOUE[1], Kunishige KATAOKA[1], Takeshi SAKURAI[1], Yamagutchi SHOTARO[2]

1) Faculty of Science, Kanazawa University, Kanazawa, Japan 2) Amano Enzyme, Kamigahara, Japan

Lecture 25

Reduction of Cytchrome *c* with Tyrosyltyrosylphenylalanine

○Shun HIROTA[1], Hisano OKUMURA[2], Noriaki FUNASAKI[1], Yoshihito WATANABE[2]

1) Department of Physical Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan 2) Department of Chemistry, Graduate School of Science, Nagoya University, Nagoya, Japan

Session XI Metal-Associated Bio-Chemical Reaction－2 (16:00－16:40)

Chairperson: **Hideki MASUDA** (Nagoya Engineering University)

Lecture 26

Relationship between the formation of singlet oxygen and substrate oxygenation in ethanol-induced rat liver microsomes

○Seiko HAYASHI[1], Hiroyuki YASUI[1], Hiromu SAKURAI[1]

1) The Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan

Lecture 27

Transesterification and cycle reaction using polynuclear zinc-peptide dendrimer

○Kazuhiko ICHIKAWA[1], TARUNAI MATE[1]

1) GRADUATE SCHOO L OF ENVIRONMENTAL EARHT SCIENCE, HOKKAIDOU UNIVERSITY, SAPPORO, JAPAN

2) GRADUATE SCHOO L OF ENVIRONMENTAL EARHT SCIENCE, HOKKAIDOU UNIVERSITY, SAPPORO, JAPAN

Session XII Structure and Property of Metal-Complex (16:40－17:20)

Chairperson: **Masahiko CHIKUMA** (Osaka University of Pharmaceutical Sciences)

Lecture 28

Optical Properties of Zinc Complex of Chlorophyll-a and Its Application for Photoinduced Hydrogen Production

○Yumi TAKEUCHI[1], Yutaka AMAO[1]

1) Department of Applied Chemistry, Oita University, Oita, Japan

Lecture 29

Structural Basis of Human Cytoglobin for Ligand Binding

○Masatomo MAKINO[1,2], Hiroshi SUGIMOTO[1], Hitomi SAWAI[1,2], Norifumi KAWADA[3], Katsutoshi YOSHIZATO[4], Yoshitsugu SHIRO[1]

1) Biometal Science Laboratory, Riken Harima Institute, Hyogo, Japan

2) Department of Life Science, Graduate School of Science, Himeji Institute of Technology, Hyogo, Japan 3) Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka, japan 4) Department of Biological Science, Graduate School of Science, Hiroshima University, Hiroshima, Japan

Poster Sessions

Friday June 11, 2004 ; Second day (13:00−14.40)

GranShip・Exhibition Gallery-1 (6F)

Session I Development of New Metal Drug

Poster1

Evaluation of Zn-DTS complexes as oral antidiabetic agents

○Jun ABE[1], Yasuhiko IIDA[2], Yasushi KIYONO[3], Youji KITAMURA[4], Hideo SAJI[5]

1)Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan 2)Faculty of Medicine, Gunma University, Maebashi, Japan 3)Kyoto University Hospital, Kyoto, Japan 4)Faculty of Pharmaceutical Sciences, Kobegakuin University, Kobe, Japan 5)Graduate School of Pharmaceutical Science, Kyoto University, Kyoto, Japan

Poster2

Development of inhibitors of metallo-β-lactamase (IMP-1 and VIM-2)

○JIN WANCHUN[1], Yoshichika ARAKAWA[2], Yosihiro YAMAGUCHI[1], Hiromasa KUROSAKI[1], Yuriko YAMAGATA[3], Michio OHTA[4], Masafumi GOTO[1]

1)Department of Structure-Function Physical Chemistry, Graduate School of Pharmaceutical Sciences, Kumamoto University 2)Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases 3)Department of Structural Biology, Graduate School of Pharmaceutical Sciences, Kumamoto University 4)Department of Molecular Bacteriology, Nagoya University Graduate School of Medicine

Poster3

Biological effects of novel binuclear platinum (II) complexes in human ovarian tumor cell lines

○Noriko AOKI[1], Takaji SATO[1], Maiko KODA[1], Aiko SAKIYAMA[1], Rei KOMAKI[1], Seiji KOMEDA[1], Yoshihiro SAITO[1], Masahiko CHIKUMA[1]

1)Osaka University of Pharmaceutical Sciences, Osaka, Japan

Poster4

Interactions of Biomolecules with Platinum(II)-Polypyridine Complexes Containing an Amino Acid. Adduct Formation and Its Effect

○Minoru TASHIRO[1], Tatsuo YAJIMA[1], Takeshi MOTOYAMA[1], Akira ODANI[2], Yasuo NAKABAYASHI[1], Osamu YAMAUCHI[1]

1)Unit of Chemistry, Faculty of Engineering, Kansai University, Osaka, Japan

2) Reserch Center for Materials Science, Nagoya University, Nagoya, Japan

Session Ⅱ Design of Functional Metal Molecule

Poster5

DNA binding selectivity of artificial 6-zinc finger proteins

○Miki IMANISHI[1], YAN WEI[1], Yukio SUGIURA[1]

1) Institute for Chemical Research, Kyoto University, Kyoto, Japan

Poster6

Interactions of glucose oxidase with dipolar ruthenium ammine complexes as electron transfer mediators of glucose oxidation

○Yasuo NAKABAYASHI[1], Yuri HIROSAKI[1], Masahiro KINOSHITA[1], Osamu YAMAUCHI[1]

1) Unit of Chemistry, Faculty of Engineering, Kansai University, Osaka, Japan

Poster7

Hetero metal-assemblage in artificial DNA

○Yusuke TAKEZAWA[1], Kentaro TANAKA[1,2], Tatsuhisa KATO[3], Mitsuhiko SHIONOYA[1]

1) Department of Chemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan 2) PRESTO, Japan Science and Technology Corporation (JST), Tokyo, Japan 3) Institute for Molecular Science, Okazaki, Japan

Poster8

Syntheses of cyclometalated ruthenium(Ⅱ) complexes and application for electron transfer mediators

○Masahiro KINOSHITA[1], Yasuo NAKABAYASHI[1], Osamu YAMAUCHI[1]

1) Unit of Chemistry, Faculty of Engineering, Kansai University, Osaka, Japan

SessionⅢ Ecosystem and Metals

Poster9

Effects of metals on post-embryonic development and reproduction in *C. elegans*

Koji ARIZONO[1,4], ○Yukari KOGA[1,4], Fumiyo MORITA[1], Takashi NAKAMOTO[1], Hirokazu KIMURA[1], Kazuhiro URA[2], Taisen IGUCHI[3,4]

1) Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto, Japan 2) Graduate School of Fisheries Sciences & Faculty of Fisheries, Hokkaido University, Hokkaido, Japan 3) Center for Integrative Bioscience, Okazaki National Research Institutes, Aichi, Japan 4) Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama, Japan

Poster10

Research on purification of soil and water pollution by the heavy metals using *Lotus japonicus*

○Rieko HIRUNUMA[1], Kaori IGARASHI[1], Hiroyuki SAITO[1], Yutaka MIYAZAWA[2], Hiromitsu HABA[1], Hiroshige TAKEICHI[1], Tomoko ABE[1], Shuichi ENOMOTO[1]

1)RIKEN 2)Graduate School of Life Sciences, Tohoku University, Miyagi, Japan

SessionⅣ Development of New Metal-Complex・Metal-Probe

Poster11

Design and Synthesis of Zinc Selective Chelators

○Eri KAWABATA[1], Kazuya KIKUCHI[1,2], Akira ODANI[3], Tetsuo NAGANO[1]

1)Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan 2)Presto, JST Corporation, Kawaguchi, Saitama, Japan 3)Department of Chemistry, Graduate School of Science, Nagoya University, Nagoya, Aichi, Japan

SessionⅤ Metal and Biological Function

Poster12

Susceptibility to excitotoxity of glutamate under zinc deficiency and effect of saiko-ka-ryukotsu-borei-to, a herbal medicine, on the excitotoxity

○Haruna TAMANO[1], Atsushi TAKEDA[1], Naoto OKU[1]

1)Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Poster13

Bioavailability of zinc yeast in rats: Stimulatory effect on bone calcification in vivo

○Satoshi UCHIYAMA[1], Aki IGARASHI[1], Masayoshi YAMAGUCHI[1]

1)Graduate School of Nutritional Sciences, University of Shizuoka

Poster14

The role of glutathione in the stability of biliary arsenic metabolites, arsenic triglutathione [$As(GS)_3$] and methylarsenic diglutathione [$CH_3As(GS)_2$]

○Yayoi KOBAYASHI[1,2], Seishiro HIRANO[1]

1)Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan 2)Japan Foundation for Aging and Health, Chita, Aishi, Japan

Poster15

Distributions of ^{67}Cu in hepatoma rats and Cu chaperone knock-down HepG2 cells

○Akihiro NAKAYAMA[1], Hiromitsu HABA[1], Shuichi ENOMOTO[1]

1)Cyclotron Center, RIKEN, Saitama, Japan

SessionⅥ Metal Analysis

Poster16

Elution behavior of elements from insoluble minerals as food additives in the manufacturing process of liquid food

○Takashi AMAGASA[1], Rie ITO[1], Koichi INOUE[1], Yoshihiro YOSHIMURA[1], Makiko YAMADA[2], Teruhisa FUJIMAKI[3], Masakazu HORIE[4], Hiroyuki NAKAZAWA[1]

1)Faculty of Pharmaceutical Science, Hoshi University 2)SaneigenFFI, Tokyo, Japan 3)Kanagawa Institute of Health, Kanagawa, Japan 4)Saitama Institute of Health, Saitama, Japan

Poster17

NMR analysis of mouse urine: application to toxicity evaluation of trace elements

○Hideki MIYATAKA[1], Toshiyuki OZAKI[1], Seiichiro HIMENO[1]

1)Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

SessionⅦ Metal-Associated Bio-Chemical Reaction

Poster18

Radical-Scavenging Reactions of 4-Hydroxycinnamic Acid Derivatives in the Presence of Metal Ions

Ikuo NAKANISHI[1,2], Yoshihiro UTO[3], ○Tomonori KAWASHIMA[1,4], Haruko YAKUMARU[1], Hideko KANAZAWA[4], Toshihiko OZAWA[1], Hideko NAGASAWA[3], Hitoshi HORI[3], Nobuo IKOTA[1]

1)Redox Regulation Research Group, National Institute of Radiological Sciences, Chiba, Japan 2)Department of Material and Life Science, Graduate School of Engineering, Osaka University, CREST, Japan Science and Technology Agency, Osaka, Japan 3)Department of Biological Science & Technology, Faculty of Engineering, The University of Tokushima, Tokushima, Japan 4)Department of Physical Chemistry, Kyoritsu College of Pharmacy, Tokyo, Japan

Poster19

Effects of Metal Ions on the Stability of Oxygen and Nitrogen Radicals

○Ikuo NAKANISHI[1,2], Tomonori KAWASHIMA[1,3], Hideko KANAZAWA[3], Kei OHKUBO[2], Kiyoshi FUKUHARA[4], Toshihiko OZAWA[1], Shunichi FUKUZUMI[2], Nobuo IKOTA[1]

1)Redox Regulation Research Group, National Institute of Radiological Sciences, Chiba, Japan 2)Department of Material and Life Science, Graduate School of Engineering, Osaka University, CREST, Japan Science and Technology Agency 3)Department of Physical Chemistry, Kyoritsu College of Pharmacy 4)Division of Organic Chemistry, National Institute of Health Sciences

SessionⅧ Structure and Property of Metal-Complex

Poster20

Control of geometric stability and spin state of the iron(III) atom by isomeric porphyrins

○Saburo NEYA[1], Tyuji HOSHINO[1], Masayuki HATA[1]

1)Department of Physical Chemistry, Graduate School of Phramaceutical Sciences, Chiba University

Poster21

Increased binding affinity of aluminum in the presence of oxalate for asialo-transferrin compared with native-transferrin studied by HPLC/HR-ICP-MS

○Megumi HAMANO NAGAOKA[1], Hiroshi AKIYAMA[1], Tamio MAITANI[1]

1)Division of Foods, National Institute of Health Sciences

Poster22

The Metal Binding Motif of Dipeptidyl Peptidase III Directly Influences the Enzyme Activity in the Copper Derivative of Dipeptidyl Peptidase III

○Junzo HIROSE[1], Hiroshi KAMIGAKIUTI[1], Hiroyuki IWAMOTO[1], Satoru YAMAMOTO[2], Kayoko FUKASAWA[3]

1)Department of Applied Biological Science, Fukuyama University, Fukuyama, Japan 2)Department of Biotechnology, Fukuyama University 3)Department of Oral Biochemistry, School of Dentistry, Matsumoto Dental University

Poster23

Roles of Heme Axial Ligands in the Regulation of CO Binding to CooA

○Taku YAMASHITA[1]

1)The Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan

Poster24

Study on Structures and Characterizations of Linear Au(I) Thiolato Complexes

○Yuki MATSUNAGA[1], AMIR NAGINA[1], Yoshitaro MIYASHITA[1], Kiyoshi FUJISAWA[1], Ken-ichi OKAMOTO[1]

1)Department of Chemistry, University of Tsukuba, Ibaraki, Japan

Poster25

Syntheses of dinuclear ruthenium(II)-2,2'-bipyridine complexes and characterization of their DNA-binding activities

○Nobuaki IWAMOTO[1], Yasuo NAKABAYASHI[1], Osamu YAMAUCHI[1]

1)Faculty of Engineering, Kansai University, Osaka, Japan

The 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04
Thursday, June 10 – Friday, June 11, 2004

Abstracts

Special Lectures

Topic Lecture

Special Lecure I

Isogenies of drugs and food, and metals

○Yoshinori ITOKAWA

Professor Emeritus, Kyoto University, Medicine, Graduate School of Nursing and Social Welfare Science, Fukui Prefectural University, Japan

1. Isogenies of drugs and foods

Drugs are used for recovery of people suffering from various diseases and foods are taken for maintaining good health. Both drugs and foods are indispensable substances to keep the health for humans. This notion is expressed in the words "drugs and foods are isogeny". This lecture aims to discuss the role of metals in isogenous functions of drugs and foods.

This notion is easily understandable when one took a case of "health food". Health foods are defined as the foods that possess functions to promote the health and prevent the diseases beyond nutritional functions of usual foods. Because of recent increase in aged population in Japan, life-style related diseases increased. This circumstance arouses people's interest in health food, and activities of traders and makers concerning health food are growing. Annual market economy of health food has exceeded on hundred million yen. However, there are a flood of unreliable nutritional information and inappropriate health food. In these situations, Ministry of Health, Labour and Welfare devised measures to meet the situation. These measures include a food with nutrient function claims which imposes a duty to demonstrate nutritional constituent of foods and a food for specified health uses which is helpful to health maintenance for patients, pregnant women, etc.

Currently two sorts of health foods exist in Japan, one is specified in the law, and the other is not specified in the law. Problems on health foods in Japan are now in disorder and should be arranged politically. Regarding metals and minerals, there are several minerals, i.e. calcium, magnesium, iron, zinc, copper and selenium, which are widely fortified in many health foods. The speaker will discuss the problems on metals in health foods.

In regard to metals in isogenies of drug and foods, metal and minerals could be classified into the following two categories. Essential minerals, which intake amounts in Japanese dietary habit are less than recommended dietary allowance of the relevant minerals. Supplement of these minerals from foods may prevent deficiency diseases. Minerals which have an effect of medicine (pharmacological function). Supply of foods containing high amount of these minerals may be useful to remedy the diseases.

2. Metals in which deficiency assumed to exist in Japanese (Category 1)

In 1999, recommended allowances of twelve minerals are estimated and in National nutrition survey 2001 intake amounts of seven minerals: calcium, magnesium, sodium, potassium, iron, zinc and copper were calculated. In comparison with recommended dietary allowance and intake amount of each mineral, grade of sufficiency of each mineral in Japanese could be estimated. The ratios(%) of intake amount of each mineral for men and women aged 30-49 years are shown in Fig. 1 and 2.

From these results calcium, magnesium, iron, zinc and copper could be included in this category.

(1) Calcium

By national nutrition survey 2001, it was found that intake amount was lower than recommended calcium allowance in people aged 15 and older. As bone mineral density increases most rapidly around age 15 in young generation, calcium deficiency in this age group may bring osteoporosis in future. In contrast, calcium deficiency in aged people may accelerate the parathyroid hormone activity and release calcium from bone to blood. This will also worsen the osteoporotic symptoms.

(2) Magnesium

Similarly as in the case of calcium, magnesium is deficient from age 15 and up. Various experimental and epidemiological studies suggested that magnesium deficiency causes cardiovascular diseases. A reduction of dietary magnesium will be associated with reduced activity of ATPase which in turn uses magnesium as an essential cofactor. Reduction of this enzyme activity may cause an increase in intracellular calcium and a decrease in intracellular magnesium. These changes in blood vessels may cause vasoconstriction and increased vascular resistance. Thus, magnesium deficiency enhances the susceptibility to ischemic heart diseases.

(3) Iron

National nutrition survey in 2001 revealed that intake of iron did not reach dietary allowance in every age group both in make and female. Especially, iron intake of female in age 18-29 is only about 60% of the recommended allowance. National nutrition survey in 2001 revealed that 23.2% of male and 18.1% of female were anemic (hemoglobin concentrations in blood were lower than the normal range) and this fact could be based on the low intake of iron.

(4) Zinc

It was found that zinc is deficient in age group over 18 in male and over 15 in female. Zinc deficiency is associated with the delayed healing of injuries, taste blindness, a condition in which food cannot be tasted, immunodeficiency, et al. As zinc is contained in various enzymes playing an important role in metabolism, including alcohol dehydrogenase, alkaline phosphatase, DNA polymerase, RNA polymerase, or superoxide dismutase, deficiency of zinc may induce a variety of symptoms in human body.

(5) Copper

Intake amount of copper was below the recommended allowance of copper in all age groups both in male and female. Anemia is the most common symptoms in copper deficiency. Copper is contained in serum ceruloplasmin, which plays a role in oxidizing bivalent iron to trivalent iron and in transporting oxidized trivalent iron. Thus, anemia is probably induced by copper deficiency because the function of ceruloplasmin is inhibited and it becomes impossible for iron to be utilized biologically. Some of the anemic persons mentioned above could be attributed to copper deficiency.

(6) Sodium and potassium

Sodium is usually available as sodium chloride (salt). Sodium hyperalimentation can therefore be regarded as salt hyperalimentation. It is known that excessive intake of salt results in development and progress of hypertension. Excessive salt intake is a risk factor for cardiovascular diseases. For prevention, it is essential to properly limit the salt intake. The minimum requirement of salt is less than 1g/day for adults, and the threshold of salt for elevation of blood pressure is assumed to be 3-5g/day. However, in view of the Japanese dietary habit with higher salt intake, it would be difficult to prepare meals appreciated by the Japanese with only 3-5g/day of salt. Consequently, 10g/day or less of salt is recommended as the safe and adequate intake for Japanese.

Average intake of sodium is 4,500 mg/day/person (11.5g as salt) in 2001 and this amount exceeds the above recommended target. Many studies have shown that an increase in blood pressure due to sodium hyperalimentation can be inhibited by the administration of potassium. Excessive potassium administration will increase urinary sodium excretion and lead to sodium loss. Thus, sodium hyperalimentation can be modified by altering the potassium intake.

In 2001, intake amount of potassium exceeded the recommended allowance of potassium in all age groups both in male and female. However, when comparison is made between sodium and potassium intakes, average sodium intake is 1.8 times as much as potassium intake. Therefore, an increase in potassium intake in excess of the current intake may be benefit to prevent cardiovascular diseases.

3. Pharmacological function of metal or mineral (Category 2)

(1) Magnesium as a laxative

Ingestion of unabsorbable compound of magnesium, such as magnesium sulfate, causes and increase in intestinal osmotic pressure. This prevents intestinal absorption of water and helps

bowel movement (vermiculation). Therefore, excessive amount of magnesium ingestion may cause diarrhea. Using this function magnesium can be used as laxative.

(2) Zinc and prevention of bedsore (Jokuso)

Tanaka et al.(Tanaka Y:Proc 16th Symp Trace Nutr Res 16:165-170, 1999) studied nutritional status of the bedridden elderly people with bedsore and those without bedsore. Although significant change was not observed on intake amount of zinc between them, concentration of zinc in serum was significantly lower in elderly with bedsore as compared to elderly without bedsore. This result suggested that elderly people with bedsore lost the ability to utilize zinc and nutritional status of zinc in bedridden elderly is related to occurrence of bedsore.

(3) Zinc treatment for taste disorders

Tomita examined 5,000 patients with taste disorder for 25 years and found that 70% of these patients were in a state of zinc deficiency. As taste buds in tongue, receptors of taste, require lots of zinc to fulfill their function, zinc deficiency may cause taste disorders. Treatment with zinc administration to these patients was very successful. (Tomita H: A Cyclopedia of Minerals. Ed: Itokawa Y., Asakura Shoten, p621, 2003).

(4) Chromium for treatment of diabetes mellitus

A low molecular compound containing chromium which has a function curable impaired glucose tolerance was isolated from pig kidney and named glucose tolerance factor (GTF). (Schwarz K, Merts W: Arch Biochem Biophys 85:292, 1959) Precise composition of GTF was not elucidated. Various studies revealed that administration of GTF or chromium to animal or human improves the symptoms of diabetes mellitus. In addition, some patients receiving total parenteral nutrition for long term using infusion fluid not fortified with chromium showed impaired glucose tolerance. Although accurate mechanism of this chromium function on diabetes mellitus is not clarified yet, chromium was considered to be essential metal as an anti-diabetic factor.

(5) Selenium and viral infections

Coxackieviruses have been implicated as possible co-factors in the etiology of the selenium responsive cardiomyopathy known as Keshan disease. Beck et al. reported that Coxackieviruses, which cause no pathology in the heart of selenium adequate mice, induce extensive cardiac disease in selenium deficient mice. The virus recovered toxicity from the heart of selenium deficient mice induced significant heart damage into selenium sufficient mice by inoculation. (Beck MA et al: J Infect Dis 170:351-357, 1994). This study suggested that selenium status of host has significant influence on the severity of viral infection.

(6) Lithium and mental conditions

Schrauzer and Shrestha reported that the incidence rates of suicide, homicide, robbery, burglary and theft were higher in areas supplied with drinking water containing low lithium as compared to ones with drinking water containing high concentration of lithium. (Schrauzer GN, Shrestha KP: Biolog Trace Ele Res 25:105, 1990). Also Schrauzer et al reported that concentration of lithium in scalp hair of violent criminals was significantly lower than normal people. These studies suggested that lithium has a sedate effect on manic status. Actually, lithium is used clinically as medicine to patients with manic-depressive psychosis.

(7) Antitumor effect of tin

Arakawa and Wada found that dialkylin compounds exhibited andtitumor activity in vivo towards various tumor in animals and inhibited the proliferation of cultured tumor cells in vitro. They suggested that tin compounds may inhibit the growth of malignant cell population by preventing the mitotic division of the tumor cells. These compounds may be used as anticancer agents. (Arakawa Y, Wada O: Tin and Malignant Cell Growth (Ed: Zuckerman JJ) CRC Press, p83, 1988).

Fig 1　Ratio of Intake Amount to Dietary Allowance
(Age:30-49, Male)

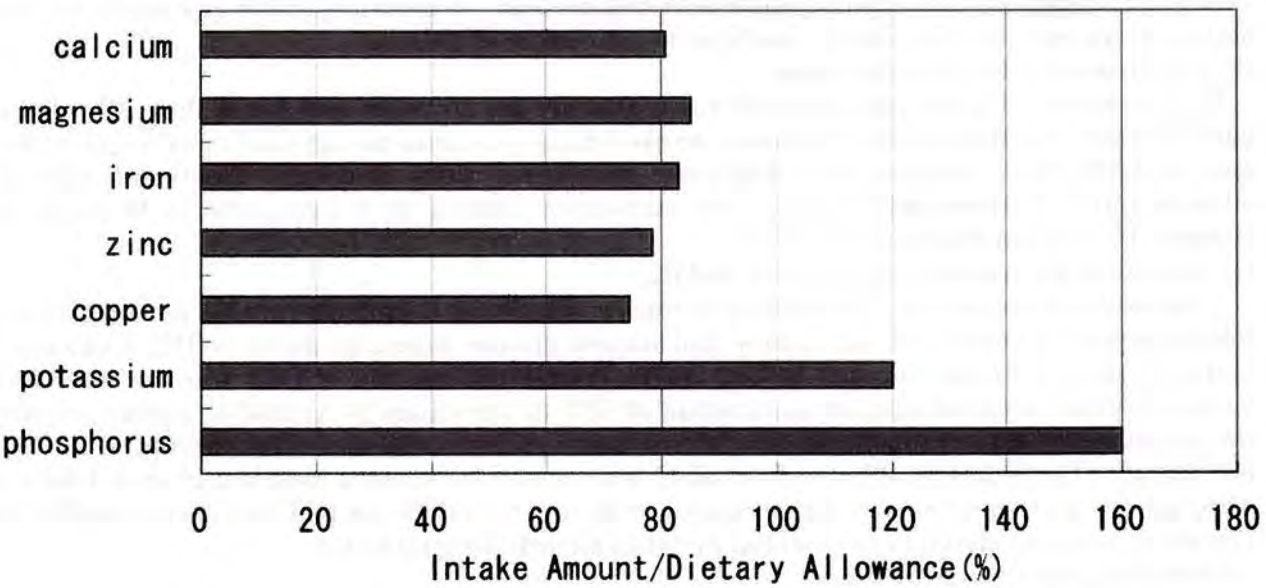
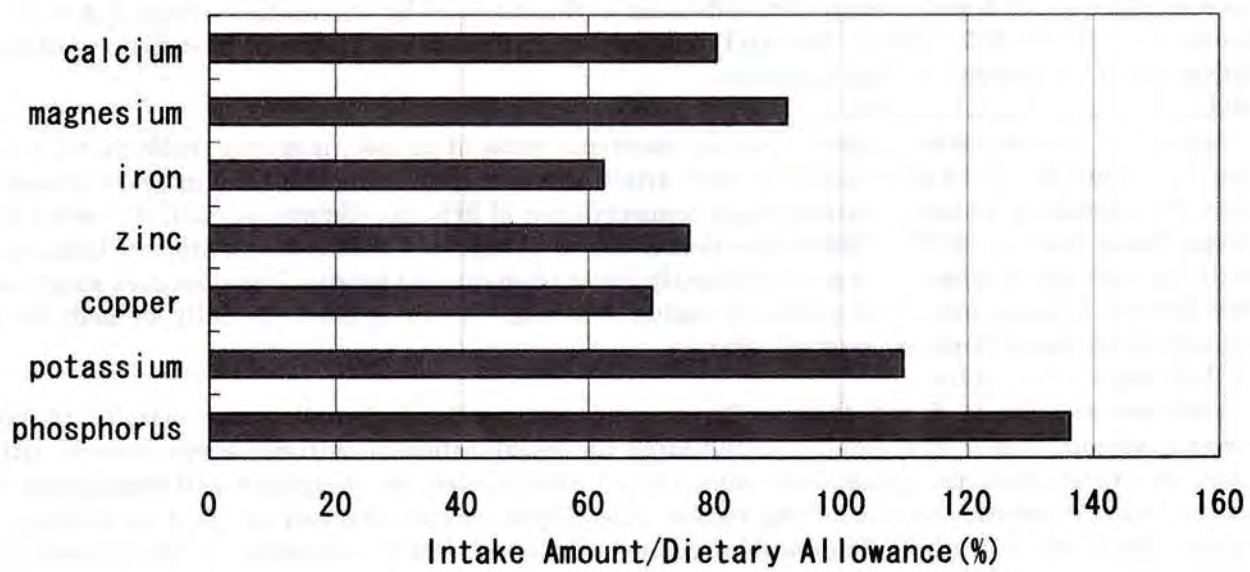


Fig 2　Ratio of Intake Amount to Dietary Allowance
(Age:30-40, Female)



薬食同源と金属

○糸川 嘉則
京都大学名誉教授　医学部、福井県立大学　看護福祉学研究科

Special Lecture II Essentiality and Toxicity of Metals -Fundamental and Practical considerations-

○Osamu WADA[1], Hiroyuki YANAGISAWA[2]

[1]Professor Emeritus, The University of Tokyo, Medicine, Tokyo, Japan, [2]Department of Hygiene, Preventive Medicine, Saitama Medical School, Saitama, Japan

Abstracts

In an attempt to promote scientific argument and to attain a consensus on the evaluation of essentiality and toxicity of metals, this lecture discusses both the present status of recommended dietary allowance (RDA) and reference dose (RfD) in Japan and other countries and their associated problems with an emphasis on how to overcome the confusion observed between evaluations on essentiality and toxicity of metals.

Fundamental concept of essentiality and toxicity

It is generally accepted that either inadequate or excessive intake of a metal can result in disease and death. This means that every metal has both essential and toxic properties at its inherent levels. Only the dose does make the metal nutrient or poison, as proposed by Paracelsus already in 16th century.

Dose-response relationship

Dose-response relationships are well founded in the evaluation and practical application of essentiality and toxicity. The information available for dose-response assessment ranges from well documented human data to a lack of human and animal data.
For example, there are many kinds of doses; exposure levels, levels in blood or urine, levels in organs and concentrations in target points in an easily measurable, but inaccurate order. Also, endopoints of responses include death, diseases, specific or non-specific biochemical and pathological changes and reactions. The latter responses are more sensitive, but they may not express real hazards or health disorders. In any way, the data concerning doses and responses are conflicting and need confirmation.

RDA and RfD and their comparison

RDA represents levels of intake of metals that are judged to be adequate to meet the known nutrient needs of practically all healthy persons. To ensure the aim stated above, an intake of two standard deviations above the estimated mean requirement is usually recommended. This value satisfies nutrient needs for 97.5% of healthy general people.
In the toxicity side, many countries including Japan establish the daily exposure levels that are likely to be without appreciable risk of deleterious effects during a lifetime for all sensitive and non-sensitive persons. However, human toxicity data adequate for use in the estimation of RfD of metals are seldom available. Usually, RfD is composed of the experimentally determined non-observed-adverse-effect levels (NOAEL) divided by the composite uncertainty factor and modifying factor.
Thus, basis, principle and methodology for establishing RDA and RfD for metals differ widely. Also, confidence, assumptions and limitations are not the same among the two indices. These result in the confusion between the evaluation of essentiality and safety of metals.
Generally speaking, uncertainty provides a conservative estimate. It tends to increase RDA,

whereas it decreases final RfD. The interval between the required and toxic amounts of metals becomes narrow. In fact, in some metals, RfD actually equals or is lower than RDA.
Usually, daily intakes of essential metals by healthy average Japanese are found to be almost identical with RDA. Furthermore, RDA and RfD values are usually within an order of magnitude. The daily doses of intake, NOAEL, LOAEL, therapeutic use, RDA and RfD of some metals for Japanese are identified without considering the inconsistency of concept and interpretation of these doses (Table 1).

Ultra-trace metals and therapeutic use of metals

Two recent scientific and practical topics present a confusion worse confounded.
The ultra-trace metals such as arsenic, nickel, cadmium, lead, tin and vanadium, all of them were considered as a purely toxic metals with no nutritional requirement, have been recognized as potentially important nutrients. Although deficiency in humans has not been established for any of these metals, evidence for a requirement in laboratory animals has been presented for all of these metals.
Furthermore, extremely high doses of some metals have been administered to effectively improve immunological disorders, cancer, cardiovascular diseases, nervous disorders, genetic disorders and smell and taste disorders. The dose used is far more than RfD of the metal.
The present status of dose-response relationships and RDA/RfD levels of metals grouped by their characteristics is illustrated in Fig.1, as compared with usual Japanese intakes, RDA/RfD and therapeutic doses.

Case studies

Case studies concerning the essentiality and toxicity of some metals are presented and discussed in the lecture. As a representative, an outline of the present status of zinc is as follows.
There is obvious evidence of zinc deficiency and poisoning in humans. RDA is determined as 15mg/day for male adults in USA, based on the amount needed to replace losses of 2.5mg/day using an absorption data of 20%. RDA in Japan is 9.6mg/day, which was estimated from the maintenance doses for intravenous alimentation. RfD is 0.3mg/kg/day in USA. This derives from a decrease in erythrocyte SOD activity resulting from Cu deficiency due to zinc dosing. RfD in Japan is 30mg/day, based on human NOAEL clinically observed form daily intakes via foods or supplements or therapeatical administrations for various biochemical or clinical conditions. There are many problems concerning these values. These include an overlap of RDA and RfD in some age groups in USA and far more amounts of supplementation taken by healthy people or of effective administration to some diseased patients without any adverse effect. Questions are also posed as to the implication concerning the decreased SOD activity or clinically observed NOAEL.
The present status of essentiality and toxicity of zinc is shown in Fig.2. Obvious confusion and conflict are observed among the values of both properties.

Conclusions and recommendations

1. Perhaps most important point of view is that neither RDA nor RfD should be construed as an absolute, rigid number. Both have an inherent degree of uncertainty surrounding them that must be taken into account in comparing and interpreting these values.
Furthermore, it should be noted that the basis, approach, conceptual purpose, inherent assumption and included meaning of essentiality and toxicity, expressed as RDA and RfD, differ widely for each of metals. As the methodology to define them progresses, RDA values tend to increase and RfD levels are likely to decrease. Finally, RDA equals RfD in some metals. Moreover, in these situations, they may represent only the conservative risk estimates without defining any deficient or toxic hazards.

2. Although the published RDA and RfD values might occasionally appear to approach each other, this does not necessarily imply incompatibility of the two methodological approaches, nor does it indicate inherent inaccuracy or error. Rather, it is far probable that the databases on which the two numbers are derived are simply not adequate to accurately define benchmark values for essentiality or toxicity.

3. To improve the databases, types of data used, synergistic / antagonistic interactions, route of exposure, correct exposure doses, bioavailability, test species and individual variability should be introduced into them in the future evaluation of essentiality and toxicity of metals.

4. The derivation and determination of RDA and RfD should be based on both updated scientific evidence and human hazard assessment rather than experimentally induced animal risk assessment, as illustrated in Table 2.

5. The concept of chronic diseases or ageing prevention should be included in the development of future RDA and RfD.

6. Greater coordination is needed between policy makers in both nutrition and toxicology to avoid unnecessary confusion. In reality, the approaches of nutritionists and toxicologists actually address opposite ends of the physiological tolerance range in a complementary manner. As the state of nutritional and toxicological knowledge advances and interactions between members of these two scientific communities increase, any problem between RDA and RfD for metals should eventually be resolved.

7. The process of translating scientific findings into public policy is urgently needed to promote better risk management and risk communication.

Fig.1. Dose-Response-Relationships and Various Indices of Metals

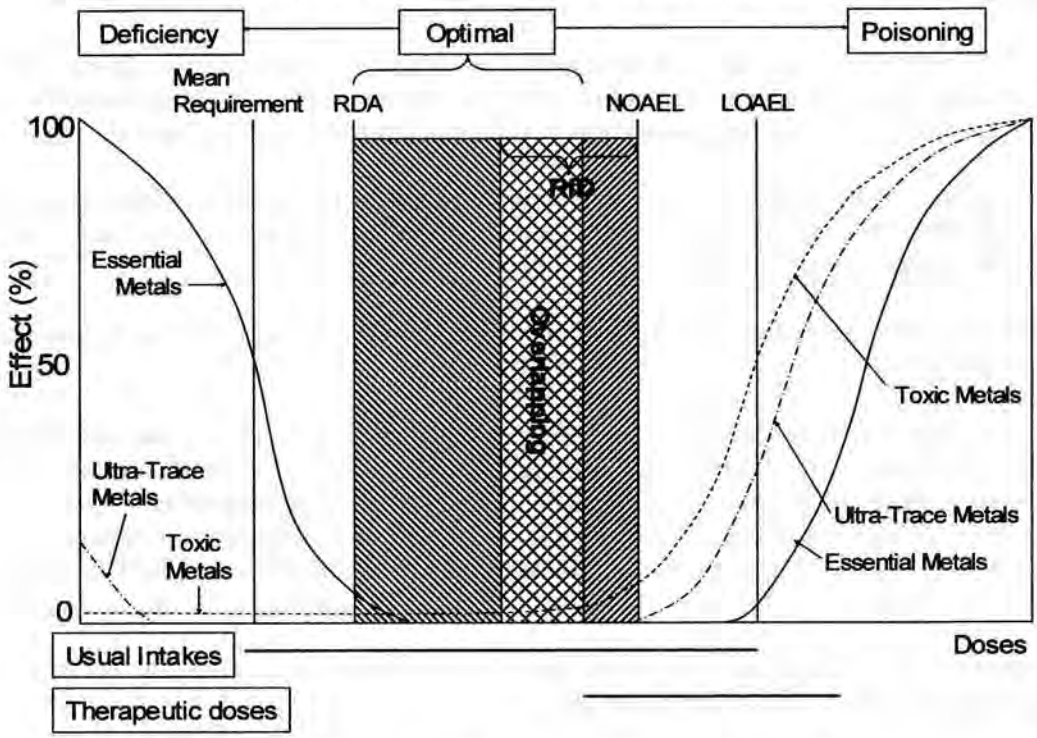


Fig.2. Comparison of Effective and Toxic Doses of Zinc

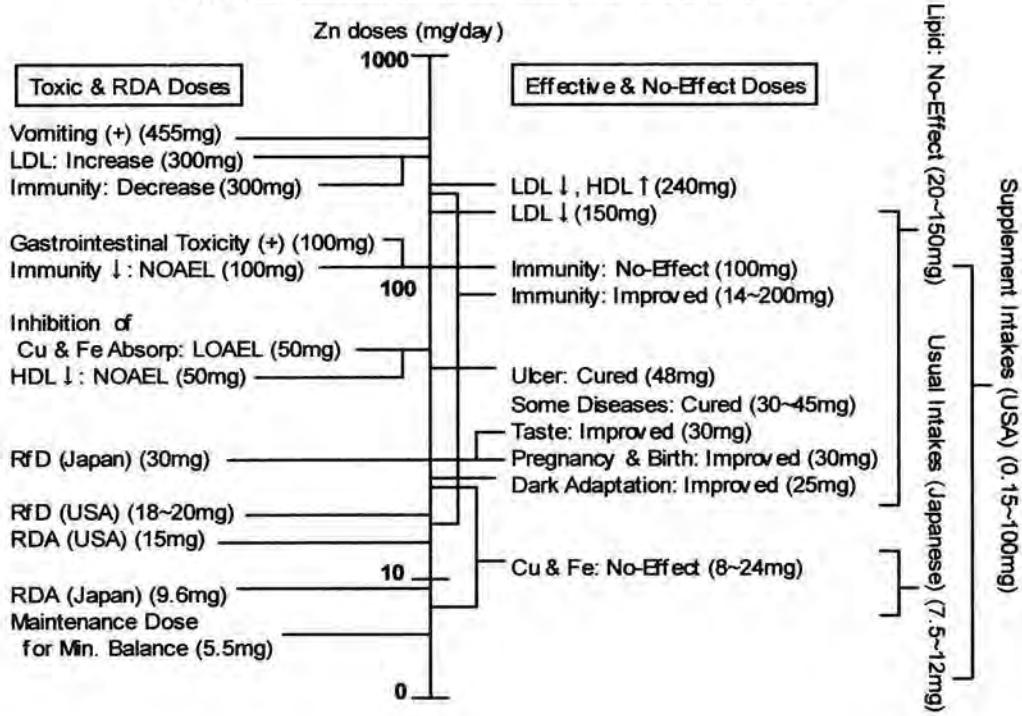


Table1. Comparison of Daily Doses of Trace Metals (Japanese male adult:50kg BW)

	Intake from food etc	NOAEL	LOAEL	Therapeutic dose	RDA	RfD	RfD/RDA ratio
Zinc	7-11mg	30mg*	60mg	~200mg	9.6mg	30mg	3
Copper	1-4mg	9mg*	10mg	—	1.8mg	9mg	5
Chromium (Ⅲ)	28-62μg	1000μg*	—	150~1000μg	35μg	250μg	7
Selenium	41-168μg	400μg	750μg*	~200μg	55μg	250μg	4.5
Manganese	3-4mg	10mg*	—	—	4mg	10mg	2.5
Molybdenum	135-215μg	350μg	7mg*	—	30μg	250μg	8
Iodine	200-300μg	3000μg*	23000μg	—	150μg	3000μg	20
Arsenic	10-34μg	40μg*	700μg	—	10~34μg	140μg	4

* Values used for RfD determination

Table 2. Comparison of Risk Assessment and Hazard Assessment

	Risk Assessment	Hazard Assessment
Methodology	Animal NOAEL divided by uncertainty and modifying factors	Human actual NOAEL measured
Safety margin	Very wide	Actual estimates with almost no safety margin
Values	Likely to be too low to use for practice	Practical, actual and easily usable
Interpretation	Conservative estimates for ease, not a boundary between safety and toxicity	Human actual estimates for regulating action indicating safety or toxicity
Objectives	Goal for complete prevention	Evaluation of actual safety
Faults	Difficult to use for metals with both essentiality and toxicity	Sometimes insufficient for far sensitive subgroups

金属の有用性と毒性 －基本的及び実践的考察－

○和田 攻[1]、柳澤 裕之[2]

[1]東京大学名誉教授 医学部、[2]埼玉医科大学 医学部 衛生学教室

Special Lecture III Elucidation and Application of Intrinsic Properties of Molecular Recognition by Zinc Ions in Biology

○Eiichi KIMURA

Department of Integrated Arts and Science, Hiroshima University, Hiroshima, Japan (ex-President, The Pharmaceutical Society of Japan)

・Introduction

The role of molecular recognition is a central issue in zinc enzymes. Typically, carbonic anhydrase(CA), where the zinc ion is ligated by three histdine residues, recognizes not only its regular substrates (and its products) CO_2 and HCO_3^-, but also irregular substrates such as phosphoesters, carboxyesters, sulfonamides, and carboxyamides as inhibitors. Another ubiquitous zinc enzyme carboxypeptidase A (CPA), where the zinc ion is bound to two histidines and a glutamate residue, recognizes substrates peptides, carboxyesters, and substrate-hydrolyzed products.

Needless to say, model studies using zinc complexes cannot serve to explain all aspects of the complex enzymatic processes. Still, they could prove useful in elucidating some of the intrinsic chemical roles played by zinc. Zinc-macrocyclic polyamine complexes offer a substantial advantage as zinc enzyme models. They are kinetically and thermodynamically stable in aquesou solution at biological pH. Since the molecular recognition properties of zinc enzymes are deliberately modulated as a function of pH in aqueous solution, this special feature of the macrocyclic complexes is extremely useful(Ref 1).

・Recogniton of Hydroxide Ion

To a first approximation, in both CA and CPA, zinc-bound hydroxide anions act as nucleophiles to attack at the bound substrates. Toward this end, water molecules are activated by zinc at neutral pH. The acidity of these zincs are thus of fundamental concern. In CAII, a pKa value of 6.8 for the zinc-bound water was reported. In CPA, a pKa value of 6.2 was assigned. However, these pKa values are fluctuated greatly in accodance to the catalytic conditions. Hence, the intrinsic acidity of zinc remained incompletely understood.

The Zn-1,5.9-triazacyclononane (abbreviated as [12]aneN3) complex **1** was a potentially attractive model for CA (Ref 2). It possesses a firm tetrahedral structure with a H_2O at the fourth coordination sites, which deprotonates ($Zn\text{-}H_2O \rightarrow Zn\text{-}OH^-$) with a pKa value of 7.3, as determined by pH-potentiometric titration. Namely, **1** has a strong affinity of OH^- with a log K(assoc) value of 6.4. The $Zn\text{-}OH^-$ in **1** proved to act as a strong nucleophile toward carbonyl groups.

・Recogniton of CO_2 and HCO_3^-

Our CA model **1** provides for the first time a convincing chemical mechanism that illustrates the important role that Zn plays in the reversible hydration of CO_2 and dehydration of HCO_3^- ($CO_2 + H_2O \rightarrow HCO_3^- + H^+$). The kinetic studies demonstrated this reversible reactions catalyzed by **1** at acidic and basic pH's (Ref 3).

・Recognition of Sulfonamides

Neutral inhibitors of CA comprise weak acids such as sulfonamides ($-SO_2NH_2$) or carboxamides ($-CONH_2$). A typical CA inhibitor is medicinally important acetazolamide (pKa = 7.5), which has a Ki value of 0.1 micromol. It was reported that roughly 60% of the CA-sulfonamide interaction energy comes from the direct coordination of the sulfonamide to Zn. The first unequivocal model in support of the proposed Zn-sulfonamide interaction was constructed by synthesizing a solfonamide-pendant [12]aneN3 (Ref 4). This model study further led to development of a selective and efficient fluorescent Zn indicator, dansylamide-pendant 1,4,7,10-tetraazacyclododecane

(abbreviated as [12]aneN4 or cyclen) (Ref 5), which is now applicable to detection of cellular zinc ion and ,moreover, detection of early stage of apoptosis of cancer cells HeLa or HL60 (Ref 6).

・Recogniton of Carboxamides

Carboxamides are inhibitors for CA, while they are substrates for CPA. For these models, several kinds of carboxamides were attached to [12]aneN4 to see if the carboxamides placed in the vicinity of Zn can bind to Zn (a CA-inhibition model) or can be hydrolyzed (a CPA-catalysis model) (Ref 7). We found a CA-inhibition model, where some carboxamides strongly bind to Zn sequested by the [12]aneN4 as neutral ligands (-$CONH_2$) at neutral pH or as anionic ligands (-$CONH^-$) at alkaline pH. We also found a possible CPA-catalysis model with other kind of carboxamide pendants, where instead of the pendant amide interacting, Zn-OH^- sepcies are more favorably formed at neutral pH to attack at electrophilic sites, e.g. externally added carboxyesters.

In relevance to the strong carboxamidate anion coordination to Zn, we have later discovered thymidine strongly coordinating (as N5-deprotonated species) to the fifth coordination site of the Zn-[12]aneN4. This finding was later extended to study of novel biochemical probe for selective and efficient recognition of thymidyl and uridyl residues in nucleotides, DNA, and RNA (Ref 8).

Exploitation of the imide anion coordination to Zn-[12]aneN4 led to discovery of a new type of supramolecular chemistry with creation of nanometer-size cage molecules that can accommodate cavity-matching hydrophobic organic guest molecules such as adamantane in aqueous solution at neutral pH (Ref 9).

・Conclusion

Our model studies using macrocyclic polyamine complexes provide the first detailed chemical support for the contention that the molecular recognition properties of CA and CPA are governed in large measure by the intrinsic properties of the central zinc ions. The pH dependence of substrate or inhibitor (X) binding affinity to the enzyme arises essentially from a competition between OH^- and X for the zinc sites. The affinity of X for Zn-OH_2 + X → Zn-X + H_2O and the affinity of OH^- for Zn-OH_2 → Zn-OH^- can be accurately determined using our model. The results from our studies proved useful in elucidating the underlying chemistry of CA, CPA, and other zinc enzymes, as well as in the design of new inhibitors and development of new supramoleuclar chemistry(Ref 10).

・References

1. "Model Studies for Molecular Recognition of Carbonic Anhydrase and Carboxypeptidase", E. Kimura, Acc. Chem. Res., 34, 171-179 (2001)
2. "A Zinc(II) Complex of 1,5,9-Triazacyclododecane as a Model for Carbonic Anhydrase", E. Kimura, T. Shiota, T. Koike, M. Shiro, and M. Kodama, J. Am. Chem. Soc., 112, 5805-5811 (1990)
3. "Kinetics and Mechanism of the Hydration of CO_2 and Dehydration of HCO_3^- Catalyzed by a Zinc(II) Complex of 1,5,9-Triazacyclododecane", X. Zhang, R. von Eldic, T. Koike, and E. Kimura, Inorg. Chem.32, 5749-5775 (1993)
4. "The First Anionic Sulfonamide-Binding Zinc(II) Complexes with a Macrocyclic Triamine; Chemical Verification of the Sulfonamide Inhibition of Carbonic Anhydrase", T. Koike, E. Kimura, I. Nakamura, Y. Hashimoto, and M. Shiro, J. Am. Chem. Soc., 114, 7338-7345 (1992)
5. "A Novel Biomimetic Zinc(II) Fluorophore, Dansylaminoethyl-Pendant Macrocyclic Tetraamine 1,4,7,10-Tetraazacyclododecane" , T. Koike, T. Watanabe, S. Aoki, E. Kimura, and M. Shiro, J. Am. Chem. Soc., 118, 12696-12703 (1996)
6. "A Macrocyclic Zinc(II) Fluorophore as a detector of Apoptosis", E. Kimura, S. Aoki, E. Kikuta, and T. Koike, Proc. Nat. Acad. Sci. U.S.A., 100, 3731-3736 (2003): "Monitoring Apoptosis with Fluorescent Zn(II)-Indicators", E. Kimura, R. Takasawa, S. Tanuma, S. Aoki, Science STKE Protocol, 2004, p17 (2004)
7. "Study of pH-Dependent Zinc(II)-Carboxamide Interactions by Zinc(II)-Carboxamide-Appended Cyclen Complexes", T. Koike, T. Gotoh, M. Shiro, and E. Kimura, Inorg. Chem. 41, 3239-3248 (2002)
8. "Zinc-Nucleic Acid Interaction", S. Aoki and E. Kimura, Chem. Rev. 104, 769-787 (2004)

9. "Three-Dimensional Supermolecules Assembled from a Tris(Zn-cyclen) Complex and Di- and Trianionic Cyanuric Acid in Aqueous Solution", S. Aoki, M. Shiro, T. Koike, and E. Kimura, J. Am. Chem. Soc., 122, 576-584 (2000): "A Cuboctahedral Supramolecular Capsule by 4:4 Assembly of Tris(Zn-Cyclen) and Trianionic Trithiocyanurate in Neutral pH Aqueous Solution", S. Aoki, M. Shiro, and E. Kimura, Chem. Eur. J., 8, 929-939 (2002): "New Supramolecular Trigonal Prisms from Zinc(II)-cyclen Complexes and Trithiocyanate in Aqueous Solution", S. Aoki, M. Zulkefeli, M. Shiro, and E. Kimura, Proc. Nat. Acad. Sci. U.S.A., 99, 4894-4899 (2002)
10. "From New Molecular Science to New Supramolecular Science with Macrocyclic Polyamines"(Special Review in the occasion of E.K's Retirement), E. Kimura, Yakugaku Zasshi, 122, 219-236 (2002)

生体亜鉛イオンによる分子認識機構とその応用

○木村 榮一
広島大学 総合科学部（前・日本薬学会会頭）

Topic Lecture

Various skin disease induced by metal allergy: metal contact allergy and systemic metal allergy

Atsuko ADACHI

Derpartment of Dermatology, Hyogo Prefectural Kakogawa Hospital

According to the patch test positive rate of this country enforced by Japanese contact dermatitis society in 1994, the first place the second place the fourth place the fifth place cobalt nickel chromium mercury and four kinds of metal occupied it within the fifth place superior. The elevation of current metal positive rate is remarkable in woman. It is assumed that this reflects increase of accessories wearing frequency by a woman.

1. Metal contact allergy

Metal is included in things of most around the body such as accessories, a coin, a clock, leather products, cement, stainless steel, paint. This metal causes contact dermatitis toward contact with skin.

2. Systemic metal allergy

With some patients of a metal allergy, various skin disease is brought about by micro dose metal absorbed through oral mucosa and alimentary canal in the body included in dental metal and a meal. When it is affection to develop when allergen was absorbed in meridian except a percutaneous route in the body by the individual that, contact sensitization was concluded, it is defined as systemic contact-type dermatitis of Fisher. However, at time, the case that an oral provocation test shows positive is presented though metal patch test is negative and disease state of this superscription is not systemic contact-type dermatitis in a correct meaning. Therefore we were not concerned with presence of contact sensitization for a case to raise allergic symptoms by metal taken for a whole body and proposed to call systemic metal allergy. Nickel, chromium, cobalt are included much in chocolate, cocoa, legume, spice, shellfish, a lever, an embryo. On the other hand, dental metal often contains palladium, gold, mercury, tin and includes nickel, chromium, and cobalt at time. This metal is taken in by percutaneous, oral mucosa, a digestive tract or a transendothelial air duct course, and it is excreted within perspiration, milk, lacrima, urine and feces. Metal, with the patient having systemic metal allergy, the metal is in vivo, and the onset or aggravation does pustulosis palmoplantaris, dyshidrotic eczema, lichen planus, nummular eczema, subacute prurigo, polymorphic chronic prurigo, erythroderma, pseudo-atopic dermatitis by what is absorbed and retrogrades by confinement of the administration.

3. Treatment of systemic metal allergy

1) Contact avoidance with metal allergen

The prohibition of contact with metal to which patient was proved to have allergy must to be done.

2) The avoidance of oral administration of metal allergen

For the case that does not retrograde by only prohibition of contact with metal allergen, we suggest the patient to limit metal administration absorbed from food and dental metal which include metal allergen. (Table 1). Because a strict metal diet can cause trace element deficiency symptom, we must cancel it immediately if null and void. For a case contained within dentist metal clearly, we explain necessity of the removal of the dentist metal to the patient, and patient compliance asks dentist for the dentist metal ablation for a provided case.

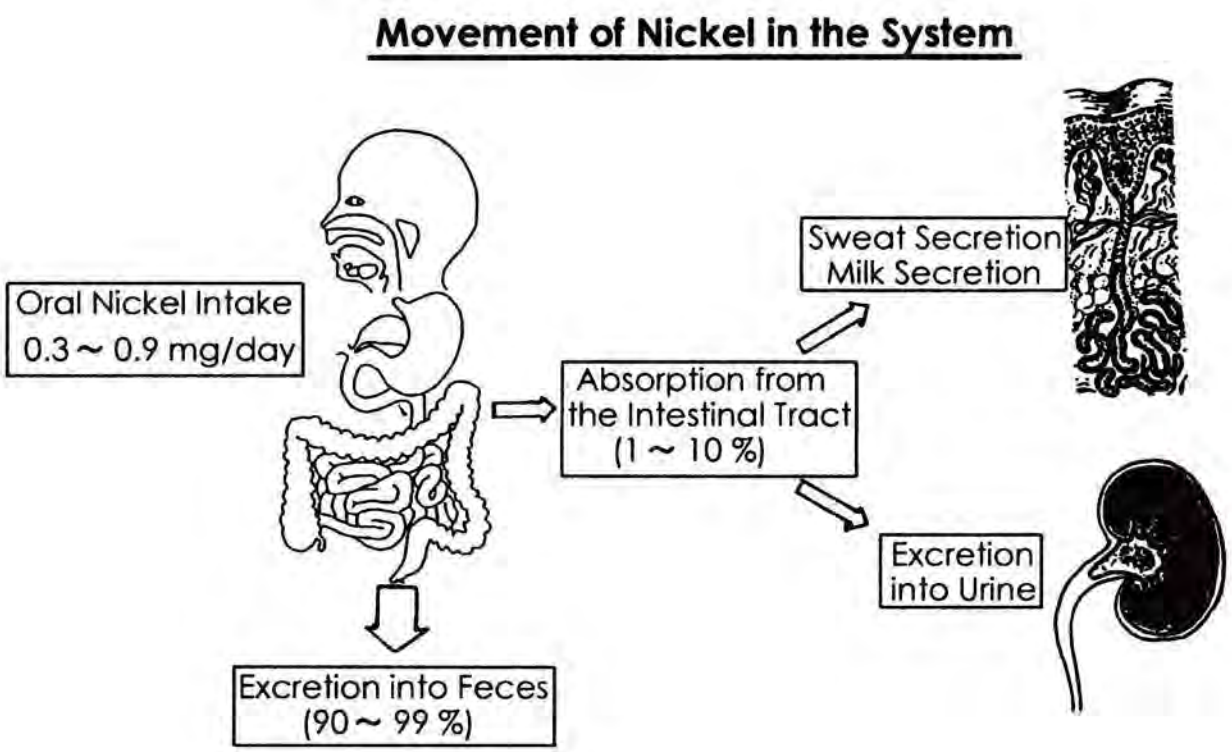
3) Drug therapy

In addition to metal elimination therapy described above, administration of oral Intal (DSCG) which inhibits metal transendothelial absorption in alimentary canal, disulfiram (TETD) and minocycline hydrochloride which are metal chelating agent may be effective.

Table 1. Metal diet guidance list (the food which includes a lot of metal)

	Ni	Co	Cr	Mn	Zn	Cu
Legume	All	All		All	All	All
Nut	All	All		All	All	All
Cereal	Unpolished rice Buckwheat noodles Oatmeal			Unpolished rice Wheat	Unpolished rice Wheat Wheat embryo	
Vegetables	Spinach, Lettuce Pumpkin, Cabbage	Cabbage	Potato, Onion	Warabi Parsley Lotus root		
Mushroom	Mushroom		Mushroom	Shiitake	Shiitake	Shiitake
Seaweed	All			Laver	Laver	
Meat		Lever		Lever	Lever/Beef	Lever
Egg					Egg yolk	
Fishery products	Oyster, Salmon, Herring	Scallop			Oyster, Crab, Octopus Dried cod	Oyster, Giant clam
Spice	All	All	All	All	All	All
Drink	Tea, Cocoa, Wine	Tea, Cocoa, Beer, Coffee	Tea, Coffee	Tea, Japanese green tea	Japanese green tea	Tea、 Japanese green tea
Sweats	Chocolate	Chocolate	Chocolate	Chocolate	Chocolate	Chocolate
Luxury goods	Cigarette					
Drug	Rhubarb					

1. Not use for five minutes of the beginning tap water.
2. Not taking canned food, canned drink.
3. Not use a stainless steel product and a plating product to cooking device.
4. Do a diet with iatric guidance by all means so that required metal is include.



金属と皮膚：金属接触アレルギーと全身型金属アレルギー

○足立 厚子
兵庫県立加古川病院　皮膚科部長

The 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04
Thursday, June 10 – Friday, June 11, 2004

Abstracts

Lectures

Lecture 01 A ^{99m}Tc-labeled hydrazino nicotinamide derivatized bisphosphonate for bone scintigraphy

○Kazuma OGAWA[1,2] , Takahiro MUKAI[1,3] , Yasuyuki INOUE[1] , Hideo SAJI[1]

[1]Department of Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan , [2]Central Institute of Radioisotopes Science, Division of Tracer Kinetics, Advanced Science Research Center, Kanazawa University, Kanazawa, Japan , [3]Department of Biomolecular Recognition Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

INTRODUCTION

Polynuclear complexes of ^{99m}Tc with methylenediphosphonate (^{99m}Tc-MDP) and hydroxymethylene-diphosphonate (^{99m}Tc-HMDP) are widely used as radiopharmaceuticals for bone scintigraphy. However, these agents required a time interval of 2-3 hours between injection and imaging. The time interval is unfavorable for patients from the points of view of the total time spent examining and the absorbed radiation dose. The phosphonate groups of ^{99m}Tc-MDP and ^{99m}Tc-HMDP participate not only in binding to bone minerals but also in complexation of ^{99m}Tc, which may cause a decrease in bone affinity. In the present study, based on the concept of bifunctional radiopharmaceuticals, we designed a ^{99m}Tc-labeled hydrazino nicotinamide derivatized bisphosphonate. All of the phosphonate groups of this compound can act on binding to the bone. Furthermore, previous studies demonstrated the low plasma protein binding of the ternary ligand complex, [^{99m}Tc](HYNIC)(tricine)(pyridine derivative) [1,2]. Therefore, the bisphosphonate conjugated with the ternary ligand complex is expected to show higher accumulation in the bone and rapid clearance from the blood. In this study, we prepared ^{99m}Tc-HYNIC-labeled bisphosphonate using tricine and 3-acetylpyridine as coligands (^{99m}Tc-HYNIC-HBP, Fig. 1a). The ability of ^{99m}Tc-HYNIC-HBP as a bone imaging agent was evaluated in comparison with ^{99m}Tc-HMDP and ^{99m}Tc-MAG3-conjugated bisphosphonate (^{99m}Tc-MAG3-HBP, Fig. 1b).

MATERIALS AND METHODS

Synthesis of ^{99m}Tc-HYNIC-HBP.

Boc-HYNIC-HBP was synthesized by coupling Boc-HYNIC-tetrafluorophenol and 4-amino-1-hydroxybutylidene-1,1-bisphosphonate. After deprotection of the Boc group, coligand solutions (tricine and 3-acetylpyridine), $^{99m}TcO_4^-$ and $SnCl_2$ were added. After the mixture was allowed to react at 95°C for 35 min, ^{99m}Tc-HYNIC-HBP was purified by reversed phase HPLC.

Synthesis of ^{99m}Tc-MAG3-HBP.

r-MAG3-HBP was synthesized as reported previously [3]. After deprotection of the trityl group, ^{99m}Tc-labeling was performed by reaction with $^{99m}TcO_4^-$ and $SnCl_2$. ^{99m}Tc-MAG3-HBP was purified by reversed phase HPLC.

Hydroxyapatite Binding Assay.

^{99m}Tc-labeled compounds were mixed with hydroxyapatite suspension. After centrifugation, the radioactivity of the supernatant was measured.

Biodistribution Studies.

Biodistribution studies were performed by the intravenous administration of ^{99m}Tc-labeled compounds to male Wistar rats (200-230 g).

Assessment of Serum Protein Binding.

Serum samples were collected at 3 min postinjection of ^{99m}Tc-labeled compounds to rats. The protein binding ratios of the radioactivity in the serum were evaluated by the ultrafiltration method.

RESULTS AND DISCUSSION

Since HYNIC serves as a monodentate or bidentate ligand, coligands are necessary for complexation with ^{99m}Tc. In this study, based on the results of preliminary studies, we chose the ternary ligand system using tricine and 3-acetylpyridine as coligands. ^{99m}Tc-HYNIC-HBP was obtained with a radiochemical yield of 39% and a radiochemical purity of over 95%.

In hydroxyapatite binding assays, ^{99m}Tc-HYNIC-HBP and ^{99m}Tc-MAG3-HBP showed a greater affinity for bone minerals than ^{99m}Tc-HMDP. When administered to rats, ^{99m}Tc-HYNIC-HBP and ^{99m}Tc-MAG3-HBP exhibited a significantly higher accumulation in the bone than ^{99m}Tc-HMDP. These results indicated that the drug design based on the concept of bifunctional radiopharmaceuticals would be advantageous for enhancing the bone accumulation of ^{99m}Tc-labeled bisphosphonates.

The high affinity of ^{99m}Tc-MAG3 for serum proteins is well known [4,5], and so we prepared a ^{99m}Tc-MAG3-conjugated bisphosphonate for use as a control. As expected, the binding of ^{99m}Tc-HYNIC-HBP to serum protein was significantly lower than that of ^{99m}Tc-MAG3-HBP. This result was reflected in the radioactivity clearance from the blood. The blood clearance of the radioactivity after administration of ^{99m}Tc-MAG3-HBP in rats was much slower than that of ^{99m}Tc-HMDP. On the other hand, there was no significant difference in blood clearance between ^{99m}Tc-HYNIC-HBP and ^{99m}Tc-HMDP. These findings suggest that the rapid blood clearance of ^{99m}Tc-HYNIC-HBP was due to the low affinity of the ternary ligand complex, [^{99m}Tc](HYNIC)(tricine)(3-acetylpyridine), for serum proteins.

CONCLUSION

We developed a ^{99m}Tc-labeled bisphosphonate with a high affinity for bone minerals and a low affinity for serum proteins, ^{99m}Tc-HYNIC-HBP. This compound showed a high bone accumulation and rapid blood clearance, leading to high bone-to-blood ratios of radioactivity. Gathered data indicated that ^{99m}Tc-HYNIC-HBP holds great potential for bone scintigraphy.

REFERENCES

1. Ono, M., Arano, Y., Mukai, T., *et al. Nucl Med Biol,* 2001;**28**:215-224.
2. Ono, M., Arano, Y., Mukai, T., *et al. Nucl Med Biol,* 2001;**28**:155-164.
3. Mukai, T., Ogawa, K., Arano, Y., *et al. J Label Compd Radiopharm,* 2003;**46**:S363.
4. Verbruggen, A.M., Nosco, D.L., Van Nerom, C.G., *et al. J Nucl Med,* 1992;**33**:551-557.
5. Taylor, A., Jr., Eshima, D., Christian, P.E., *et al. J Nucl Med,* 1988;**29**:616-622.

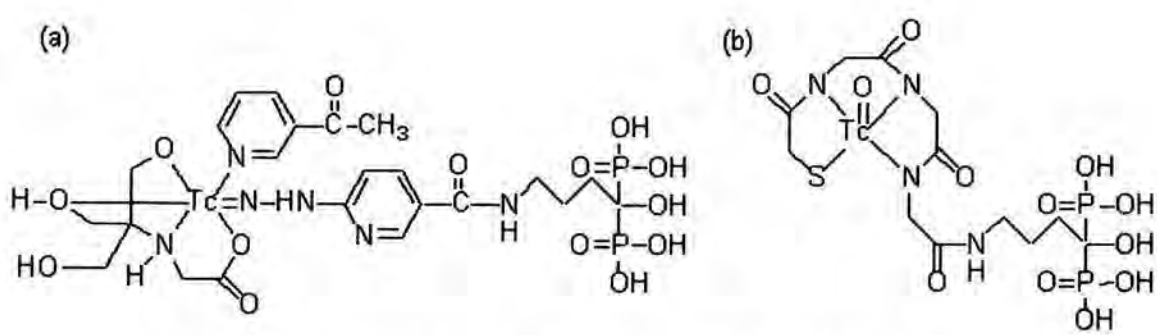


Figure 1. Structures of Tc-HYNIC-HBP (a) and Tc-MAG3-HBP (b).

新規骨シンチ薬剤、^{99m}Tc-hydrazino nicotinamide 結合ビスホスホネートの開発

○小川 数馬[1,2]、向 高弘[1,3]、井上 靖之[1]、佐治 英郎[1]

[1]京都大学大学院 薬学研究科、[2]金沢大学学際科学実験センター、[3]九州大学大学院薬学研究院

Lecture 02 A new potent insulin-mimetic vanadyl(IV)-allixin complex

○Yusuke ADACHI[1] , Jiro YOSHIDA[2] , Yukihiro KODERA[2] , Akira KATOH[3] , Hiromu SAKURAI[1]

[1]Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan , [2]Healthcare Institute, Wakunaga Pharmaceutical Co., Ltd., Hiroshima, Japan , [3]Department of Applied Chemistry, Faculty of Engineering, Seikei University, Tokyo, Japan

Introduction

The worldwide number of patients suffered from diabetes mellitus (DM) was estimated to be around 173 million in 2002. Although several types of insulin preparations for type 1 DM and those of synthetic drugs for type 2 DM have been developed and clinically used, they have several problems such as physical and mental pain due to daily insulin injections and defects involving side effects, respectively. For these reasons, it is extremely important to create and develop new type anti-diabetic agents in place of insulin injections and synthetic drugs. Since the finding of insulin-mimetic effects of vanadium, several insulin-mimetic vanadyl(IV) complexes have been proposed for clinical use [1, 2]. Bis(maltolato)oxovanadium(IV) complex ($VO(ma)_2$) prepared in 1992 was found to normalize blood glucose levels of both type 1 and 2 diabetic animals [3, 4]. To develop a higher insulin-mimetic vanadyl(IV) complex than $VO(ma)_2$, we examined *in vitro* and *in vivo* structure-activity relationship for $VO(ma)_2$ and its related complexes. On the basis of the results, we propose here a new potent vanadyl(IV) complex with allixin, which was isolated from garlic [5].

Methods

1. Preparation and characterization of vanadyl(IV) complexes

$VO(ma)_2$ and its related complexes were prepared according to the method to the preparation of $VO(ma)_2$ [3]. Briefly, vanadyl(IV) complexes were prepared in hot water (70-80°C) by mixing maltol related compounds and vanadyl sulfate ($VOSO_4$) at pH 8-9. Structures of vanadyl(IV) complexes were estimated by infra-red (IR) spectra, elemental analyses, visible absorption spectra, ESR spectra, and magnetic susceptibilities.

2. Evaluation of *in vitro* insulin-mimetic activity of vanadyl(IV) complexes

In vitro insulin-mimetic effects of vanadyl(IV) complexes were estimated by the inhibitory activity of free fatty acid (FFA)-release and the enhancing activity of glucose-uptake in the isolated rat adipocytes [6, 7].

3. Evaluation of *in vivo* insulin-mimetic activity of vanadyl(IV) complexes

Type 2 diabetic KKA^y mice with obesity were given vanadyl(IV) complex at the doses of 1 mg (20 mmol) V/kg at the first day and then 0.1 mg (2 mmol) V/kg for the following 13 days by daily intraperitoneal (*i.p.*) injections, and their blood glucose levels, body weights, food intakes, and water consumptions were daily monitored. After the period of treatment, serum concentrations of insulin, leptin, GOT, GPT, and uric nitrogen (UN) were measured, and oral glucose tolerance tests (OGTT) were performed.

Results and Discussion

Structures of $VO(ma)_2$ and its related complexes were determined to have a $VO(O_4)$ coordination mode at the binding ratio of VO^{2+}:ligand=1:2. Vanadyl(IV) complex with allixin ($VO(alx)_2$) was found to exhibit the highest insulin-mimetic activity among others as evaluated by both the inhibition of FFA-release and glucose-uptake in the isolated rat adipocytes. The insulin-mimetic activity of vanadyl(IV) complexes strongly correlated with the partition coefficients ($\log P$) of both the complexes and their ligands, indicating that insulin-mimetic activity of the vanadyl(IV)

complexes depends on the lipophilicity of the complexes and ligands. Then, we compared the blood glucose lowering effects of $VO(alx)_2$ and $VO(ma)_2$ in type 2 diabetic KKA^y mice. The blood glucose levels of KKA^y mice treated with both complexes lowered from the hyperglycemic levels (approximately 20 mM) to the normal range (below 8 mM) at the first day after the start of injection. Then KKA^y mice were given lower doses (0.1 mg V/kg) of the complex for the following 13 days. During the period of treatment, the blood glucose levels of KKA^y mice treated with $VO(alx)_2$ maintained the normal range, however, those of KKA^y mice treated with $VO(ma)_2$ increased within a week (Fig. 1). Moreover, $VO(alx)_2$ exhibited higher improving effect of DM as observed on the glucose tolerance than $VO(ma)_2$. In addition, no changes in the serum GOT, GPT, and UN by the treatment of vanadyl(IV) complexes were observed.

In conclusion, we propose a new potent $VO(alx)_2$, which has more potent pharmacological activity to treat type 2 DM in KKA^y mice at lower doses than $VO(ma)_2$. It was revealed that the high insulin-mimetic activity of $VO(alx)_2$ depends on the high lipophilicity of allixin, which relates to the incorporation of the complex into the cells. $VO(alx)_2$ complex was thus indicated to be a possible agent to treat type 2 DM.

References

1) H. Sakurai *et al., Expert Opin. Investig. Drugs*, **12**, 1189 (2003). 2) H. Sakurai *et al., Coord. Chem. Rev.*, **226**, 187 (2002). 3) J.H. McNeill *et al., J. Med. Chem.*, **35**, 1489 (1992). 4) V.G. Yuen *et al., Diabetes Res. Clin. Pract.*, **43**, 9 (1999). 5) Y. Kodera *et al., Chem. Pharm. Bull.*, **50**, 405 (2002). 6) M. Nakai *et al., Biol. Pharm. Bull.*, **18**, 719 (1995). 7) Y. Adachi *et al., Chem. Pharm. Bull.*, in press.

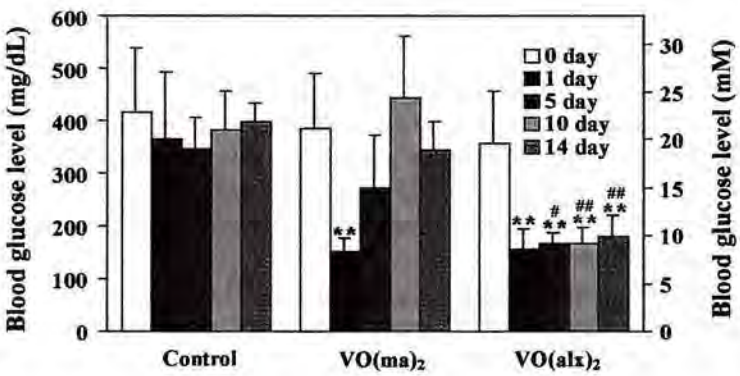


Fig. 1. Changes of blood glucose levels in control KKA^y mice and KKA^y mice treated with $VO(ma)_2$ and $VO(alx)_2$ complexes by intraperitoneal injections for 14 days. Doses were 1 mg (20 μmol) V/kg at first day and 0.1 mg (2 μmol) V/kg for the following 13 days. Each column is expressed as the mean ± S.D. for 4-8 mice. **Significance at $p < 0.01$ *vs.* control, #Significance at $p < 0.05$ *vs.* $VO(ma)_2$, ##Significance at $p < 0.01$ *vs.* $VO(ma)_2$.

バナジル-アリキシン錯体のインスリン様作用

○安達 祐介[1]、吉田 治郎[2]、小寺 幸広[2]、加藤 明良[3]、桜井 弘[1]

[1]京都薬科大学 代謝分析学教室、[2]湧永製薬(株)ヘルスケア研究所、[3]成蹊大学工学部応用化学科

Lecture 03 Non-covalent interactions of an antitumor dinuclear platinum(II) complex with DNA

○Ryuhei WADA , Sachie WADA , Takaji SATO , Katsuhiko MINOURA , Keiko NOGUCHI , Keiko FUJII , Seiji KOMEDA , Yoshihiro SAITO , Masahiko CHIKUMA

Osaka University of Pharmaceutical Sciences

[Introduction]

Cisplatin is one of the most effective anticancer drugs. Cisplatin binds to DNA primarily as a GpG bifunctional adduct and this is believed to be responsible for its pharmacological action. The overall effectiveness of cisplatin is limited by the emergence of drug resistant tumor cells. In an attempt to search for more active platinum complexes and to overcome problems with drug resistance, we have developed a dinuclear platinum(II) compound, $[\{cis\text{-}Pt(II)(NH_3)_2\}_2(\mu\text{-}OH)(\mu\text{-}pz)](NO_3)_2$ (**1,2-μH-Ampz,Fig.1**). The dinuclear complex is effective against cisplatin-resistant tumor cells. Effectiveness to the cisplatin-resistant tumor cells is based on its different binding mode with DNA. We have examined the binding modes between 1,2-μH-Ampz and DNA from that of cisplatin. At this symposium we reports that 1,2-μH-Ampz shows non-covalent interactions with DNA. The reaction of 1,2-μH-Ampz with DNA was pursued with time, and the interaction modes were examined in detail by agarose gel electrophoresis, binding analysis using Scatchard plot and NMR spectroscopy.

[Experimental]

Determination of platinum bound to DNA

1,2-μH-Ampz (0.3 per DNA base pair) was added to calf thymus DNA solution containing 0.3mM citric buffer (pH 7.0) and 3mM NaCl. The mixture was allowed to stand at 37°C. The reaction mixture was ultrafiltrated periodically in the presence and absence of NaCl. The platinum amounts in the filtrates and the DNA fractions were determined by ICP-MS.

Electrophoresis

1,2-μH-Ampz was added to 10μg/ml ColE1 DNA and the volume of reaction mixture was adjusted to 25μl with MilliQ water. The reaction mixture was incubated for 0, 2, or 48 hours at 37°C and provided for agarose gel electrophoresis.

Binding analysis using Scatchard plot

DNA was reacted with fluorescence dyes EtBr and DAPI in the various concentrations and the fluorescence intensity were measured in the presence and absence of 1,2-μH-Ampz. The data were analyzed by Scatchard plot for binding of the dye.

NMR experiments

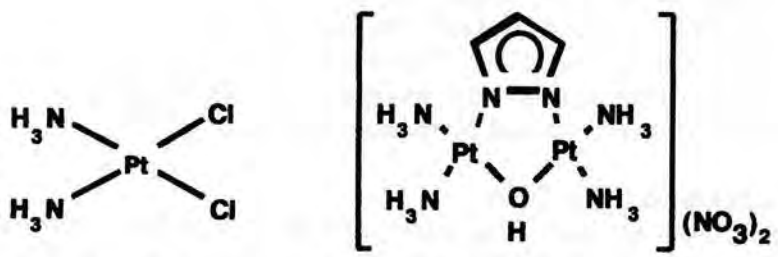
A dodecanucleotide $d(CAATCCGGATTG)_2$ (0.7μg) was dissolved in 10mM phosphate buffer (0.7ml, pH 7.0) in D_2O (99.96%) containing 20mM NaCl and 0.1mM EDTA. DNA was titrated with small aliquots of 1,2-μH-Ampz solution.The samples were measured by 1D and 2D ^{1}H NMR methods at 35°C.

[Results and Discussion]

The amount of platinum binding to DNA was found to reach plateau immediately after the reaction. The considerable amount of platinum complex was bound to DNA. Almost all platinum bound to DNA was removed by the addition of NaCl, but a small amount of platinum was not removed. The binding mode immediately after the reaction was supposed not covalent bond but an electrostatic interaction since the bound platinum was removed by the addition of NaCl.

As the reaction ratio of platinum to DNA became larger when the 1,2-μH-Ampz was reacted with plasmid DNA for 2 hours, the mobility shit of the super coil DNA became shorter gradually. It is thought that the unwinding of DNA which is found in the presence of an intercalator took place. On the other hand, immediately after the reaction the unwinding of DNA was not found.

Therefore, the reaction with the unwinding of DNA is based on not electorostatic interaction but other binding mode.
The effect of 1,2-μH-Ampz on the binding of DNA with both EtBr which is an intercalator and DAPI which is a minor groove binder was investigated. The results show that 1,2-μH-Ampz interferes with the binding of EtBr and DNA competitively and with the binding of DAPI and DNA non-competitively. ^{1}H NMR (1D and 2D) spectroscopy investigation is now underway.



cisplatin **1,2-μH-Ampz (Complex I)**

Structures of cisplatin and platinum(II) complexes

制癌活性を有する白金(II)二核錯体の DNA との非共有結合的相互作用

○和田 隆平、和田 幸恵、佐藤 卓史、箕浦 克彦、野口 恵子、藤井 景子、米田 誠治、齊藤 睦弘、千熊 正彦
大阪薬科大学

Lecture 04 Structure-based design of the irreversible inhibitors to metallo-β-lactamase (IMP-1)

○Yoshihiro YAMAGUCHI[1], Kimitaka SOGA[1], Toshihiro HIGASHI[1], Shogo MISUMI[2], Hiromasa KUROSAKI[1], Yuriko YAMAGATA[3], Yoshichika ARAKAWA[4], Masafumi GOTO[1]

[1]Department of Structure-Function Physical Chemistry, Graduate School of Pharmaceutical Sciences, Kumamoto University, [2]Department of Biochemistry, Graduate School of Pharmaceutical Sciences, Kumamoto University, [3]Department of Structural Biology, Graduate School of Pharmaceutical Sciences, Kumamoto University, [4]Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases

Metallo-β-lactamases (MBLs) are zinc metalloenzymes and can hydrolyze almost all the β-lactams which are most widely applicable drugs to cure infectious diseases. MBLs-producing bacteria will pose a serious global problem in the future because there are no clinical relevant inhibitors for MBLs in spite of several efforts. For this reason, the development of inhibitors to these enzymes is important.We reported that thiol compounds are competitive inhibitors to a MBL, IMP-1. If the thiol group acts as a strong coordinating group to a zinc(II) ion, the introduction of good leaving group for nucleophilic attack will lead to irreversible inhibitors because several amino acid side chains are available as nucleophilic center in the active site (Figure 1).We prepared 3-(3-Mercaptopropionylsulfanyl)propionic acid pentafluorophenyl ester, MPAP, having a thiol group and pentafluorophenyl ester. This compound acts as a inhibitor, with an IC_{50} of 3.8 nM. Next, to verify the irreversible inhibition, after IMP-1 was preincubated with MPAP at various times, the treated protein was separated free inhibitors by gel-filtration and was measured the activity. The activity of the treated protein was not recovered at all.The MALDI-TOF mass spectra of the untreated IMP-1 showed the parent peak at *m/z* of 25112.9 while that treated with MPAP showed the peak at *m/z* of 25290.1. The difference between them is 176.9, indicating that segment of MPAP bonds to IMP-1 covalently. We also succeeded in crystallization of IMP-1-MPAP complex and determined the binding mode by X-ray crystallography.

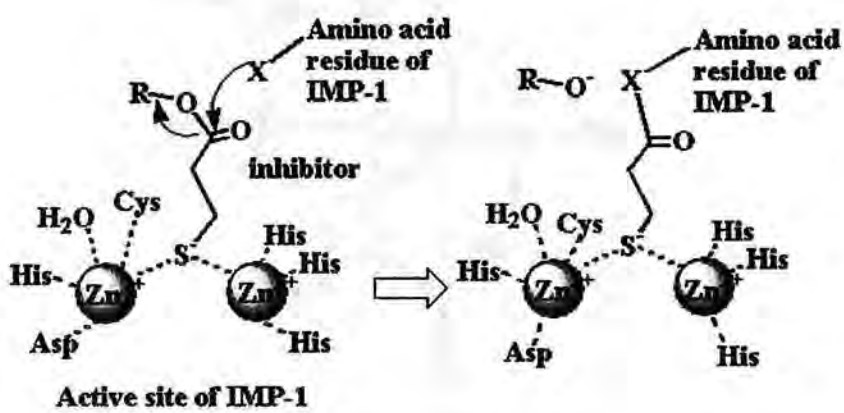


Figure 1. Irreversible inhibition mechanism.

メタロ-β-ラクタマーゼ（IMP-1）への非可逆的阻害剤の構造に立脚した設計

○山口 佳宏[1]、曽我 公孝[1]、東 俊博[1]、三隅 将吾[2]、黒崎 博雅[1]、山縣 ゆり子[3]、荒川 宜親[4]、後藤 正文[1]
[1]熊本大学大学院 薬学教育部 構造機能物理化学分野、[2]熊本大学大学院 薬学教育部 薬学生化学分野、[3]熊本大学大学院 薬学教育部 機能分子構造解析学分野、[4]国立感染症研究所 細菌第二部

Lecture 05 Co(III) complexes of an N2S3-type ligand designed as an active site model of nitrile hydratase: Influences of the oxygenated sulfurs on the central metal ion

○Tomohiro OZAWA , Tomohiro IKEDA , Yasuhiro FUNAHASHI , Koichiro JITSUKAWA , Hideki MASUDA

Graduate School of Engineering, Materials Science & Engineering, Nagoya Institute of Technology, Nagoya, Japan

Co-type nitrile hydratase (NHase) hydrolyzes nitrile derivatives to the corresponding amides, which have a non-corrinoid Co(III) ion as the active site. Recently, X-ray structural analysis revealed that two amide nitrogens from main chain of the peptide backbone and three sulfurs from cystein residues coordinated to the metal center. Two of three sulfurs are posttranslationally oxygenated to a sulfenic and a sulfinic acids. Thus, influences of the oxygenated sulfurs on the structure and properties of the metal complex are interesting in connection with the enzymatic hydration mechanisms. An N2S3-type of ligand, 3,3'-bis{(2-mercapto-2-methyl)propioylamido}-dipropylsulfide (DPDS), was designed and two Co(III) complexes with the ligand were synthesized: One has non-oxygenated sulfurs and another is fully oxygenated to sulfinic acids. An X-ray structure of the latter complex is shown in Figure, which is a distorted square-pyramidal structure with τ value of 0.37. A ^{1}H-NMR spectral measurement showed that all the signals appeared within the range of 0-10 ppm in $CDCl_3$ as usually shown in an octahedral Co(III) complex, indicating the complex, $PPh_4[Co(dpds\text{-}O_4)]$, has a low-spin state. On the other hand, the ^{1}H-NMR spectrum of the Co(III) complex containing non-oxygenated sulfurs showed a paramagnetic shift in the range of -10-20 ppm, indicating that it has a high spin or an intermediate spin state. Here, we will discuss the influence of the oxygenated sulfurs on the central metal ion by comparing with the two Co(III) complexes.

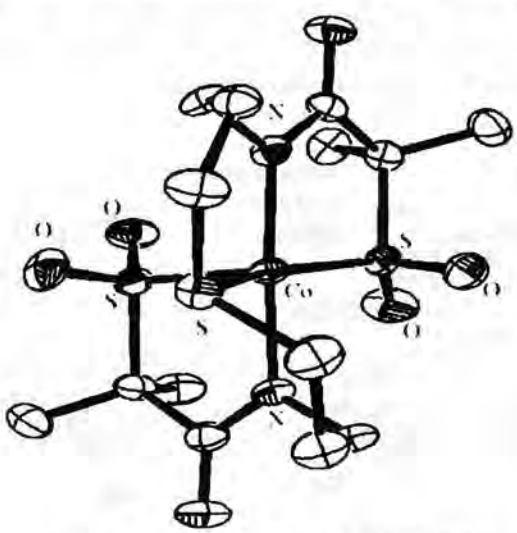


Figure Crystal structure of $[Co(dpds\text{-}O_4)]^-$

ニトリルヒドラターゼ活性部位として設計したN2S3型配位子を有するCo(III)錯体：酸化したチオールの中心金属イオンに対する影響

○小澤 智宏、池田 友宏、舩橋 靖博、実川 浩一郎、増田 秀樹

名古屋工業大学大学院 工学研究科 物質工学

Lecture 06 Syntheses and Functional Analyses of Artificial Zinc Finger Proteins Linked with Metal Complex Moiety

○Akira ONODA , Nozomi ARAI , Shigenori KOBAYASHI , Kenji KANEKO , Takeshi YAMAMURA

Department of Chemistry, Faculty of Science, Tokyo University of Science, Tokyo, Japan

Zinc Fingers are attractive motif for the design of DNA-binding proteins with sequence specificity and controlling the binding availability by further improvement on zinc fingers are promising method for the regulation of gene expression. Various modifications on zinc fingers, such as mutagenesis on several residues, domain swapping, and fusion with other proteins, has been progressively studied. Strategy for creating zinc finger proteins with novel DNA-binding specificity are established Cys2His2-type zinc finger (ZF) is an abundant DNA binding motif that recognizes specific three base pairs of DNA in the presence of Zn^{2+} ion. This type of ZF motif obtains strong and specific DNA binding ability by forming tandem repeat structures. In this study, we aimed at the creation of new zinc finger proteins having additional metal binding domains for further functionality (as shown below).

1. YQCRICGRNFSRSDHLTRHIRTHTG-**DKNNDGRIDAEEALEILRAKG** (ZF1-Tn)
2.CRICGRNFSRSDDLTRHIRTHTGEKPYGCRICGRNFSRSDHLTRHIRTHTG-**DKNNDGRIDAEE ALEILRAKG** (ZF1-ZF2-Tn)
3. **Tbp**$^{Ru(II)}$-GCRICGRNFSRSDDLTRHIRTHTG (Tbp$^{Ru(II)}$-F1)
4.**Tbp**$^{Ru(II)}$-GCRICGRNFSRSDDLTRHIRTHTGEKPY-GCRICGRNFSRSDHLTRHIRTHTG (Tbp$^{Ru(II)}$-F1F2)

1. Ca Responsive DNA Binding in Artificial Troponin-Fused Zinc Finger Protein

We created novel zinc finger fusion proteins to develop Ca responsive regulation of DNA-binding. For the purpose, we focused on representative Ca-binding protein, troponin, which contains helix-loop-helix binding site. Novel zinc finger fusion proteins composed of zif268 finger2 and following a loop-helix region of troponin. Herein we report the characterization of our fusion proteins with single finger, ZF2-Tn. Ca dependant DNA-binding affinity was also investigated using troponin fusion with two fingers, ZF1-ZF2-Tn.

ZF2-Tn and ZF1-ZF2-Tn were synthesized by the combination of solid phase and chemical ligation methods. The final products were purified by reverse phase HPLC and analyzed by MALDI-TOF mass spectroscopy. All experiments were performed under nitrogen atmosphere to avoid oxidation of cysteine residues. The coordination ability of Cys2His2 part of ZF2-Tn was investigated by Co^{2+} ion titration using absorption spectroscopy. Folding structure of Zn^{2+}-bound form, ZF2-Tn/Zn^{2+}, was investigated by CD spectroscopy. Detailed structural analyses of ZF2-Tn/Zn^{2+} using NMR experiments clarify that our fusion protein gives native zinc finger folding with the artificial Ca binding domain. Two significant stretches of $d_{\alpha}N(i,i+3)$ and $d_{\alpha}N(i,i+4)$ connectivities for residues 13-23 and 35-46 suggest that helical conformation is present in both of these region. The three dimensional structure of the protein was computed form ROE and $^3J_{NH\alpha H}$ constraints by the simulated annealing method with X-PLOR program. NMR structure of ZF2-Tn is illustrated in Figure. The result indicates the formation of the α-helix in zinc finger domain and in the following part of Ca^{2+} binding loop, which does not perturb the native folding.

Ca responsive DNA-binding affinity of troponin-fused protein with two fingers, using of ZF1-ZF2-Tn/Zn^{2+}, was investigated by electrophoretic mobility shift assay (EMSA). EMSA analyses of ZF1-ZF2-Tn/Zn^{2+} were performed under the condition with various concentration of Ca ion.

ZF1-ZF2-Tn/Zn^{2+} has Kd value of 34 nM in the absence of Ca ion and shows higher Kd values

with the addition of Ca ion, up to 66 nM (in 15 equiv. Ca ion). Thus, our zinc finger protein with troponin Ca binding domain has potential for Ca responsive DNA binding affinity.

2. Zinc finger proteins linked with Ruthenium complex

We combined the Cys2His2-type zinc finger motif with the unnatural amino acids having tris(2,2'-bipyridine)ruthenium complex in side chains, $Tbp^{Ru(II)}$. New fingers, $Tbp^{Ru(II)}$-F1, and $Tbp^{Ru(II)}$-F1F2, were designed based on the amino acid sequence of Zif268 finger2. (2-tert-Butoxycarbonylamino-3-(4'-methyl-[2,2']bipyridin-4-yl)propanoic acid)bis-(2,2'-bipyridine)Ru(II), abbreviated as Boc-$Tbp^{Ru(II)}$, was prepared according to the reported method. $Tbp^{Ru(II)}$-F1 and $Tbp^{Ru(II)}$-F1F2 were synthesized by Fmoc method. All peptides were purified by reversed phase HPLC and characterized by MALDI-TOF mass spectrometry.

Folding structure of zinc finger part of TbpRu(II)-F1 was investigated by Zn^{2+} and Co^{2+} titration experiments in HEPES buffer, monitoring by UV-vis. and CD spectroscopies. The results showed that 1) $Tbp^{Ru(II)}$-F1 forms 1:1 complexes with Zn^{2+} and Co^{2+} ion, 2) Co^{2+} ion in $Tbp^{Ru(II)}$-F1 gives a Cys2His2 coordination, and 3) the helicity of $Tbp^{Ru(II)}$-F1 increases with the amount of Zn^{2+}.

Gel mobility shift assay was performed to clarify the binding ability of $Tbp^{Ru(II)}$-F1F2 to the DNA containing GGGGCG. The assay indicate that $Tbp^{Ru(II)}$-F1F2 has a DNA binding ability similar to the F1F2 without $Tbp^{Ru(II)}$ moiety. Dissociation constant (Kd) of $Tbp^{Ru(II)}$-F1F2 for the DNA was 3.6 nM, while that of F1F2 was 9.5 nM. Our zinc finger protein with $Tbp^{Ru(II)}$ moiety at N-terminus has similar DNA recognition ability to native zinc finger



金属錯体部位を付与した新規人工亜鉛フィンガーの合成と機能

○小野田 晃、荒井 望、小林 茂則、金子 健二、山村 剛士
東京理科大学 理学部 化学科

Lecture 07 Effect of tributyltin on reproduction of fish and shellfish

○Yuji OSHIMA[1] , Shimasaki YOHEI[1] , Suguru INOUE[2] , Kei NAKAYAMA[2] , Tsuneo HONJO[1]

[1]Faculty of Agriculture, Kyushu University, Fukuoka, Japan , [2]Center for Marine Environmental Studies, Ehime University, Ehime, Japan

Tributyltin (TBT) is a typical endocrine disrupting chemical that can induce imposex in mollusks. However, few studies addressing the effect of TBT in bivalves and fish have been reported. We examined the effect of TBT on reproduction of the pearl oyster (*Pinctada fucata martensii*). In a maternal exposure test, the embryo developmental success was measured. The embryo developmental success was significantly decreased in the 0.191-µg/L treatment group, and concentrations of TBT in the ovary reached 0.088 µg/g. In a waterborne exposure test, the embryo developmental success was also significantly decreased in the 0.192-µg/L treatment group and no development at all was observed in the 0.374-µg/L treatment group. These results clearly demonstrate that TBT accumulating in the bodies of bivalves has the potential to inhibit reproduction.We examined the effect of TBT on the sex differentiation process in genetically female Japanese flounder (*Paralichthys olivaceus*). The fish were fed an artificial diet containing tributyltin oxide at concentrations of 0.1 and 1.0 mg/g diet from 35 to 100 d after hatching, which includes the sex differentiation period. The ratio of sex-reversed males significantly increased to 25.7% of the flounder fed the 0.1 µg/g diet and to 31.1% of those fed the 1.0 µg/g diet compared with the control (2.2%). From morphological and histological examination of the fish in the TBT-treated groups, normal females had typical ovaries and sex-reversed males had typical testes. These results clearly demonstrated the masculinization of flounder exposed to TBT. We also found that TBT accumulates in the serum or plasma of some fishes and is bound to a high molecular weight compound in the serum of the Japanese flounder. We succeeded in purifying the TBT-binding protein (TBT-bp) from the serum of Japanese flounder. The molecular mass of TBT-bp was approximately 46.5 kDa and it contained 42% N-glycan. From cDNA nucleotide sequence of TBT-bp, we deduced a sequence of 191 amino acids of mature TBT-bp. Furthermore, we evaluated the effects of TBT, polychlorinated biphenyls (PCBs), and a mixture of TBT and PCBs on reproduction in Japanese medaka (*Oryzias latipes*). We administered TBT (1 mg/g body weight [b.w.] daily), PCBs (1 mg/g b.w. daily), or both to medaka for 3 weeks. The profiles of fertility developed a bimodal distribution in the TBT and TBT+PCBs groups, and the sexual behavior were significantly decreased in the TBT and TBT+PCBs group. Furthermore, frequencies of abnormal eye were significantly increased in hatched larvae spawned from the TBT and TBT+PCBs group. Significant interaction was observed in next generation when parent fish were exposed to mixtures of TBT and PCBs.

トリブチルスズが魚介類の再生産に及ぼす影響

○大嶋 雄治[1]、島崎 洋平[1]、井上 英[2]、仲山 慶[2]、本城 凡夫[1]

[1]九州大学大学院 農学研究院、[2]愛媛大学 沿岸環境研究センター

Lecture 08 Imposex induced by organotin compounds in gastropods: from molecular mechanism to population-level effects

○Toshihiro HORIGUCHI[1] , Hiroaki SHIRAISHI[1] , CHO HYEON-SEO[2] , Yasuhiko OHTA[3] , Taisen IGUCHI[4] , Tomohiro NISHIKAWA[1] , Fujio SHIRAISHI[1] , Masatoshi MORITA[1] , Jun-ichi NISHIKAWA[5]

[1]Endocrine Disruptors & Dioxin Research Project, National Institute for Environmental Studies, Tsukuba, Japan , [2]Yosu National University, Yosu, Korea , [3]Faculty of Agriculture, Tottori University, Tottori, Japan , [4]Center for Integrative Bioscience, Okazaki National Research Institutes, Okazaki, Japan , [5]Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

Imposex (a superimposition of male reproductive tracts, such as penis and vas deferens, on female gastropods) is known to be typically caused by organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), from antifouling paints for vessels and fishing nets. Here, we show the current status of imposex and organotin contamination as well as populations decline due to reproductive failure involved by imposex in Japanese gastropods, such as the rock shell (Thais clavigera) and the ivory shell (Babylonia japonica). The fourth countrywide survey on imposex and organotin contamination in the rock shell was conducted in 174 locations along the Japan coast between January 1999 and November 2001. The percentage occurrence of imposex was still high in many locations whereas no or rare instances of imposex were found in eight locations. Severe imposex symptoms were observed in the rock shell populations from 41 locations. Temporal decreases in the tissue concentrations of tributyltin (TBT) were not very distinct although those of triphenyltin (TPT) were clearly observed in almost all surveyed locations. Decreased catches of the ivory shell were thought to be caused mainly by the dysfunction of ovaries due to suppressed ovarian maturity including spermatogenesis, accompanied by imposex possibly induced by TBT and/or TPT from antifouling paints. Endocrine disruption resulting in the masculinization of females was also observed in abalone, and ovarian spermatogenesis was observed to be caused by organotins, such as TBT and TPT in laboratory flow-through exposure experiments. Masculinization of female abalone seems resemble to imposex in meso- and neogastropods. To clarify the mode of action of TBT and/or TPT inducing imposex in gastropods, we also studied basic biology of gastropods, in terms of steroid hormones, their receptors, cytochrome P450arom (CYP19A1, aromatase), neuropeptides and biogenic amines. The results obtained in the basic study are also shown, and the possible mode of action of TBT and/or TPT on the development of imposex in gastropods is discussed. Finally, the new hypothesis about imposex induction mechanism is presented: the retinoid X receptor (RXR) has an important role on the development of imposex in gastropods.

有機スズ化合物による腹足類のインポセックス：分子メカニズムから個体群レベルの影響まで

○堀口 敏宏[1]、白石 寛明[1]、趙 顕書[2]、太田 康彦[3]、井口 泰泉[4]、西川 智浩[1]、白石 不二雄[1]、森田 昌敏[1]、西川 淳一[5]

[1]国立環境研究所 環境ホルモン・ダイオキシン研究プロジェクト、[2]麗水大学校、[3]鳥取大学 農学部 獣医学科、[4]岡崎国立共同研究機構 統合バイオサイエンスセンター、[5]大阪大学 大学院薬学研究科

Lecture 09 Triphenyltin (TPT) chloride increased production of interleukin-1β (IL-1β) by murine macrophages after subacute oral exposure

○Masashi TSUNODA[1] , Ken NAKANO[2] , Nobuhiro KONNO[3]

[1]Department of Preventive Medicine and Public Health, Kitasato University School of Medicine, Kanagawa, Japan , [2]Fukushima Institute of Public Health, Fukushima, Japan , [3]Koriyama Women's University & College, Koriyama, Japan

Triphenyltin (TPT) compounds were known as environment pollutant. The immune system is a target of TPT intoxication. In our previous data, relative spleen weight decreased dose-dependently after subacute oral administration of TPT chloride. We evaluated immunotoxicity of TPT on splenocytes by the determination of cytokines in the supernatant of splenocytes. Male BALB/c mice were exposed to 0, 25, 50, 100 and 200 ppm of TPT chloride in the feed for a month. Following the treatment period, mice were euthanized, liver, spleen and kidney were removed and weighed. Splenocytes were divided into adherent cells (macrophages) and non-adherent cells (lymphocytes) and incubated in a cell culture plate. The macrophages were activated by lipopolysaccharide (LPS) and lymphocytes were activated by concanavalin A. After the incubation, the concentrations of tumor necrosis factor α(TNFα) and interleukin-1β (IL-1β) in the supernatant of macrophages were determined by ELISA. The concentration of interferon-γ (IFN-γ) in the supernatant of lymphocytes was also determined by ELISA. The mean body weight of the 200 ppm group was significantly lower compared to the other groups. The mean values of relative spleen and kidney weights were significantly lower among the 100 and 200 ppm groups compared to the control. There were no significant differences in the concentrations of TNFα and IFN-γ, however, the concentration of IL-1β was significantly higher compared to the other groups (Figure). The production of IL-1β by splenic macrophages may be increased by a compensatory manner.

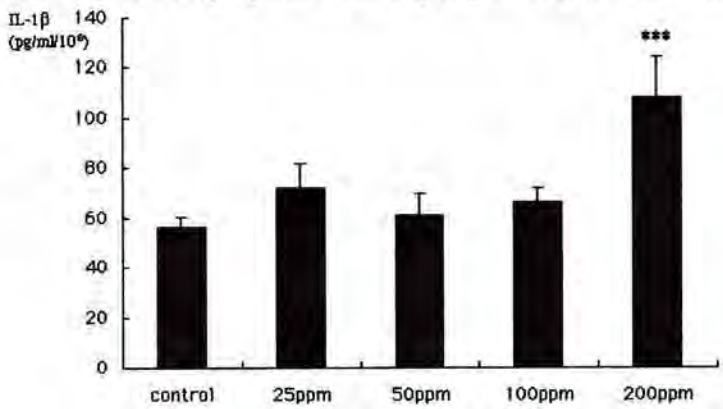


Figure. IL-1β concentrations in the supernatant of the splenic adherent cells from mice exposed to TPT chloride

***; p<0.001 compared to the control

塩化トリフェニルスズ亜急性経口投与による脾臓マクロファージ上清中インターロイキン-1bの濃度の増大

○角田 正史[1]、中野 憲[2]、紺野 信弘[3]

[1]北里大学 医学部 衛生学公衆衛生学教室、[2]福島県衛生研究所、[3]郡山女子大学短期大学部

Lecture 10 Metabolism of triphenyltin

○Shuji OHHIRA , Mitsunori ENOMOTO , Yoshikazu MIURA , Hisao MATSUI

Department of Hygiene, Dokkyo University School of Medicine, Tochigi, Japan

Triphenyltin compounds have been used mainly as agricultural biocides and antifouling paints for ship hulls and fishery firm nets. On the basis of our recent studies, the objective of this lecture is to provide information on the metabolism of triphenyltin in mammals. Triphenyltin is metabolized by cytochrome P-450 (CYP) system enzymes, and this metabolic process can generally produce metabolites that are usually less toxic than the parent triphenyltin. Triphenyltin toxicity induces the diabetogenic effects, such as hyperglycemia and hypertriglyceridemia, in hamsters and rabbits, but these signs of intoxication were not found in rats and mice. Similarly, species differences in the metabolic fate of triphenyltin exist for susceptibility between hamsters and rats. Triphenyltin accumulated more and was metabolized slower in hamsters than in rats. Furthermore, the diabetogenic actions of triphenyltin to hamsters were suppressed by pretreatment with phenobarbital (PB), which increased levels of cytochrome P-450 and accelerated metabolism of triphenyltin. Although mice are insusceptible to the hyperglycemic action by triphenyltin, the chemical produced marked hyperglycemia in the mice after the pretreatment with α-phenyl-α-propylbenzeneacetic acid 2-[diethylamino]-ethyl ester hydrochloride (SKF-525A), which is an inhibitor of CYP of PB-inducible forms. These results suggest that the metabolic fate of triphenyltin may contribute to the toxicity of the chemical. Since triphenyltin compounds produced a concentration-related in vitro functional inactivation of CYP through interaction with critical sulfhydryl groups of the hemoprotein, the metabolic reaction using the microsome-NADPH system usually conducted was not applicable to in vitro metabolism of triphenyltin. We therefore examined the effects of dithiothreitol (DTT), one of the antioxidant for sulfhydryl group, to determine the in vitro metabolism of triphenyltin with hepatic microsomes. As a result, the DTT was significantly effective in preventing the triphenyltin-induced inactivation of CYP-mediated triphenyltin metabolism. The in vitro model system for triphenyltin metabolism was applied to a comparative study of the metabolism of triphenyltin in rats, hamsters and humans. There is a marked difference in the metabolic capacity for triphenyltin with hepatic microsomes between rats and other species, the largest dearylation of the organotin was observed in rats (Fig. 1). In contrast, the least amounts of metabolites of triphenyltin were detected in humans, in which in vitro metabolic reaction induced no significant sex-related differences in the capacity, and the in vitro metabolic pattern resembled that with hamsters, which were susceptible to in vivo triphenyltin toxicity as well as incompetent to the metabolism. It is possible that hamsters are suitable animal model for exploring the influence of triphenyltin in humans. Moreover, we also identified the principal CYP in triphenyltin metabolism in rats, because the metabolic capacity for triphenyltin in rats is relatively high and many types of cDNA-expressed rat CYP enzymes can be easily obtained. No significant sex differences occurred between the in vivo and in vitro metabolic patterns of the chemical, indicating that the principal CYP for triphenyltin metabolism in rats is not a sex-specific form of CYP. In addition, seven types of cDNA-expressed rat CYPs, typical PB-inducible forms (Fig. 2A) and CYP2C subfamily (Fig. 2B), were tested to determine the activity of triphenyltin metabolism. Among the CYP isoforms studied, although CYP2B1 had a small metabolic capacity, a marked dearylation of the chemical was induced by CYP2C6. Furthermore, anti-rat CYP2B1 and CYP2C6 antibodies and cimetidine, one of a CYP2C6 selective inhibitor, inhibited triphenyltin dearylation activity in hepatic microsomes of rats. Taken together, these findings suggest that CYP2C6 is specific CYP for triphenyltin metabolism in rats. On the other hand, although the complexity of the situation regarding regio- and stereo-selectivity in metabolism of drugs via both rat and human CYP2C isozymes is reported, these CYP2C subfamilies have common substrate

specificities due to homology between rat and human orthologous proteins. In vitro metabolism of triphenyltin was also studied using predominant cDNA-expressed CYP2C subfamily of human: CYP2C8, 2C9, and 2C19 (Fig. 3). Although these metabolic capacities for triphenyltin were relatively poor, significant dearylation of the chemical was observed in CYP2C9 and CYP2C19, particularly CYP2C19. The CYP2C subfamily may play a key role in triphenyltin metabolism in humans.

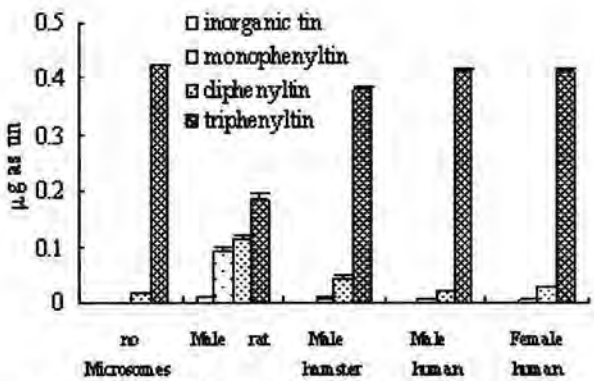


Fig. 1 In vitro metabolism of triphenyltin with rat, hamster, and male and female human hepatic microsomes (0.5 mg protein). The reaction mixtures (triphenyltin was added as 0.5 mg tin) were incubated at 37°C for 180 min. Values represent the mean ± S.D. of four assays. No microsome, without microsomes.

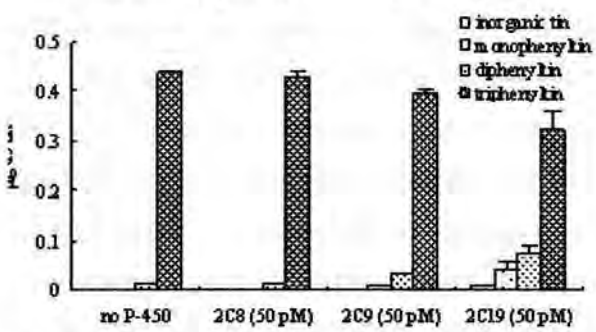


Fig. 3 In vitro metabolism of triphenyltin with cDNA-expressed human CYPs of the 2C subfamily. No P-450 (vehicle control), containing baculovirus vector only. Values represent the mean ± S.D. of four assays.

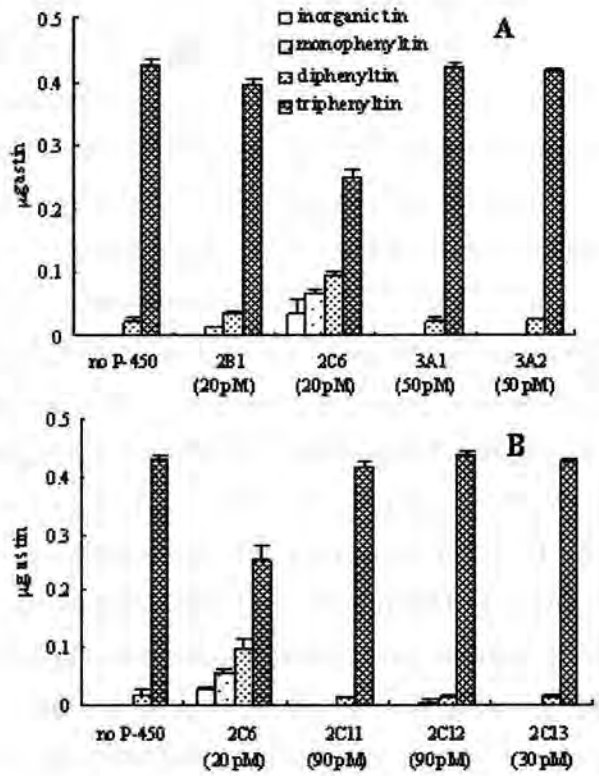


Fig. 2 In vitro metabolism of triphenyltin with cDNA-expressed rat CYPs of typical PB-inducible (A) and the 2C subfamily (B). No P-450 (vehicle control), containing baculovirus vector only. Values represent the mean ± S.D. of four assays.

トリフェニル錫の代謝

○大平 修二、榎本 光紀、三浦 善憲、松井 寿夫
獨協医科大学 衛生学教室

Lecture 11 Effects of organoltin compounds on endocrine functions of human placental cells

○Tsuyoshi NAKANISHI[1] , Jun-ichi NISHIKAWA[2] , Keiichi TANAKA[1]

[1]Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan , [2]Laboratory of Environmental Biochemistry, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), are known to induce an irreversible sexual abnormality of female neogastropod snails which is termed "imposex". Human exposure to these organotin compounds may result from the consumption of organotin-contaminated meat and fish products. However, in humans, it remains unclear whether organotin compounds disturb the sexual development and reproductive. The estrogens and human chorionic gonadotropin (hCG) the principal hormones produced by the placenta in human pregnancy. These hormones are essential to several important events in establishment and maintenance pregnancy. Thus such endocrine functions of placenta might be at high risk due to the developmental and reproductive toxicity of environmental contaminants which have endocrine-disrupting effects. Previously, we reported that TBT and TPT enhance hCG secretion and aromatase activity in human choriocarcinoma cells **(J. Clin. Endocrinol. Metab. 87, 2830, 2002)**. In order to extend the knowledge on correlation between the structure of organotin compounds and their endocrine-disrupting effects, we assessed the effects of 17 alkyltin compounds on hCG secretion, aromatase activity and the both mRNA level of human choriocarcinoma Jar cell, suggesting that the potency of disrupting effects induced by organotin compounds have relevance to both the number and the length of their side chain with regularity. Moreover, these results lead us to hypothesize that trialkyltin compounds may interact with a certain molecule, such as a nuclear receptor, to affect these placental endocrine functions similar to environmetal estrogenic chemicals which interact with esrogen receptors. In this symposium, the potential ability of organotin compounds as a ligand of nuclear receptor will be discussed.

有機スズ化合物のヒト胎盤機能修飾による内分泌撹乱作用とその作用メカニズム

○中西 剛[1]、西川 淳一[2]、田中 慶一[1]

[1]大阪大学 大学院薬学研究科 毒性学分野、[2]大阪大学 大学院薬学研究科 微生物動態学分野

Lecture 12 Radiometal Labeled Probes for in vivo Redox Imaging

○Takako FURUKAWA[1] , Takeshi TANAKA[2] , Yasuhisa FUJIBAYASHI[1]

[1]Biomedical Imaging Research Center, University of Fukui, Fukui, Japan , [2]Department of Otorhinolaryngology

In our search for radioactive Cu-labeled radiopharmaceuticals with unique in vivo characteristics, we found that some small molecular weight lipophilic Cu-chelates can be metabolized by redox enzymes in electron transport chains and show reductive retention in living cells in redox-dependent manner. Based on these findings, we surveyed various Cu-complexes for Cu-radiopharmaceuticals which can selectively visualize the tissues in normal redox state as well as in abnormal redox state. Hypoxia can be specified as an abnormally reduced state and hypoxic tissues can be visualized by Cu-complex with low redox potential. Most of the Cu-complexes studied showed hypoxia selective reduction, though the levels of selectivity varied. Copper-pyruvaldehyde bis(N-methylthiosemicarbazone)(Cu-PTSM), which serves as a blood flow imaging reagent, was reduced both in hypoxic and normoxic cells. Copper-diacetyl-bis (N4-methylthiosemicarbazone)(Cu-ATSM), which has a lower redox potential than Cu-PTSM, was reduced and retained in hypoxic cells but not in normoxic cells. We selected Cu-ATSM as a hypoxia imaging agent and could specifically visualize hypoxic tissues including acute ischemic heart and hypoxic tumors. Hypoxic tumors are known to be resistant to conventional chemotherapy and radiotherapy and the specific diagnosis would help to select more efficient treatment protocol.Studies on Cu-ATSM metabolism revealed that the reduction was enzyme dependent and that the dominant redox systems of Cu-ATSM in non-tumor cells and tumor cells were completely different; the former being mitochondrial electron transport chain and the latter being microsomal electron transport chain. In non-tumor cells the complex I of mitochondrial electron transport chain mainly reduced Cu-ATSM, and in tumor cells NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase played an important role in the reduction. The significance of microsomal electron transport chain in tumor cells is still unclear but it could be related to the tolerance of tumor cells to hypoxia.Redox imaging agents like Cu-ATSM will bring us new insights in energy metabolism in tumor cells and might become a key in tumor treatment.

インビボ Redox イメージングのための放射性金属標識プローブ

○古川 高子[1]、田中 健[2]、藤林 靖久[1]

[1]福井大学 高エネルギー医学研究センター、[2]耳鼻咽喉科学

Lecture 13 Design and Synthesis of a Novel Lanthanide-based Zinc Ions-Sensitive Chemosensors for Magnetic Resonance Imaging (MRI) and/or Luminescence Measurement

○Kenjiro HANAOKA[1] , Kazuya KIKUCHI[1,2] , Tetsuo NAGANO[1]

[1]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan , [2]Presto, JST Corporation, Kawaguchi, Saitama, Japan

Zinc (Zn^{2+}) is the second most abundant heavy metal ion after iron in the human body. It is an essential component of many enzymes, transcription factors and synaptic vesicles in excitatory nerve terminals, and present in serum at a concentration of ~12 μM (total Zn^{2+}). So, imaging of chelatable zinc is of interest, recently. Here, we have designed and synthesized two types of novel lanthanide(Gd^{3+} or Eu^{3+})-based chemosensors designed for detecting zinc ions.

(i) Gadolinium (Gd^{3+}) complexes have been widely used as MRI contrast agents. Gd^{3+} complexes enhance the T_1 (spin-lattice) and T_2 (spin-spin) relaxation rates of water protons by rapid exchange of inner-sphere water molecules with bulk solvent. This increase in relaxation rate is due in part, to the direct interaction of water molecules (inner sphere) with the unpaired electrons of the paramagnetic metal ion Gd^{3+}. The longitudinal and transverse relaxivity value R_1 and R_2 refer to the amount of increase in $1/T_1$ and $1/T_2$, respectively, per millimolar concentration of agent. Light-based microscopy imaging techniques using fluorescence sensor molecules sometimes suffer from photobleaching and light scattering, whereas magnetic resonance imaging (MRI) can provide a three-dimentional imaging without these problems. From above background, we have designed and synthesized the first Zn^{2+}-sensitive MRI contrast agents.

(ii) Luminescent lanthanide complexes such as europium ion (Eu^{3+}) and terbium ion (Tb^{3+}) possess extremely long luminescent lifetimes in the order of milliseconds, very large Stoke-s shifts (>200 nm) and high water-solubilities. This property of long-lived luminescence can be utilized to introduce a delay before emission measurement, thus allowing the short-lived background fluorescence, which typically has a lifetime of nanosecond order, to decay to negligible levels resulting in measurement with low background noise level. From these spectroscopic properties as a result of a unique luminescent pathway, lanthanide chelates have been recently exploited as a fluorophore for many purposes, such as time-resolved assays and the luminescence imaging microscopy, etc. We have designed and synthesized the novel luminescent lanthanide probe designed for detecting Zn^{2+}, which is the Eu^{3+}-complex.

新規亜鉛イオン応答性 MRI 及び蛍光ランタノイド錯体の開発

○花岡 健二郎[1]、菊地 和也[1,2]、長野 哲雄[1]

[1]東京大学大学院 薬学系研究科、[2]科学技術振興機構さきがけ

Lecture 14 A Versatile Strategy for the Synthesis of Crown Ether Bearing Heterocycles: Discovery of Calcium Selective Fluorescent Probe

Yuko AOKI , Naoki UMEZAWA , Yuko ASANO , Kei-ichiro HATANO , Shin-ichi NAGAI , ○Tsunehiko HIGUCHI

Graduate School of Pharmaceutical Sciences, Nagoya City University

This paper deals with the versatile synthesis of crown ether-incorporated heterocycles and the discovery of a novel Ca^{2+} selective fluorescent probe. A variety of modifications of crown ether structure has been achieved to search for new functions, however, relatively few examples are known about crown ethers incorporating heterocycles in macro ring systems. We found a simple and versatile synthesis of acylurea-type heterocyclic compounds bearing various sizes of crown ring. Since these molecules are expected to have many functions, we examined fluorescence spectroscopy upon titration with various metal ions and discovered a Ca^{2+} selective fluorescent probe (LM3). Compound LM3 has only 11-membered ring, which is rather small for metal ion inclusion. In order to examine the coordination mode, we synthesized various related compounds. Their fluorescence response to metal ions suggested that the coordination at both crown ether moiety and heterocyclic amine site are important for selective fluorescence enhancement. X-Ray crystal structure analysis of Ca^{2+}-LM3 complex has been achieved. Infrared carbonyl stretching frequencies and crystal structure also supported above hypothesis. Our simple methodology provides many novel heterocyclic compounds, which are expected to have a wide variety of functions such as fluorescent probe and pharmaceuticals.

アシルウレア構造を組み込んだ新規クラウンエーテル類の合成法開発とカルシウムイオン選択的蛍光プローブ構築への応用

青木 優子、梅澤 直樹、浅野 優子、畑野 研一郎、永井 慎一、○樋口 恒彦

名古屋市立大学 大学院薬学研究科

Lecture 15 Immune disrupting effect of arsenic; Inorganic arsenite induces HLA-DR strong positive dendritic macrophage generation from human peripheral blood monocytes

○Teruaki SAKURAI , Takami OHTA , Kitao FUJIWARA

Laboratory of Environmental Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Tokyo, Japan

A trivalent inorganic arsenic, arsenite, has been recently developed as a potent therapeutic agent for acute promyelocytic leukemia (APL), while, it is also causing chronic poisoning through the consumption of contaminated well water. The total peripheral blood arsenic concentrations of two APL patients who underwent complete remission by intravenous injection of arsenite and chronic arsenic poisoning patients who had inflammatory-like immune responses are less than 1 μM, thus, nM concentrations may be very importance to induce both therapeutic and chronic toxic effects by arsenite. However, there are few reports about the biological effects of low concentrations of arsenite in mammalian cells, especially in normal immune effector cells. We demonstrate in this study the effect of low concentrations of inorganic arsenite in human peripheral blood monocytes. Arsenite significantly inhibited the cytokine induced *in vitro* maturation of monocytes into macrophages and/or dendritic cells at nM levels. Surprisingly, arsenite induced the generation of a unique nonadherent dendritic macrophage which strongly expressed HLA-DR and could stimulate T cell responses at nM levels together with the granulocyte-macrophage colony stimulating factor. These immune disrupting effects of arsenite at nM concentrations may suggest the important mechanistic basis for both arsenic chemotherapy and chronic arsenic poisoning.

ヒ素の免疫攪乱作用；極微量の無機ヒ素によるヒト末梢血単球から HLA-DR 強陽性樹状マクロファージへの分化誘導

○櫻井 照明、太田 宇海、藤原 祺多夫
東京薬科大学 生命科学部 環境衛生化学研究室

Lecture 16 Molecular mechanism of cytolethality of dimethylated trivalent arsenic compound

○Chikara KOJIMA , Teruaki SAKURAI , Kitao FUJIWARA

Laboratory of Environmental Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Tokyo, Japan

1. Introduction

Inorganic arsenic is clearly a toxicant and carcinogen in humans. Inorganic arsenic is enzymatically methylated in human body, and dimethylarsinic acid ($DMAs^{V}$) is the major pentavalent organic arsenic metabolite in human urine. It has been believed that the methylation of inorganic arsenic is not a universally detoxifying event, because dimethylated trivalent arsenic compound ($DMAs^{III}$), which is thought to be an intermediary metabolite of inorganic arsenic, is much more cytotoxic *in vitro* than inorganic arsenic. $DMAs^{III}$ exists as the conjugate with reduced glutathione (GSH) ($DMAs^{III}$-SG) in the liver which is a major site of arsenic methylation, and is excreted into the blood. Therefore, defining the mechanisms of arsenic poisoning requires further studies on the toxicity of $DMAs^{III}$-SG. In this study, we demonstrated the molecular mechanism of cytolethality of $DMAs^{III}$-SG using rat epithelial liver TRL1215 cells.

2. Materials and Methods

$DMAs^{III}$-SG was synthesized as described elsewhere. Cytolethality of arsenic was determined by AlamarBlue assay, and cellular arsenic concentration was analyzed by hydride generation coupled with atomic absorption spectrometry.

3. Results and Discussions

$DMAs^{III}$-SG showed strong cytolethality in TRL1215 cells and the lethal concentration *in vitro* in 50 % of a population (LC_{50}) after 48 h exposure was 200 nM, although the LC_{50} values of inorganic arsenite and $DMAs^{V}$ were 35 μM and 1.5 mM, respectively. $DMAs^{III}$-SG was taken up into the cells approximately 35-fold more than $DMAs^{V}$. Thus, it was suggested that the strong cytolethality of $DMAs^{III}$-SG is depended on the high affinity for the cells. However, the addition of exogenous GSH (1 mM, which is the same as the concentration in human blood) significantly reduced both cytolethality and cellular uptake of $DMAs^{III}$-SG. These results indicate that GSH maintains the form of $DMAs^{III}$-SG conjugate and this conjugate cannot be transported into the cells. $DMAs^{III}$-SG might separate the SG and become $DMAs^{III}$-OH when it showed the significant cytolethality. The results of the present study suggested that the significant cytolethality of $DMAs^{III}$-SG cannot be appeared under the normal GSH concentration in the blood, and arenic methylation in the human body may be essentially a detoxifying event in order to excrete arsenic.

ジメチル 3 価ヒ素の毒性発現機構とグルタチオンによる防御

○小島 力、櫻井 照明、藤原 祺多夫

東京薬科大学 生命科学部 環境衛生化学研究室

Lecture 17 Role of zinc in the regulation of protein tyrosine phosphatase activity in osteoblastic MC3T3-E1 cells

Masayo FUKAGAWA , ○Satoshi UCHIYAMA , Masayoshi YAMAGUCHI

Graduate School of Nutritional Sciences, University of Shizuoka

Zinc is known to be an essential trace element for the growth of humans and other animals. Zinc deficiency results in a retardation of bone growth, suggesting that the element is required for the growth, development, and maintenance of healthy bone. There is growing evidence that zinc plays a role in the regulation of bone metabolism; the metal can stimulate osteoblastic bone formation and inhibit osteoclastic bone resorption [1,2]. Moreover, the pathophysiologic role of zinc in osteopenia and psteoporosis has been shown [3]. Bone zinc content is reduced with increasing age and skeletal unloading in rats. Osteoporosis patients have been shown to have lower levels of skeletal zinc than normal individuals. Woman with osteoporosis excrete a great amount of zinc in urine. Zinc supplementation has been shown to have a preventive and therapeutic effect on bone loss. Zinc may have a role as a nutritional and pharmacologic tool in the prevention of osteoporosis with increasing age [4,5]. Bone growth factor is important in the physiologic mechanism of bone growth. Zinc may play a role as a stimulatory factor in bone growth. Zinc may play a role as a stimulatory factor in bone growth. Zinc deficiency has been shown to decrease in serum IGF-I levels and skeletal growth in young rats. Also, zinc has been demonstrated to have a stimulatory effect on IGF-I production in osteoblastic MC3T3-E1 cells in vitro and IGF-I mRNA expression in femoral tissues of newborn rats in vivo. Zinc may play a special role in bone growth in collaboration with IGF-I. The cellular mechanism by which zinc stimulates bone growth, however, has not been fully clarified. The present study was undertaken to determine the effect of zinc on protein tyrosine phosphatase activity in osteoblastic MC3T3-E1 cells. This enzyme plays an important role in intracellular signaling pathway which is related to the stimulation of hormone and cytokine in cells. Cells were cultured for 72 h in medium containing 10% fetal bovine serum (FBS) to obtain subconfluent monolayers, and then exchanged to culture medium containing either vehicle, zinc sulfate or various hormones in the absence of 10% FBS. After medium change, cells were cultured for 48 h. Protein tyrosine phosphatase activity in the lysate of cells was significantly increased by culture with zinc (10^{-6}-10^{-4} M). The effect of zinc in increasing the enzyme activity was completely blocked by culture with cycloheximide (10^{-7} M), an inhibitor of protein synthesis, or 5, 6-dichloro-1-b-D- riboifuranosylbenzimidarzole (DRB) (10^{-6} M). an inhibitor of translational activity. Addition of calcium chloride (10 mM) into the reaction mixture caused a significant increase in protein tyrosine phosphatase activity; this increase was completely blocked

in the presence of trifluoperazine (50 mM), an antagonist of calmodulin. Culture with zinc caused a significant increase in Ca^{2+}/calmodulin-dependent protein tyrosine phosphatase activity in osteoblastic cells. Protein tyrosine phosphatase activity was significantly raised by culture with parathyroid hormone (human PTH [1-34]; 10^{-7} M), 17β-estradiol (10^{-9} M), insulin-like growth factor-I (IGF-I; 10^{-8} M) or insulin (10^{-8} M). The enzyme activity was not significantly enhanced by the addition of calcium (10 mM) into the reaction mixture. The effect of PTH or IGF-I in increasing protein tyrosine phosphatase activity was completely blocked by culture with DRB. The IGF-I-induced increase in enzyme activity was significantly enhanced by culture with zinc. Such an effect was not seen in the case of PTH. Moreover, the effect of IGF-I in increasing proliferation of osteoblastic cells was significantly enhanced by culture with zinc. The effect of PTH was not enhanced by zinc. This study demonstrated that protein tyrosine phosphatase activity in osteoblastic cells was enhanced by various bone anabolic factors, and that zinc modulates IGF-I's effect on protein tyrosine phosphatase activity and cell proliferation.[References] 1. Yamaguchi M: Curr Med Chem 1: 356-365 (1995) [review]. 2. Yamaguchi M: General Pharmacol 26: 1179-1183 (1995) [review]. 3. Yamaguchi M: J Trace Elem Exp Med 11: 119-135 (1998) [review]. 4. Yamaguchi M, Uchiyama S: Int J Mol Med 12: 755-761 (20039. 5. Uchiyama S, Yamaguchi M: Int J Mol Med 12: 949-954 (2003).

骨芽細胞培養系におけるプロテインチロシンホスファターゼ活性調節に及ぼす亜鉛の作用

深川 雅代、○内山 聡志、山口 正義
静岡県立大学 大学院 生活健康科学研究科

Lecture 18 Inhibitory zinc action against excitation of hippocampal glutamatergic neurons

○Akira MINAMI[1] , Atsushi TAKEDA[1] , Jun-ichi KOSUGE[2] , Noboru FUKASAKU[2] , Tetsuo NAGANO[3] , Naoto OKU[1]

[1]Department of Medical Biochemistry, School of Phrmaceutical Sciences, University of Shizuoka, Shizuoka, Japan , [2]Daiichi Pure Chemicals Co., LTD., Ibaraki, Japan , [3]Laboratory of Bioorganic and Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

Zinc is present at high concentrations in the synaptic vesicles of hippocampal mossy fibers (MFs). However, physiological role of zinc in synaptic neurotransmission is poorly understood. An in vivo mycrodialysis experiment in the hippocampal CA3 region demonstrated that glutamate concentration in the extracellular fluid is decreased by perfusion with zinc, suggesting that zinc suppressively acts against glutamate release from MF terminals[1,2]. MF long-term potentiation (LTP) is initiated and expressed presynaptically, and calcium is required for this presynaptic form of LTP. Thus, calcium influx into MF terminals is a trigger of effective glutamate release. In the present study, zinc action in calcium influx into MF terminals was examined in hippocampal slices prepared from mice and rats. Zinc released from MF terminals was monitored with extracellular zinc indicator, ZnAF-2, and excitability of MFs was monitored with intracellular calcium indicators, fura-2 AM and Fluo-4 AM.

The dentate hilus and stratum lucidum, in which MF terminals exist, was evidently imaged with ZnAF-2 immediately after addition of ZnAF-2 to hippocampal slices. When dentate molecular layer was stimulated for 1 s with 100 mM KCl, ZnAF-2 signal was increased with fura-2 signal in the stratum lucidum. These results indicate that zinc is released from MFs along with synaptic activity and instantaneously detected with ZnAF-2. When endogenously released zinc was chelated with 1 mM CaEDTA, a zinc chelator, by using in vivo microdialysis, extracellular glutamate concentration was increased, suggesting that endogeneous zinc released into synaptic cleft suppress presynaptic glutamate release[1]. Therefore, the effect of the zinc chelator on calcium influx was examined in the stratum lucidum by electrical stimulation (100 Hz for 5 S) of dentate granule cells. Fluo-4 signal was increased immediately after electrical stimulation. This increase was enhanced by addition of CaEDTA (1 mM) to hippocampal slices, but inhibited by addition of $ZnCl_2$ (10-300μM). The effects of CaEDTA and $ZnCl_2$ were also observed in the CA3 pyramidal cell layer. The present study demonstrates that zinc released from MF terminals may inhibit not only calcium influx into MFs, but also calcium influx into the postsynaptic pyramidal cells.

1) J. Neurosci. Res., 75, 225-229 (2004), 2) Epilepsy Res., 57, 169-174 (2004)

亜鉛による海馬グルタミン酸作動性ニューロンの興奮抑制

○南 彰[1]、武田 厚司[1]、小菅 順一[2]、深作 昇[2]、長野 哲雄[3]、奥 直人[1]

[1] 静岡県立大学 薬学部 医薬生命化学教室、[2] 第一化学薬品株式会社、[3] 東京大学大学院 薬学系研究科 薬品代謝化学教室

Lecture 19 Studies on the valid utilization of abundant zinc contained in Oyster (*Crassostrea gigas*)

○Yoshikazu MATSUDA[1] , Natsumi SUMIDA[2] , Sachiko KITANI[2] , Shoko KITANI[2] , Mamiko ABE[1] , Nami KOMURA[1] , Tomoe KAWASHIMA[1] , Kenji FUKUNAGA[2] , Munehiro YOSHIDA[2]

[1]Central Research Institute, Japan Clinic Co., Ltd., Kyoto, Japan , [2]Department of Biotechnology, Faculty of Engineering, Kansai University, Osaka, Japan

The oyster extract which was extracted from the oyster (*Crassostrea gigas*) with hot water is marketed as dietary supplement. Even if an oyster is food including extremely a large quantity of zinc compared with other food, only about 20% of total content of zinc in oyster are collected and utilized by commercial hot water extraction products. This shows that most of zinc included in oyster is present in insolubility form.Thus solubility of zinc fraction in oyster for fluid and digestive enzyme was examined. As a result, the most of zinc fraction in oyster were insoluble in neutrality, but it was soluble in acid. And about in vitro protease digestion test, in oyster homogenate under acid condition less than pH 3.0, more than 90% of the oyster zinc fraction was solubilized with irrelatively to pepsin digestion. However, in pepsin digestion on pH 5.0, the ratio of the solubilization zinc fraction decreased remarkably. These results suggested that when elevation of gastric juice pH by meal block (pH three to four) is considered, insolubility zinc fraction is not dissolved enough in a stomach, and therefore may not be absorbed and digested in small intestine.Then, solubilization and extraction method with hydrochloric acid of zinc rich fraction was examined. The oyster homogenate was extracted by 0.1N hydrochloric acid. The precipitation formed after neutralization was collected, and was lyophilizated. As a result, zinc content of obtained zinc rich fraction was 76mg/g. Zinc content in this fraction corresponds to about 70% of oyster immanence zinc. Furthermore, about obtained zinc rich fraction, digestion and absorption in alimentary canal and tissue distribution were examined. As a result, about in vitro digestion test, solubility was more superior to zinc carbonate. And, about in vivo ingestion test, zinc transition to tissue (especially, tibia) increased in significance. It was suggested that intestinal absorption and distribution to tissue were superior.In comparison feeding test with four zinc sources, liver alcohol dehydrogenase and serum alkaline phosphatase activity which were zinc content enzyme were increased in significance by zinc rich fraction compared with other zinc form.These finding were identified that obtained zinc rich fraction had excellent solubility and absorptivity on digestion and enhancement of the bioactivity which zinc participated.

牡蠣に内在する亜鉛の有効利用に関する研究

○松田 芳和[1]、隅田 奈都美[2]、木谷 祥子[2]、木谷 祥子[2]、安部 麻美子[1]、小郁 奈未[1]、川島 朋絵[1]、福永 健治[2]、吉田 宗弘[2]

[1]日本クリニック株式会社 中央研究所、[2]関西大学工学部生物工学科

Lecture 20 Activation of Iron Regulatory Protein 1 through Heme Oxygenase 1 Signaling Pathway after Oxidative Stressful Stimuli

○Satoru OSHIRO[1] , Koji NOZAWA[2] , CHUN ZHANG[3] , Tshio SONE[4] , Ken-ichi KAWAMURA[5]

[1]Biochemical Genetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan , [2]Showa College of Pharmaceutical Sciences, Tokyo, JAPAN , [3]Van Andel Research Institute, Michigan, USA , [4]Allergy and Immunology, Saitama Medical School, Saitama, Japan , [5]TK Craft Co., LTD, Gunma, Japan

The iron regulatory protein 1 (IRP1) is known as a cytosolic aconitase and is one of the central regulators of iron homeostasis. IRP 1 regulates the expression of iron metabolism-related proteins by interacting with the iron responsive element (IRE) in the untranslated regions of the transcripts of these proteins. We previously reported that IRP 1 responds at the levels of various metals including toxic metals (1). Therefore, we presented that IRP1 is a metal sensor protein (2). In in vitro experiment, the metal treatment decreased the affinity of purified IRP1 to IRE and increased the aconitase activity, demonstrating that these metals mimic the Fe effect except manganese. In human PLC hepatoma cells, the metal loading inactivated the IRE binding activity and activated the enzyme activity. Northern blot analysis also showed that these metals strongly suppressed the expression of transferrin receptor (TfR) mRNA in the cells. In contrast, various oxidative stress stimuli increased the IRE-binding activity but decreased the aconitase activity. These stimuli activated IRP 1 by 24h after treatment. Though the cadmium (Cd)-induced IRP1 activation was not inhibited by okadaic acid (type I/IIa protein phosphatase inhibitor), H_2O_2-induced IRP1 activation was inhibited by the phosphatase inhibitor. Both of oxidative stressors also induced heme oxygenase 1 (HO1) known as heat shock protein 32 involved in iron metabolism. Therefore, HO1 seems to be activated by at least two pathways; type I/IIa protein phosphatase-dependent or -independent pathway. Expression of HO1 mRNA and its protein peaked at 8 and 12h prior to IRP1 activation. When HO 1 protein was maximally expressed, iron release reached to the maximum and cellular iron was minimum level at that time. Dominant negative mitogen activated protein kinase kinase 4, 7 and antisense HO-1 DNA abolished this HO-1 effect. It was also shown that iron distributed in non-ferritin fraction as well as reactive oxygen species (ROS) decreased at that time. Therefore, we hypothesized that HO-1 activated through type I/IIa protein phosphatase-dependent or JNK/SAPK HO1 pathway promotes iron excretion resulting in the suppression of ROS production (3). Thereafter, IRP1 activation through the HO1 signaling pathway after oxidative stressful stimuli may normalize cellular iron level by expression of iron metabolism related proteins. **[REFERENCES]** (1) Oshiro, S., Nozawa, K., Hori, M., Zang, C., Hashimoto, Y., Kitajima, S. and Kawamura,K.: Modulation of iron regulatory protein-1 by various metals. *Biochem. Biophys. Res. Commun.* 290, 213-218, 2002. (2) Oshiro, S. Structure and Bonding, A new effect of aluminum on iron metabolism in mammalian cells. 104, 60-77, Roesky, H.W. and Atwood, D.A., Group 13 Chemistry II Springer-Verlag. 2003. (3) Oshiro, S., Nozawa, K., Zhang, C., Kawamura, K., Sone, T.: Iron regulatory protein-1 acts not only as a metal sensor protein but as a stress sensor protein. *Mol. Cell. Proteom.* 2, 878, 2003

酸化ストレス刺激後の Heme Oxygenase 1 シグナル伝達系による Iron Regulatory Protein 1 の活性化

○大城 聰[1]、野澤 浩司[2]、張 純[3]、曽根 敏雄[4]、川村 憲一[5]

[1]東京医科歯科大学 難治疾患研究所 遺伝生化学、[2]昭和薬科大学 生化学、[3]Van Andel Research Institute, Michigan, USA、[4]埼玉医科大学 免疫学、[5]TK クラフト 研究開発

Lecture 21 Implications of calcium homeostasis and neurotrophic factors in neurotoxicity of aluminum on primary cultured neurons

Keiko KONOHA[1] , Yutaka SADAKANE[1] , Lisa IMAMURA[2] , Masaaki TSUDA[2] , ○Masahiro KAWAHARA[1]

[1]Department of Analytical Chemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, Miyazaki, Japan , [2]Department of Biological Chemistry, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama, Japan

Aluminum (Al) is widely distributed in our environment. However, it is not an essential element and harmful to many living things. Al toxicity has been linked to various diseases. In particular, the relationship between Al and Alzheimer's disease has been argued for decades, but is still controversial. We have investigated the effects of Al on primary cultured rat cerebral cortical neurons or hippocampal neurons. Chronic exposure of Al for more than 1 month caused the accumulation of tau protein and β-amyloid protein. Although acute exposure of Al for 3-7 days did not cause significant cell death, co-existence of maltol, which formed the complex termed aluminum maltolate, enhanced the neurotoxicity of Al and caused apoptosis-like death of cultured neurons. We investigated the detailed characteristics of aluminum maltolate-induced neurotoxicity, and found that preadministration of brain-derived neurotrophic factor (BDNF) markedly attenuated its neurotoxicity. Aluminum maltolate-induced neurotoxicity was also blocked by addition of high concentration of K^+ and glial cell line-derived neurotrophic factor (GDNF). BDNF is a member of neurotrophin family and plays significant roles in the neuronal survivals. It is widely known that various functions of BDNF are accompanied with the BDNF-induced elevations in intracellular calcium levels ($[Ca^{2+}]i$). GDNF and K^+ also cause the elevations in $[Ca^{2+}]i$. Furthermore, aluminum maltolate inhibited BDNF-induced elevations in $[Ca^{2+}]i$ rise, but did not affect glutamate-induced $[Ca^{2+}]i$ in cultured hippocampal neurons. Our results suggest the involvement of neuretrophic factor and calcium homeostasis in the molecular mechanism underlying Al neurotoxicity.

アルミニウムによる培養神経細胞の細胞死における神経栄養因子とカルシウム・ホメオスタシスの寄与

木葉 敬子[1]、定金 豊[1]、今村 理佐[2]、津田 正明[2]、○川原 正博[1]

[1]九州保健福祉大学 薬学部 分析学講座、[2]富山医科薬科大学 薬学部 分子神経生物学研究室

Lecture 22 A robust speciation method for arsenic in biological matrices by ion-exclusion chromatography-ICPMS using reaction cell and hydride generation techniques

○Tetsuya NAKAZATO , Hiroaki TAO

Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan

Liquid chromatography combined with inductively coupled plasma mass spectrometry (LC-ICPMS) is a powerful hyphenated method for arsenic speciation in biological samples. However, in the case of analyzing high matrix samples, such as human urine, the analytical performance drastically decreases because a large amount of saline deteriorates the LC separation and chloride ions due to the matrix cause spectroscopic interference of ArCl on 75As measurement by ICP-MS. Therefore, the sample pretreatment for removing the matrix by dilution and/or extraction was necessary. However, the dilution operation frequently decreases the concentration of minor arsenic species below the detection limits for most LC-ICPMS systems. Moreover, the extraction often causes conversion of the species in the original sample. In order to accurately determine the arsenic species in biological matrices, the samples should be directly injected to LC-ICPMS.

In this study, we developed robust and sensitive methods for determination of arsenic species in biological matrices by ion-exclusion chromatography combined with ICPMS, which allows direct injections of the high matrix samples. The optimized chromatographic condition achieved not only separating arsenic species, but also effectively eluting or separating the matrix, even when non-diluted human urine was repeatedly injected. The ArCl interference on ICPMS detection was eliminated efficiently by collision or decomposition of ArCl molecules with gases in a reaction cell. Another technique, hydride generation, was also useful for reducing the interference and additionally lowered the detection limits of five arsenicals to 10 pg ml-1 level. The proposed methods were successfully applied to determine five and six arsenic species in human urine and an extract from tuna fish tissue, respectively. The advantage and disadvantage of both methods using the reaction cell and hydride generation were evaluated by analyzing certified reference materials. The robustness of our methods for long term use was demonstrated by the fact that no serious deterioration in analytical performance was observed in the experimental period: eighteen months.

反応セルおよび水素化物発生技術を用いたイオン排除クロマトグラフィ／誘導結合プラズマ質量分析法による生体マトリックス中ヒ素の高耐久性化学形態別分析法

○中里 哲也、田尾 博明

産業技術総合研究所 環境管理研究部門

Lecture 23 Analysis of inorganic antimicrobial agents used in antimicrobial products

○Harunobu NAKASHIMA[1] , Tomoko OOSHIMA[2]

[1]Osaka Prefectural Institute of Public Health, Osaka, Japan , [2]Osaka City Institute of Public Health and Environmental Sciences, Osaka, Japan

Antimicrobial agents used in antimicrobial products are classified into inorganic, organic, and natural organic compounds. Inorganic agents, such as Cu, Zn, and Ag compounds, are known to be relatively safe, and these agents are used in many kinds of products. Patients with metal allergy, and infants should avoid coming in contact with products in which inorganic agents are used at a high concentration, in particular regions treated with such agents at a high concentration. However, regions treated with inorganic agents and their concentrations are not indicated in most commercially available products. In this study, to establish a simple method of measuring the concentration of metals in product regions, we evaluated screening methods using non-destructive X-ray fluorescence spectrometry (XFS). Qualitative analysis by XFS and quantitative analysis by inductively coupled plasma atomic emission spectroscopy (ICP) were performed in the same regions, and the results were compared. Inorganic antimicrobial agents used in commercially available antimicrobial products were analyzed.Methods: (1) Samples: Forty antimicrobial products with which antimicrobial and deodorizing treatment and odor-neutralizing treatment were indicated were purchased, and 96 regions of the products were also analyzed. (2) XFS analysis: Cu, Zn, Ag, Ti, Fe, Cr, Ca, S, and Ni were examined. According to the CPS value, the concentrations were ranked qualitatively. (3) ICP analysis: Samples were decomposed with acid by microwaves, and Cu, Zn, Ag, Al, Ni, Cr, Co, and Mg were analyzed.Results and discussion: (1) XFS analysis was useful for the screening of Cu and Zn. (2) XFS analysis was not suited for the screening of Ag, but in the samples in which Ag was detected by ICP analysis, Cu or Zn was detected at a higher concentration. Generally, Ag is not as a sole antimicrobial agent but together with Cu or Zn. Therefore, it is expected that Ag can be measured by quantitative ICP analysis of regions in which Cu or Zn is detected. (3) 21 products were treated with inorganic antimicrobial agents. (4) Cu was detected in 6 products. (5) In 18 products, Zn was used for the antimicrobial treatment. (6) Ag was detected in 5 products, in which Al and Cu or Zn were contained at higher concentrations. (7) Although 2 products for infants indicated the treatment with chitosan, Zn was detected.

抗菌加工製品に使用されている無機系抗菌剤の分析

○中島 晴信[1]、大嶋 智子[2]

[1]大阪府立公衆衛生研究所、[2]大阪市立環境科学研究所

Lecture 24 Reaction Intermediates Detected during the Four-Electron Reduction Process of Dioxygen by Bilirubin Oxidase Mutants

○Rieko KITAGAWA[1] , Megumi INOUE[1] , Kunishige KATAOKA[1] , Takeshi SAKURAI[1] , Shotaro YAMAGUTCHI[2]

[1]Faculty of Science, Kanazawa University, Kanazawa, Japan , [2]Amano Enzyme, Kamigahara, Japan

Bilirubin oxidase (BO) belongs to multicopper oxidase, a class of Cu-proteins containing one type I Cu, one type II Cu and a pair of type III Cus in the active site. Type I Cu site mediates the electron transfer from substrate to the trinuclear center composed of type II and type III Cus, where a dioxygen molecule is reduced to two water molecules at the turnover stage of the enzyme cycle. Since BO catalyzes the conversion of bilirubin to biliverdin, it has been widely utilized in the clinical investigation of liver. We have characterized each Cu binding site in *Myrothecium verrucaria* BO by preparing a variety of mutants using *Aspergillus oryzae* and *Pichia pastoris* as hosts. However, the reactions of BO have not been studied in detail, although the two reaction intermediates, which appear during the reduction of dioxygen, have been successfully detected in the studies of laccase. The strong absorption band at around 330 nm originating in the charge transfer from the bridged hydroxide ion to type III Cus is very strong in the wild type BO, preventing the detection of the bands coming from intermediates. The apoBO and/or denatured BO might be present in the authentic BO, while the g = 1.9 EPR signal has been detected at cryogenic temperatures similarly with the case of laccase. In order to evercome the difficulty to detect the reaction intermediates in the studies of BO, we performed mutations on the type I Cu ligand, Cys457 or Met467 and a putative proton donor to dioxygen, Asp105.

The substitution of Ser for Cys457 was performed, expecting the disappearance the charge transfer bands coming from type I Cu and the facile detection of the new bands coming from intermediates. The charge transfer bands of type I Cu including that at 600 nm disappeared in the Cys457Ser mutant as expected, although it was slightly colored blue due to the unexpectedly strong d-d transitions of the type II and type III Cus. The EPR spectrum showed only the signal due to type II Cu, indicating that the type I Cu site was vacant unexpectedly. The total content of Cu in the Cys457Ser mutant was exactly 3.0 per protein molecule. The three-electron reduced Cys457Ser mutant under anaerobic conditions was reacted with dioxygen, giving the absorption bands at 340, 470 and 680 nm derived from the reaction intermediate. This spectral feature is similar to that observed in the reoxidation process of laccace in the mixed valent state in which type I Cu is oxidized and type II and type III Cus are reduced. Another reaction intermediate observed in the reoxidation process of the fully reduced laccase gave the slightly different absorption bands, of which the strongest one appeared at 370 nm. It was considered that all Cu ions in this intermediate were fully oxidized with the bridging of the two hydroxide ions in the trinuclear center, giving a very broad EPR signal g < 2 at cryogenic temperatures. Absorption spectra of the corresponding intermediates have not been detected in the reactions of the authentic and recombinant BOs. However, the substitution of Gln for Met467 as a ligand to type 1 Cu to give the stellacyanin-like type 1 Cu with the low redox potential value as the type I Cu center and the rhombic EPR signal, afforded this type of intermediate, although it has not been characterized yet in detail. Accordingly, two intermediates detected in the reoxidation process of laccase could be also detected in the case of BO by mutations and it appears that multicopper oxidases follow the common reaction pathway in the conversion of dioxygen to water.

The decays of the laccace intermediate were accelerated with decreasing pH. Therefore, it has been supposed that an amino acid with the pKa value of 5.4 is concerned in the four-electron reduction process of dioxygen as a putative proton source. Asp105 in BO is supposed to be this amino acid from homology of the amino acid sequence among multicopper oxidases and the crystal

structures of ascorbate oxidase and fungal laccases. Therefore, Asp105 was substituted by Asn in order to minimize the size change in mutation. However, only ca. 2 Cu ions were incorporated into Asp105Asn, indicating that Asp105 is indispensable to accommodate three Cu ions in the trinuclear center probably because Asp105 interacts with the coordinated hydroxide ion or water molecule directly or indirectly via a water molecule. Therefore, it appeared that Asp105 functions as the structural factor in addition to the proton donor towards the reduced species of dioxygen. In order to ascertain this, Ala was substituted for Asp105 because of the inability to form hydrogen-bond. Asp105Ala was successfully expressed and its properties are under investigation. On the other hand, we prepared Asp105Glu, the mutant with the potential proton source. Four Cu ions were incorporated into Asp104Glu as in the case of the authentic and recombinant BOs, indicating that the amino acid carrying a carboxyl group is obligatory at the amino acid 105 in BO. Asp105Glu showed the reduced specific activity of 11unit/mg protein, lower than 1/4 of the recombinant BO.

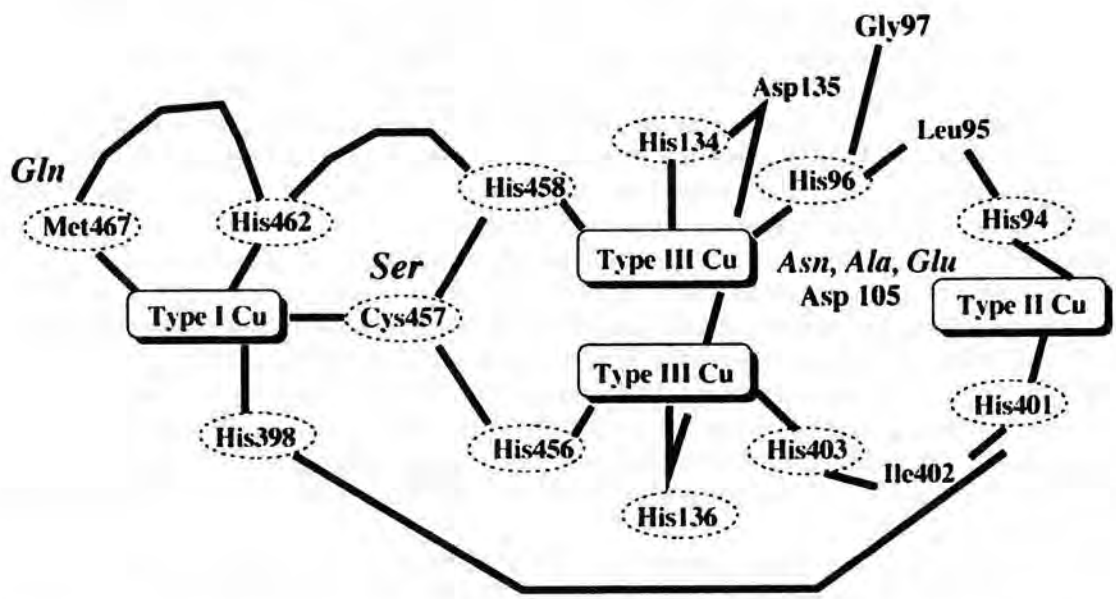


Fig. 1 Correlation of the Amino Acids around the Metal Binding Sites of BO

ビリルビンオキシダーゼの変異体による酸素還元中間体の捕捉

○北川 理映子[1]、井上 めぐみ[1]、片岡 邦重[1]、櫻井 武[1]、山口 庄太郎[2]
[1]金沢大学 理学部 化学科、[2]天野エンザイム

Lecture 25 Reduction of Cytchrome *c* with Tyrosyltyrosyl-phenylalanine

○Shun HIROTA[1] , Hisano OKUMURA[2] , Noriaki FUNASAKI[1] , Yoshihito WATANABE[2]

[1]Department of Physical Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan , [2]Department of Chemistry, Graduate School of Science, Nagoya University, Nagoya, Japan

Redox-active tyrosine plays important roles in enzymatic reactions, and its oxidative product, tyrosyl radical, is frequently formed in proteins. An indispensable tyrosine interacts with a metal in many of these proteins, such as iron-dependent ribonucleotide reductase, photosystem II, galactose oxidase, and etc. Tyrosyl radical is highly reactive, and especially phenoxyl radicals have been shown to be reactive highly towards phenols. For example, tyrosine oxidation products, 3,3'-dityrosine, 3,3',5',3"-trityrosine, 5-[4"-(2-carboxy-2-aminoethyl)phenoxyl]-3,3'-tyrosine (pulcherosine), and 3-[4'-(2-carboxy-2-aminoethyl) phenoxyl]-tyrosine (isodityrosine) are formed by post-translational modification. These crosslinking tyrosines are formed by oxidation of protein-bound tyrosyl residues in the presence of peroxidase to produce a hard fertilization membrane. Tyrosine-containing peptides have also been shown to polymerize by oxidation with compound I or compound II of horseradish peroxidase, lactoperoxidase, and myeloperoxidase. Formation of o-tyrosine and 3,3'-dityrosine in proteins by metal-catalyzed oxidation with Cu^{2+} and H_2O_2 has been observed, and dityrosine has been shown to account for the large fraction of fluorescence that accumulated in protein upon the oxidation.

Cytochrome *c* (cyt *c*) is a heme protein, which is positively charged at neutral pH. Cyt *c* functions as an electron transporting protein by shuttling electrons from cytochrome c_1 in the bc_1 complex to the Cu_A site in cytochrome *c* oxidase. It also provides electrons to cytochrome *c* peroxidase (CcP) for the catalytic reduction of hydrogen peroxide. Crystal and solution NMR structures of cyt *c* showed charged amino acids distributed around the heme edge of cyt *c*, and it is suggested that an electrostatic interaction occurs between the positively charged residues of cyt *c* and the negatively charged residues of its reaction partners. Actually, x-ray crystallographic structures of horse cyt *c*-CcP and yeast iso-1 cyt *c*-CcP complexes showed that the basic sites of cyt *c* interact with the acidic sites of CcP.

We have previously shown that charged peptides interact with electron transfer proteins, including cyt *c*.[1] Recently, we have shown that the His/His coordinated species for the heme increases at the initial stage of cyt *c* folding by interaction with trityrosine.[2] To gain insights into the interaction of cyt *c* with tyrosine-containing peptides, we investigated the interaction of cyt *c* with tyrosyltyrosylphenylalanine (TyrTyrPhe) in detail. The results demonstrate that reduction of cyt *c* occurs by interaction with a short tyrosine-containing oligopeptide at neutral pH and shows a new way of reducing a metalloprotein at neutral pH with a peptide.

Changes in the absorption spectra of oxidized cyt *c* by interaction with excess TyrTyrPhe were observed at pH 7.0 and 8.0 under air (Figure 1). By addition of TyrTyrPhe, the Soret band at 409 nm of oxidized cyt *c* shifted gradually to 412 nm for both pH conditions. New absorption bands also appeared at 520 and 550 nm, typical for reduced cyt *c*, by interaction with the peptides. These absorption changes clearly showed that oxidized cyt *c* was reduced with TyrTyrPhe, where the changes occurred faster at pH 8.0 than 7.0. Reduction of cyt *c*, however, was not observed by addition of tyrosinol, although it was reduced a little by tyrosyltyrosine.

The absorption change by interaction with TyrTyrPhe was monitored at 418 nm, since the absorption change was most prominent at this wavelength. The initial rate constants (k_{int}) were 6.8×10^2 and 2.8×10^3 s^{-1} at pH 7.0 and 8.0, respectively, for the reaction with 1.0 mM TyrTyrPhe in 10 mM phosphate buffer at 15°C. The reduction rate decreased with time for both pH (Figure 1,

inset), and the absorption changes were not possible to fit with a single exponential function. The reaction rate constant decreased gradually from 1.6 x 10^{-4} to 8.6 x 10^{-6} s^{-1} after 4 h of reaction, whereas it increased to 1.5 x 10^{-4} s^{-1} by addition of TyrTyrPhe (added peptide concentration, 0.5 mM). These results showed that the concentration of the non-reacted TyrTyrPhe decreased rapidly during the reaction with cyt *c*. Since the reaction rate decreased gradually, we measured k_{int} with different conditions for the reaction of oxidized cyt *c* with TyrTyrPhe. The reciprocal initial rate constant ($1/k_{int}$) increased linearly with a slope of 4.4 M s as the reciprocal peptide concentration increased. The effect of ionic strength on k_{int} was measured to investigate the electrostatic effect on the reaction between cyt *c* and the deprotonated peptide, $(TyrTyrPhe)^-$. The value of log kint decreased linearly against the root of the ionic strength with a slope of -1.9 M^2 log s^{-1}, which indicated that a cyt *c*-$(TyrTyrPhe)^-$ complex was formed by electrostatic interaction during the reaction.

References
1. a) Hirota, S.; Endo, M.; Hayamizu, K.; Tsukazaki, T.; Takabe, T.; Kohzuma, T.; Yamauchi, O. J. Am. Chem. Soc., 121, 849-855 (1999); b) Hirota, S.; Yamauchi, O. (review) Eur. J. Inorg. Chem., 17-25 (2002).
2. Hirota, S.; Suzuki, M.; Watanabe, Y. Biochem. Biophys. Res. Commun., 314, 452-458 (2004).

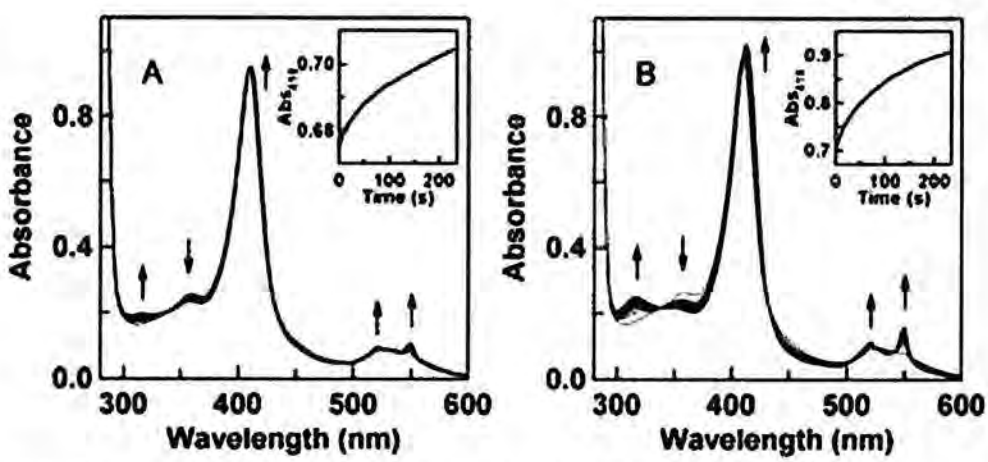


Figure 1. Absorption spectral changes of oxidized cyt *c* by reduction with TyrTyrPhe. Absorption spectra were taken each 30-min for 4 h. Inset: Time course of the absorption change at 418 nm.

チロシルチロシルフェニルアラニンによるシトクロム*c*の還元

○廣田 俊[1]、奥村 久野[2]、舟崎 紀昭[1]、渡辺 芳人[2]
[1]京都薬科大学 薬品物理化学教室、[2]名古屋大学 大学院理学研究科 物質理学専攻 化学系

Lecture 26 Relationship between the formation of singlet oxygen and substrate oxygenation in ethanol-induced rat liver microsomes

○Seiko HAYASHI , Hiroyuki YASUI , Hiromu SAKURAI

The Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan

Introduction

Cytochrome P450 is a group of enzymes that are responsible for the biotransformation of numerous endogenous and exogenous compounds. A mechanism for the P450-dependent catalytic cycle has been accepted, in which an oxo-ferrylporphyrin-π-cation radical is involved as an active oxygen intermediate. However, the precise process of dioxygen activation involved in P450-dependent substrate oxygenations has not been established. Previously, we reported that singlet oxygen ($^1O_2$) played an important role in microsomal P450-dependent oxygenations using type I substrates such as 7-ethoxycoumarin, lauric acid, *p*-nitroanisol and aminopyrin[1, 2]. In order to confirm the $^1O_2$ involvement, we investigated the relationship between the formation of reactive oxygen species (ROS) and a substrate oxygenation in the ethanol-induced rat liver microsomal P450 using a different type of substrate.

Experimental

Microsome preparation

Microsomes were prepared from the livers of male Wistar rats. CYP2E1 was induced in the livers of rats by pretreatment with oral ingestion of 15 % (v/v) ethanol for 3 weeks. The fraction of microsomes was obtained by differential ultracentrifugation. The total protein, total P450 and CYP2E1 contents were determined by the methods of Lowry *et al.*[3], Omura and Sato in terms of the CO-reduced P450 complex[4], and ELISA.

p-Nitrophenol hydroxylation

p-Nitrophenol hydroxylation activity with respect to 4-nitrocatechol formation was determined by the method of Reinke *et al.*[5].

ESR spin-trapping

ESR spectra were recorded at room temperature. TMPD was used as a $^1O_2$-trapping agent and DMPO was used to trap $\cdot O_2^-$ and $\cdot OH$.

Substrate-induced difference spectra

UV-VIS spectra at 300 to 600 nm were recorded in the rat liver microsomes with *p*-nitrophenol.

Results

p-Nitrophenol hydroxylation and effect of ROS scavengers, a P450 inhibitor and a ferric chelator

The hydroxylation of *p*-nitrophenol mediated by CYP2E1 in the rat liver microsomes was examined to specify the ROS involvement. Addition of SOD, catalase and mannitol exhibited essentially no effects. However, the hydroxylation rate of *p*-nitrophenol was suppressed by the addition of SKF525A and deferoxamine, which is a P450 inhibitor and a ferric chelator, respectively. When $^1O_2$ quenchers, β-carotene and NaN_3, were added to the microsome systems, *p*-nitrophenol hydroxylation was suppressed in concentration-dependent manners. Similarly, ethanol and DMSO, substrates for CYP2E1, restrained the hydroxylation rate in concentration-dependent manners (Fig. 1).

ESR spin-trapping

Relationship between the hydroxylation rate of *p*-nitrophenol and its $^1O_2$ quenching activity was examined in rat liver microsomes using the ESR spin-trapping method. In addition, to analyze the origin of $^1O_2$ in P450-dependent oxygenations, the action of SKF525A was examined. When *p*-nitrophenol, β-carotene and SKF525A were added to microsomes with NADPH, the signal

intensity due to 4-oxo-TEMPO was suppressed. ESR signal intensity due to 4-oxo-TEMPO decreased by additions of β-carotene and *p*-nitrophenol in their concentration-dependent manners. Both compounds also reacted with $^1O_2$ generated chemically in the HP-UVA system. However, SKF525A had no effect. Generations of $\cdot O_2^-$, and H_2O_2 and were ruled out by the experiment using DMPO as a spin trap. These results indicate that $\cdot O_2^-$, H_2O_2 and ·OH are not likely to contribute to the CYP2E1-dependent aromatic hydroxylation.

Substrate-induced difference spectra

Spectral change was characterized by the appearance of a trough at 365 nm and an absorption peak at 420 nm. This spectrum for *p*-nitrophenol with P450 indicated that the substrate coordinated to the heme iron directly, suggesting the substrate is a kind of type II substrate.

Conclusion

The suppression of *p*-nitrophenol hydroxylation by $^1O_2$ quenchers, and the detection of $^1O_2$ generation during the substrate oxygenation strongly indicated that $^1O_2$ plays an essential role in the hepatic microsomal substrate oxygenation system. Involvement of $^1O_2$ during the P450-dependent oxygenations was thus demonstrated in not only type I[1, 2] substrates but also a type II substrate like *p*-nitrophenol.

References

1. Osada, M. *et al. Biochem. Biophys. Res. Commun.*, **263**: 392 (1999). 2. Yasui, H. *et al. Drug Metabol. Pharmacokin.*, **17**: 416 (2002). 3. Lowry, O. H. *et al. J. Biol. Chem.*, **243**: 265 (1951). 4. Omura, T. *et al. J. Biol. Chem.*, **239**: 2370 (1964). 5. Reinke, L. A. *et al. Drug Metab. Dispos.*, **13**: 548 (1985).

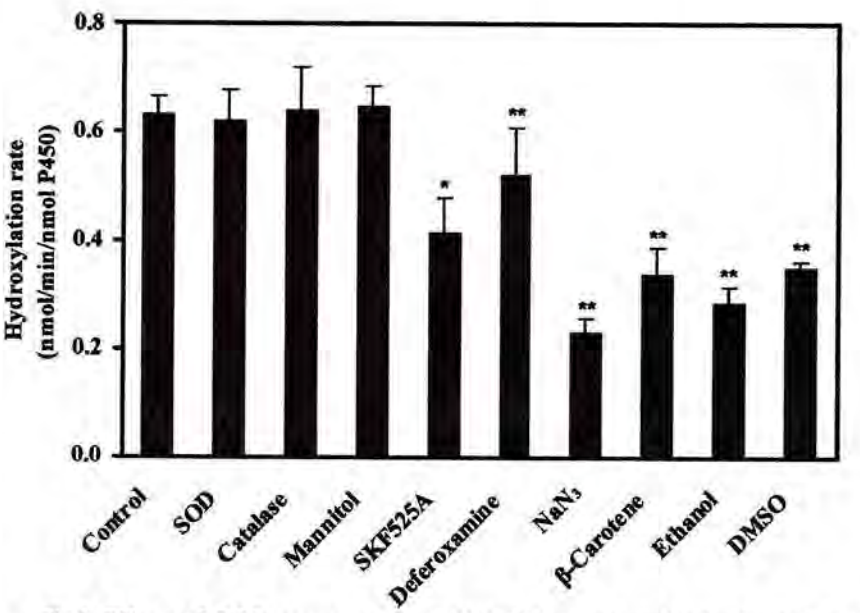


Fig.1 Effects of ROS scavengers, a P450 inhibitor and a ferric chelator on *p*-nitrophenol hydroxylation rate in the liver microsomes of rats treated with ethanol
Concentrations: SOD; 5,000 units/mL, catalase; 5,000 units/mL, mannitol; 0.5 mM, SKF525A; 0.05 mM, deferoxamine; 20 mM, NaN_3; 2 mM, β-carotene; 1 mM, ethanol; 4.3 mM, DMSO; 3.5 mM
Significance: *p < 0.05, **p < 0.01 *vs.* control

エタノール誘導ラット肝ミクロソームによる一重項酸素生成と基質酸化反応との相関

○林 聖子、安井 裕之、桜井 弘
京都薬科大学 代謝分析学教室

Lecture 27 Transesterification and cycle reaction using polynuclear zinc-peptide dendrimer

○Kazuhiko ICHIKAWA , TARUNAI MATE

Graduate School Of Environmental Earht Science, Hokkaidou University, Sapporo, Japan

Zinc complexes of histidine-contaning artificial peptides derived from (benzylhistidyl)-dipropylenetriamine and tetrakis(benzylhistidyl-3-aminopropyl))1,4-diaminobutane , L2Zn and D4Zn, effitiently promoted the transesterification of 2-hydroxypropyl p-nitrophenyl phosphate (HPNP) as RNA-type phosphodiester. Determination of turnover for the transesterification of HPNP by D4Zn has successfully been carried out .

デンドリマー型多核亜鉛ペプチドによるトランスエステル化反応及びそのサイクル反応

○市川 和彦、TARUNAI MATE

北海道大学 大学院地球環境科学研究科

Lecture 28 Optical Properties of Zinc Complex of Chlorophyll-a and Its Application for Photoinduced Hydrogen Production

○Yumi TAKEUCHI , Yutaka AMAO

Department of Applied Chemistry, Oita University, Oita, Japan

Chlorophyll-a acts as the effective photosensitizer in photosynthesis of green plant and exhibits physiological functions of the photolysis of water, the reduction of NADP+ and carbon dioxide fixation under visible light irradiation. Mg chlorophyll-a has absorption maximum in 432 and 670 nm and is attractive compound as a visible photosensitizer for the means of converting solar energy to chemical energy. However, Mg chlorophyll-a is unstable against the irradiation. On the other hand, Zn bacteriochlorophyll-a was founded in an aerobic bacterium Acidiphilium rubrum. Some studies on the preparation and characterization of the Zn chlorophylls and Zn bacteriochlorophylls have been reported. Water-soluble Zn porphyrins have been widely used as effective photosensitizers, for these porphyrins have absorption band in the visible light region (380 - 600 nm). As the Zn porphyrins are stable against the irradiation and effective photosensitizers, Zn chlorophyll-a are attractive compound as a stable visible photosensitizer. In this work we describe the preparation of Zn chlorophyll-a from Mg chlorophyll-a from spirulina, the optical properties of the photoexcited state of Zn chlorophyll-a and the effect of pH on the photostability of Zn chlorophyll-a in the surfactant micellar media. In comparison of the UV-vis absorption and fluorescence emission spectra of Zn chlorophyll-a and Mg chlorophyll-a, the blue-shift in the absorption bands and emission peak of Zn chlorophyll-a was observed. Fluorescence lifetime of Zn chlorophyll-a was 5.2 ns. The fluorescence lifetime of Zn chlorophyll-a is shorter than that of Mg chlorophyll-a (5.9 ns). The photositability of Zn chlorophyll-a was superior to that of Mg chlorophyll-a in various pH conditions. Photoinduced hydrogen production system coupling the sucrose degradation with invertase and glucose dehydrogenase (GDH) and hydrogen production with colloidal platinum as a catalyst using the visible light-induced photosensitization of artificial Zn chlorophyll-a has been developed. The continuous hydrogen gas production more than 240 min was observed when the reaction mixture containing sucrose, invertase, GDH, nicotinamide adenine dinucreotide (NAD+), Zn chlorophyll-a, methylviologen (MV2+, an electron relay reagent) and colloidal platinum was irradiated by visible light.

亜鉛クロロフィル錯体の光学的特性と光水素生産反応への応用

○竹内 由美、天尾 豊

大分大学 工学部 応用化学科

Lecture 29 Structural Basis of Human Cytoglobin for Ligand Binding

○Masatomo MAKINO[1,2] , Hiroshi SUGIMOTO[1] , Hitomi SAWAI[1,2] , Norifumi KAWADA[3] , Katsutoshi YOSHIZATO[4] , Yoshitsugu SHIRO[1]

[1]Biometal Science Laboratory, Riken Harima Institute, Hyogo, Japan , [2]Department of Life Science, Graduate School of Science, Himeji Institute of Technology, Hyogo, Japan , [3]Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka, japan , [4]Department of Biological Science, Graduate School of Science, Hiroshima University, Hiroshima, Japan

Cytoglobin (Cgb), a recently discovered member of vertebrate globin family, binds O_2 reversibly via the Fe^{2+} ion of the heme group. Cgb was found to be expressed in a broad range of mammalian tissues. Sequence comparison show about 25% identity of human Cgb with vertebrate myoglobin(Mb) and presence of unusual long amino- and carboxyl- terminal extension of about 20 residues each. Some key residues close to active site which related to ligand binding has been highly conserved among different species of Mb, hemoglobin(Hb), neuroglobin(Ngb). Spectroscopic studies show that Cgb displays a hexacoordinated hemochrome structure of the Fe^{2+} in the deoxy form and Fe^{3+} in the ferric form. Cgb binds exogenous ligand such as O_2 or CO reversibly. Thus it is thought that Cgb has a function related to oxygen binding, but its physiological role has not yet been clearly understood.

We have determined the crystal structure of recombinant human Cgb in a ferric state at 2.2 Å resolution. Cgb forms traditional globin fold consisting of 8-helices, corresponding to A-H helices of typical globins. Overall backbone structure of Cgb is basically similar to these of Mb. Cgb is a homodimer with crystallographic 2-fold axis, through two intermolecular disulfide bonds. Its subunit interface resembles that of Lamprey Hb V. The active site of ferric Cgb is significantly different from those of traditional Hb and Mb. His(E7) of Cgb directly coordinates to the sixth position, where water or O_2 binds in Hb or Mb. The oxygen affinity of Cgb is higher than Mb although His(E7) occupies sixth position. It seems that dissociation of His(E7) occurs by slight outward movement of the imidazole sidechain with concomitant bend or tilt of the E-helix when exogenous ligand binds heme iron. On the basis of the crystal structure of Ngb, which is the hexacoordinated globin as Cgb, it has been proposed that conformational flexibility of the CD regions in globins capable of heme endogenous hexacoordination may have functional roles in relation to displacement of the His(E7) upon external ligand binding. However, corresponding region of Cgb is well-ordered in the electron density and suggests that such structural flexibility of CD region might not be responsible for the ligand binding. Therefore our result indicates that ligand binding property of Cgb is controlled by different mechanism from that of Ngb

]

ヒト由来サイトグロビンのリガンド結合機構

○牧野 正知[1,2]、杉本 宏[1]、澤井 仁美[1,2]、河田 則文[3]、吉里 勝利[4]、城 宜嗣[1]

[1]理化学研究所 播磨研究所 城生体金属科学研究室、[2]姫路工業大学大学院 理学研究科 、[3]大阪市立大学大学院 医学研究科 、[4]広島大学大学院 理学研究科

The 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04

Thursday, June 10 – Friday, June 11, 2004

Abstracts

Posters

Poster 01 Evaluation of Zn-DTS complexes as oral antidiabetic agents

○Jun ABE[1] , Yasuhiko IIDA[2] , Yasushi KIYONO[3] , Youji KITAMURA[4] , Hideo SAJI[5]

[1]Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan , [2]Faculty of Medicine, Gunma University, Maebashi, Japan , [3]Kyoto University Hospital, Kyoto, Japan , [4]Faculty of Pharmaceutical Sciences, Kobegakuin University, Kobe, Japan , [5]Graduate School of Pharmaceutical Science, Kyoto University, Kyoto, Japan

[Introduction]

Healthy eating, physical activity, and medication are the basic therapies for diabetes mellitus. In the medication, insulin therapy and oral antidiabetic drugs are clinically used. But insulin therapy has a problem such as physical and mental pain because of the daily injection. On the other hand, oral antidiabetic drugs also have a deleterious side effect or difficulty keeping blood glucose levels. Thus, the development of oral antidiabetic drugs that can overcome these problems has been desired.

Recently, it has been reported that oral administration of $ZnCl_2$ to STZ-induced type I diabetic rats reduces the blood glucose[1)] and some zinc(II) complexes improve the insulin resistance in KK-A^y type II diabetic mice[2)]. So, the effect of zinc(II) ion on diabetes has been of great interest. Since the normoglycemic activities of zinc(II) complexes seems to depend on their high tissue uptake and their stability in cells, more effective drugs will be able to develop by optimizing their property. However, the systematic studies on zinc(II) complexes have not been performed.

Recently, our preliminary studies showed that some Zn-dithiosemicarbazone(DTS) complexes have high membrane permeability and tissue uptake. Therefore, in this study, to clarify the relationship between the partition coefficient (PC), the zinc(II)-ligand dissociation constant (Kd), and *in vivo* antidiabetic effect, PC and Kd, and the *in vivo* antidiabetic effect of Zn-DTS complexes were investigated.

[Experiments]

Preparation of dithiosemicarbazone(DTS) derivatives

Dithiosemicarbazone(DTS) derivatives was prepared according to the method of Gingras et al[3)]. Briefly, 25 mmol thiosemicarbazide derivative dissolved in 5% acetic acid (30 ml) was added to 50% EtOH solution (12 ml) containing 11 mmol α-keto-aldehyde derivative over 1 hr at 50 ~ 60 °C. Then, about 5 ml of EtOH was added, and reaction mixture was stirred overnight at room temperature. The product was recrystallized in EtOH.

Determination of the Zn(II)-dissociation constants (Kd)

To determine the Zn(II)-dissociation constants, spectrophotometric titrations were performed by adding $ZnCl_2$ up to 0.1 mM to the DTS derivative (10-20 μM) in NaCl-Hepes buffer (0.1 M NaCl, 30 mM Hepes, pH 7.5). Absorbance was monitored at 420 nm. The Zn(II)-dissociation constants (Kd) and molar extinction coefficient (e) were computed by fitting the experimental values of A and [Zn]t to the equation

$A^2-(Kd+[L]t+[Zn]t)*e*A+e^2*[L]t*[Zn]t=0$

where A was the measured absorbance, while [L]t and [Zn]t were respectively the total concentration of ligand and zinc(II).

Measurement of n-octanol / water partition coefficient

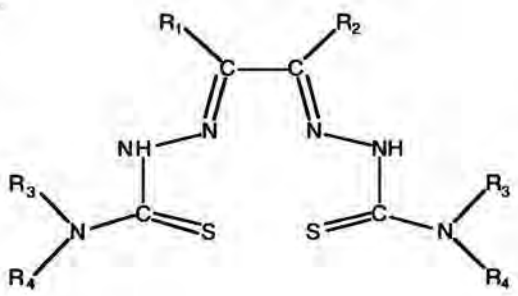
For the determination of the partition coefficient (PC), equal volume of n-octanol and 50 mM Tris buffer at pH 7.0 was vortexed and equilibrated for 24 h. Then 20 μl of ^{65}Zn-DTS complexes were added and vortexed, left standing. After centrifuged at 1,500 x g for 5 min, organic and aqueous

layers were collected and the radioactivity of both layers was measured with a well counter. Partition coefficient was evaluated by the equation.
log PC = $\log_{10}$ ([radioactivity of n-octanol layer]/[radioactivity of Tris buffer layer])
Evaluation of anti-diabetic effects of Zn-DTS complexes in KK-A^y type II diabetic mice
KK-A^y type II diabetic mice were orally administered Zn-DTS complexes and their blood glucose levels were measured for 2 weeks.

[Results and discussion]
UV-visible absorbance spectrum of Zn-DTS complexes were measured, and λ_{max} was observed approximately at 400-450 nm for all of them. Therefore, the Zn-dissociation constants were measured by the measurement of absorbance at 420 nm. The measured dissociation constants (-log Kd) of $ATSM_2$, $KTSM_2$, $GTSM_2$, $PTSM_2$ were higher than 10, which showed relative stable. The others have lower values (-log Kd = 3.7-6.5). When n-octanol / water partition coefficients of Zn-DTS complexes were measured, all of them showed higher PC values than Zn-EDTA and $ZnSO_4$.
After these interesting *in vitro* results called for the *in vivo* antidiabetic effect of Zn-DTS complexes. When KK-A^y type II diabetic mice were daily administered Zn-DTS complexes orally, Zn-ATSM and Zn-$KTSM_2$ decreased blood glucose level and improved hyperglycemia. Zn-$ATSM_2$, Zn-$GTSM_2$, Zn-$PTSM_2$, which have similar stability and lipophilicity with Zn-$KTSM_2$, did not show antidiabetic effect, while Zn-ATSM with lower affinity and lipophilicity compared with them, showed it. These results reveal that other factors except the affinity and lipophilicity are responsible for the antidiabetic effect of zinc complexes. Furthermore, oral glucose tolerance tests showed that Zn-ATSM and Zn-$KTSM_2$ improved glucose tolerance. These results suggested the investigation on the common characters of Zn-ATSM and Zn-$KTSM_2$ will be necessary to develop more effective oral antidiabetic drugs.

1) Shisheva A *et al*, Diabetes 1992, 41: 982-988.
2) Yoshikawa Y *et al*, Biochem. Biophys. Res. Commun. 2001, 281: 1190-1193.
3) Gingras BA *et al*, Can. J. Chem. 1962, 40: 1053-1059.

	R_1	R_2	R_3	R_4
ATSM	CH_3	CH_3	H	CH_3
$ATSM_2$	CH_3	CH_3	CH_3	CH_3
KTSM	H	$CH(CH_3)OC_2H_5$	H	CH_3
$KTSM_2$	H	$CH(CH_3)OC_2H_5$	CH_3	CH_3
KTSM-OAc	H	$CH(CH_3)OCOCH_3$	H	CH_3
GTSM	H	H	H	CH_3
$GTSM_2$	H	H	CH_3	CH_3
KTS	H	$CH(CH_3)OC_2H_5$	H	H
KTS-OAc	H	$CH(CH_3)OCOCH_3$	H	H
PTSM	H	CH_3	H	CH_3
$PTSM_2$	H	CH_3	CH_3	CH_3



Structure of Dithiosemicarbazone Derivatives

Zn-DTS 錯体の経口糖尿病治療薬としての可能性に関する基礎的検討

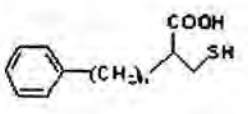
○安部 潤[1]、飯田 靖彦[2]、清野 泰[3]、北村 陽二[4]、佐治 英郎[5]
[1]京都大学大学院 人間環境学研究科、[2]群馬大学 医学部、[3]京都大学医学部附属病院、[4]神戸学院大学 薬学部、[5]京都大学大学院 薬学研究科

Poster 02 Development of inhibitors of metallo-β-lactamase (IMP-1 and VIM-2)

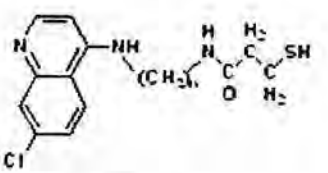
○JIN WANCHUN[1] , Yoshichika ARAKAWA[2] , Yosihiro YAMAGUCHI[1] , Hiromasa KUROSAKI[1] , Yuriko YAMAGATA[3] , Michio OHTA[4] , Masafumi GOTO[1]

[1]Department of Structure-Function Physical Chemistry, Graduate School of Pharmaceutical Sciences, Kumamoto University , [2]Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases , [3]Department of Structural Biology, Graduate School of Pharmaceutical Sciences, Kumamoto University , [4]Department of Molecular Bacteriology, Nagoya University Graduate School of Medicine

A wide variety of β-lactam antibiotics are used in the treatment of infectious diseases. However, bacteria have developed several strategies for resisting β-lactam antibiotics, including the production of a family of enzymes, β-lactamases. Metallo-β-lactamase (MBLs) containing one or two Zn(II) ions at the active site are able to hydrolyze most of the β-lactam antibiotics including carbapenems, and no clinically available inhibitors for them exist at present. For the purpose of development of metallo-β-lactamase inhibitors, two series of compounds 2-ω-phenylalkyl-3-mercaptopropionic acid (PhenylCnSH (n=1-4)) and *N*-[(7-chloro-quinolin-4-ylamino)-alkyl]-3-mercapto-propionamide (QuinolineCnSH (n=2-6)) were synthesized. PhenylCnSH (n=1-4) was found to be a potent inhibitor of both IMP-1 and VIM-2. PhenylC4SH was the strongest inhibitor of both IMP-1 (IC_{50}=1.2μM) and VIM-2 (IC_{50}=1.1μM). When the number of methylene units was varied, QuinolineC4SH showed the maximum inhibitory activity against both IMP-1 and VIM-2 (IC_{50}=2.5μM and IC_{50}=2.4μM). The relationship between the inhibitory effecting of n was different for both series of inhibitors, suggesting that IMP-1 has a tighter binding site than VIM-2.In order to investigate the binding mode, we prepared an X-ray diffractable crystal of VIM-2 complexed with PhenylC3SH by hanging-drop method and determined the molecular structure. The thiol group in PhenylC3SH bridged the two Zn(II) ions, and the phenyl group interacted face-to-face with Try67 at the end of loop1(62-67) of VIM-2.



PhenylCnSH



QuinolineCnSH

メタロ-β-ラクタマーゼ(IMP-1,VIM-2)に対する阻害剤の開発

○金 万春[1]、荒川 宜親[2]、山口 佳宏[1]、黒崎 博雅[1]、山縣 ゆり子[3]、太田 美智男[4]、後藤 正文[1]

[1]熊本大学大学院 薬学教育部 構造機能物理化学分野、[2]国立感染症研究所 細菌第二部、[3]熊本大学大学院 薬学教育部 構造分子機能解析学、[4]名古屋大学大学院 医学研究科 分子病原細菌学 耐性菌制御学分野

Poster 03 Biological effects of novel binuclear platinum (II) complexes in human ovarian tumor cell lines

○Noriko AOKI , Takaji SATO , Maiko KODA , Aiko SAKIYAMA , Rei KOMAKI , Seiji KOMEDA , Yoshihiro SAITO , Masahiko CHIKUMA

Osaka University of Pharmaceutical Sciences, Osaka, Japan

[INTRODUCTION]

Cisplatin has been one of the most widely used chemotherapeutic agents for cancer treatment. The major mechanism of cisplatin-induced damage to tumor cells is via induction of apoptosis. Ovarian tumor cells are sensitive to cisplatin, but they are likely to acquire resistance to cisplatin. Cisplatin-resistant tumors show cross-resistance to other platinum-based cancer anti agents used clinically. Therefore, the development of chemotherapeutic agents effective against cisplatin-resistant tumor has been required. In an attempt to search for more active platinum compounds and to overcome problems with acquired resistance, two novel binuclear platinum (II) complexes, [{cis-Pt(II)$(NH_3)_2$}$_2$(μ-OH)(μ-pz)]$(NO_3)_2$(1,2-μH-Ampz) and [{cis-Pt(II)$(NH_3)_2$}$_2$ (μ-OH)(μ-4-methyl-pz)]$(NO_3)_2$ (1,2-μH-Am-4Me-pz) were developed.T hese complexes were found active to cisplatin-resistant L1210 murine leukemia cell lines. We have investigated the action of the complexes on both TYK-nu human ovarian tumor cells and TYK-nu.CP-r cells with acquired resistance to cisplatin.

[MATERIALS AND METHODS]

< Cells > cisplatin-sensitive (TYK-nu) and resistant (TYK-nu.CP-r) human ovarian tumor cell lines were maintained in Eagle's minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum in an atmosphere of 5% CO_2 in air at 37°C.

< Cytotoxicity assays and determination of IC_{50}> IC_{50} were determined by analyzing dose-response plots of MTT assay. Cells (1×10^5cells/ml) were treated with platinum complexes for 72h.

< DNA fragmentation > Cells were treated with platinum complexes at the dose equivalent to IC_{50} for 24h, 48h and 72h. Adherent and floating cells were separately analyzed. Isolated genomic DNAs were analyzed by agarose gel electrophoresis.

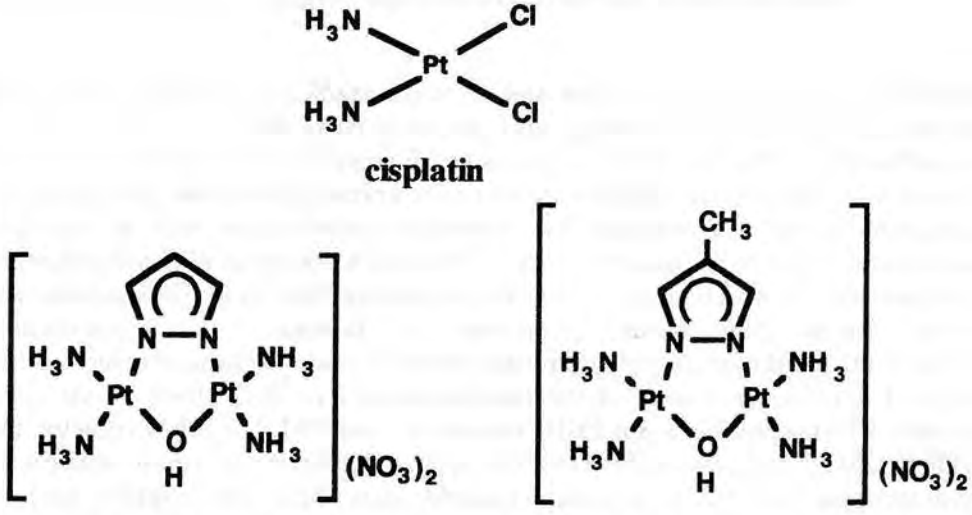
< Cell cycle analysis > Cells were treated with the respective IC_{50} doses of platinum complexes for 24h, 48h and 72h. Harvested cells were fixed in 70% ethanol, and then prepared for flow cytometric analysis by treatment with propidium iodide.

< Morphological changes in cells > Cell size and cell density are monitored by forward scatter and side scatter in flow cytometric analysis.

< Platinum accumulation > Intracellular Pt levels were measured by atomic absorption spectroscopy following 1h, 7h, 24h and 48h exposure to 2μM.

[RESULTS AND DISCUSSION]

The cell line TYK-nu.CP-r showed about 7-fold cisplatin resistance by IC_{50} when compared with the sensitive cell line TYK-nu. The cytotoxicities of 1,2-μH-Am-4Me-pz in the wild type and cisplatin-resistant cell lines were almost the same as that of cisplatin and higher than cisplatin respectively. In the sensitive cell line, DNA fragmentations were detected in floating and adherent cells in the presence of both platinum complexes after 72h-exposure. However, the extent of fragmentation was not different from each other. Sensitive cells displayed a considerable fraction of cells with sub-G_1 DNA following 48h-treatment with the two drugs. The degrees of G_2/M arrest by 1,2-μH-Ampz and 1,2-μH-Am-4Me-pz were smaller than that of cisplatin. Intracellular platinum level was increased in both 1,2-μH-Ampz and 1,2-μH-Am-4Me-pz -treated cells compared with cisplatin-treated cells. Intracellular platinum level was higher in the resistant cells following the exposure of 1,2-μH-Am-4Me-pz than in the sensitive cells. In conclusion,cellular pharmacology of the binuclear platinum complexes seems different from that of cisplatin.



Structures of cisplatin and platinum(II) complexes

シスプラチン関連白金(II)二核錯体のヒト卵巣癌細胞に対する作用

○青木 典子、佐藤 卓史、神田 麻衣子、崎山 愛子、駒木 麗、米田 誠治、齊藤 睦弘、千熊 正彦
大阪薬科大学

Poster 04 Interactions of Biomolecules with Platinum(II)-Polypyridine Complexes Containing an Amino Acid. Adduct Formation and Its Effect

○Minoru TASHIRO[1] , Tatsuo YAJIMA[1] , Takeshi MOTOYAMA[1] , Akira ODANI[2] , Yasuo NAKABAYASHI[1] , Osamu YAMAUCHI[1]

[1]Unit of Chemistry, Faculty of Engineering, Kansai University, Osaka, Japan , [2]Reserch Center for Materials Science, Nagoya University, Nagoya, Japan

Weak interactions such as hydrogen bonds and aromatic stacking interactions play important roles in substrate recognition, cofactor binding, and catalysis by proteins. In flavodoxin, for example, flavin mononucleotide (FMN) is bound in a pocket formed by the aromatic rings of tyrosyl and tryptophyl residues. The pterin cofactor for phenylalanine hydroxylase, tetrahydrobiopterin, has been revealed to be bound to a phenylalanine residue by stacking as well as hydrogen bonds with other groups around the iron at the active site. The redox potential of flavin cofactors is known to vary with the proteins in which they are bound, indicating that weak interactions with the amino acid residues change the redox properties of flavins. Our previous studies on platinum(II)-aromatic N-donor ligand complexes showed that stacking interactions with aromatic molecules affect the electron density of the central metal ion. With these points in mind, we now studied the adduct formation between Pt(II) complexes and FMN or other cofactor analogs and its electronic effects. Pt(II) complexes [Pt(AA)(PP)], where AA refers to amino acids such as alanine, arginine, and tyrosine and PP to aromatic ligands phen, bpy, and tmphen, and the complexes involving ethylenediamine in place of PP were prepared. Association between the Pt(II) complexes and FMN or a pterin cofactor analog 6,7-dimethyl-5,6,7,8-tetrahydropterin (H_4DMP) were detected by uv-visible absorption and NMR spectra. The [Pt(AA)(PP)]-FMN systems exhibited upfield shifts of the ^{1}H NMR signals for FMN and PP, and the systems with H_4DMP also showed small but similar shifts of the PP moiety, although no shifts were observed for the reduced pyrazine ring of H4DMP. The results show that the Pt(II) complexes stack with FMN and partly with H_4DMP. The redox potentials for FMN and H_4DMP in the [Pt(AA)(PP)]-FMN (or H_4DMP) systems measured by cyclic voltammetry were found to be shifted to higher potentials as compared with the values obtained in the absence of the Pt(II) complexes, indicating that the reduced form of the cofactors are stabilized by stacking the the Pt(II) complexes. The present results show that the redox activity of the cofactors are changed by the interaction with the molecule in the adduct, further suggesting that the cofactor-dependent reactions may be controlled by adjusting the molecular environment.

生体関連分子とアミノ酸含有白金(II)-ポリピリジン錯体との会合とその効果

○田代 稔[1]、矢島 辰雄[1]、元山 健[1]、小谷 明[2]、中林 安雄[1]、山内 脩[1]

[1]関西大学 工学部 教養化学教室、[2]名古屋大学 物質国際研究センター

Poster 05 DNA binding selectivity of artificial 6-zinc finger proteins

○Miki IMANISHI , YAN WEI , Yukio SUGIURA

Institute for Chemical Research, Kyoto University, Kyoto, Japan

Cys2His2 type zinc finger motif has attractive characteristics to create novel DNA binding proteins from following points, (1) recognition of 3-4 base pairs per motif, (2) mediation of base recognitions by specific amino acids, (3) a DNA binding module composed of tandemly repeated zinc finger motifs, and (4) binding to nonpalindromic sequence. Artificial zinc finger proteins with desired DNA recognition specificities or elongated binding sequences were created through selection strategies or by connecting zinc finger domains with designed linkers. In this study, we tried to create artificial proteins that have DNA binding selectivity between continuous and discontinuous target sequences.

Transcription factor Sp1 has three zinc finger motifs (Sp1ZF3) as the DNA binding domain and binds to GC rich 9 bp called GC-box. Sp1ZF6, in which two DNA binding domains of Sp1 are connected with a canonical linker, binds to continuous two GC-boxes sequence (2GC(0)). Here, we created Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8 by connecting two Sp1ZF3s with continuous 10 glycine residues ((Gly)10) and with 10 residues composed of Gly-(Arg)8-Gly, respectively. As target DNA sequences, we prepared two substrates, 2GC(0) and 2GC(10), containing continuous two GC-boxes and separated two GC-boxes by 10 bp, respectively. The DNA binding properties of Sp1ZF6(Gly)10 and Sp1ZF6(Arg)8 to 2GC(0) and 2GC(10) were investigated using gel shift assays and DNase I footprinting assays. Sp1ZF6(Gly)10 showed almost comparable DNA binding affinity between 2GC(0) and 2GC(10) and it bound to both two GC boxes in the both substrates. On the other hand, Sp1ZF6(Arg)8 bound to 2GC(10) with a higher affinity than to 2GC(0). DNase I footprinting assays demonstrated that Sp1ZF6(Arg)8 bound to the both GC boxes in the substrate 2GC(10) but to only one GC box in the substrate 2GC(0), and that it took much longer time for Sp1ZF6(Arg)8 to bind to both GC box sequences in 2GC(0). The results indicate that the poly-arginine linker causes selective binding of a 6-finger protein to 2GC(10) rather than 2GC(0), though the 6-finger protein with the flexible poly-glycine linker shows little preferences for the spacings between two GC-boxes. It is suggested that the components and the structure of a linker play an important role to determine the DNA binding selectivity of artificial 6-zinc finger proteins.

人工6-亜鉛フィンガータンパク質のDNA結合配列選択性に関する検討

○今西 未来、圀 薇、杉浦 幸雄

京都大学 化学研究所

Poster 06 Interactions of glucose oxidase with dipolar ruthenium ammine complexes as electron transfer mediators of glucose oxidation

○Yasuo NAKABAYASHI , Yuri HIROSAKI , Masahiro KINOSHITA , Osamu YAMAUCHI

Unit of Chemistry, Faculty of Engineering, Kansai University, Osaka, Japan

The enzyme glucose oxidase (GOx, EC 1.1.3.4) specifically converts glucose into glucono-δ-lactone, and this oxidation reaction is accompanied by reduction of the cofactor flavin adenine dinucleotide (FAD). The reason that reduced FAD ($FADH_2$) at the active site is not oxidized electrochemically is that the redox centers of the homodimer are located in the pocket at *ca.*1 nm from the surface of GOx from *Aspergillus niger*. Numerous artificial electron acceptors are therefore used as electron transfer mediators for coupling the glucose/GOx system to an electrode surface. Ruthenium complexes have the advantage of lower cost and higher reactivity toward GOx as compared with related osmium complexes. In this study, we describe the results of studies on the interactions between GOx and dipolar ruthenium(II) ammine complexes $[Ru(NH_3)(L^+)]^{3+}$ (L^+: pyridinium-substituted ligand), which are chemically stable in both oxidized and reduced forms. The factors to be satisfied by electron transfer mediators for amperometric glucose sensors are discussed, and evaluation of the above ruthenium(II) complexes as electron transfer mediators is made on the basis of the redox potentials of ruthenium(III/II) couples and the second-order rate constants for electron transfer between reduced GOx and the ruthenium(III) complexes. The ruthenium(II) complexes synthesized in this study were electrochemically characterized by cyclic voltammetry to give reversible redox waves with the redox potentials of ruthenium(III/II) couples in the range 0.10 - 0.18 V vs. Ag/AgCl, which indicates that each complex has a higher potential than FAD/$FADH_2$ couple at pH 7.0 and 25 °C (-0.41 V vs. Ag/AgCl). As all the ruthenium(II) complexes possess the redox potentials around 0.0 - 0.2 V, they can thermodynamically serve as superior electron transfer mediators. For evaluation of the sensitivity of the glucose sensors the second-order rate constant k_s for the electron transfer between reduced GOx and a ruthenium(III) complex was determined. The k_s values of $5.7 - 17 \times 10^6$ M^{-1} s^{-1} for the ruthenium complexes are greater than those of osmium complexes possessing similar redox potentials. These facts suggest that the electrostatic ruthenium(III/II) complex-GOx interaction and/or the "antenna effect&quot of a pyridinium-substituted ligand may lead to greater k_s values.

グルコース酸化におけるメディエータとしてのピリジニウム置換配位子を含有するルテニウム-アンミン錯体とグルコースオキシダーゼとの相互作用

○中林 安雄、広崎 結里、木下 晶広、山内 脩

関西大学 工学部 教養化学教室

Poster 07 Hetero metal-assemblage in artificial DNA

○Yusuke TAKEZAWA[1] , Kentaro TANAKA[1,2] , Tatsuhisa KATO[3] , Mitsuhiko SHIONOYA[1]

[1]Department of Chemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan , [2]PRESTO, Japan Science and Technology Corporation (JST), Tokyo, Japan , [3]Institute for Molecular Science, Okazaki, Japan

DNA hybridizes in a sequence-specific manner through hydrogen bonded base pairing. We have recently reported a discrete metal array in artificial DNA that forms stable duplexes by metal-coordinated base pairing. We report herein hetero metal-assemblage in artificial DNA containing two hydroxypyridone-bearing nucleotides (**H**), which form square-planar complexes with Cu^{2+} ions, and a pyridine-bearing one (**P**), which forms a linear complex with Ag^{+} or Hg^{2+} ion.

To align Cu^{2+} and Hg^{2+} ions inside the DNA duplex (Fig. 1a), a pentanucleotide d(5'-GHPHC-3') (**1**) was synthesized. In the photometric titration of Cu^{2+} ions with pentanucleotide **1** (Fig. 1b), with an increase in the Cu^{2+} concentration, the absorbance at 277 nm decreased while a new peak at 307 nm appeared with two isosbestic points in the range of [Cu^{2+}]/[duplex] from 0 to 2.0. This indicates that, with deprotonation of hydroxy groups of hydroxypyridone ligands upon Cu^{2+} complexation, duplex $\mathbf{1}_2Cu^{2+}{}_2$ (**2**) was quantitatively formed through Cu^{2+}-mediated base pairing (H-Cu^{2+}-H).

The CD spectral changes of **2** in the presence of Hg^{2+} ions are shown in Fig. 1c. A positive Cotton effect around 300 nm was gradually decreased upon addition of Hg^{2+} ions. The spectra changed linearly in the range of [Hg^{2+}]/[duplex] from 0 to 1.0. These results suggest that two Cu^{2+} and one Hg^{2+} ions were aligned quantitatively in each artificial DNA strand exactly as expected from the ligand sequence of pentanucleotide **1**.

The EPR study for the spin-spin interactions between Cu^{2+} centers is now underway.

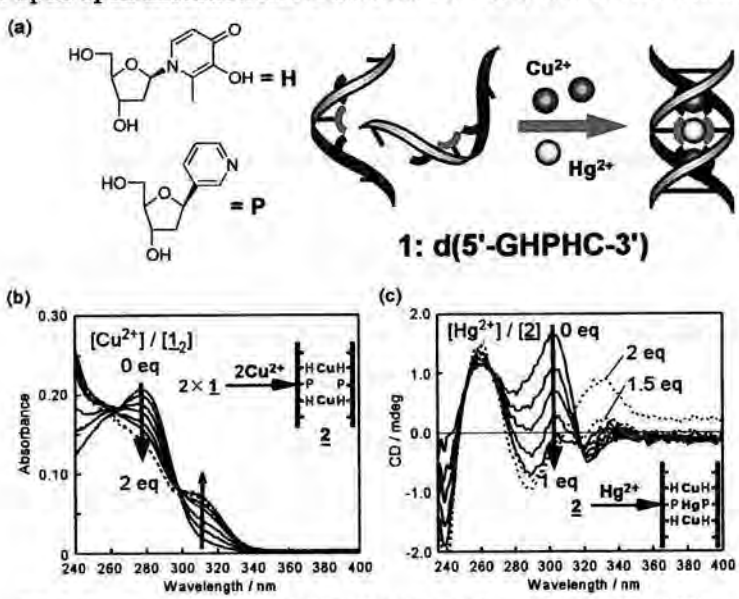


Fig. 1. (a) Schematic representation of hetero metal-assembly in artificial DNA. (b) UV absorption changes and (c) CD spectral changes of d(5'-**GHPHC**-3') at various concentrations of Cu^{2+} and Hg^{2+} ions at 25 °C. [Duplex] = 2.14 μM, 2 mM HEPES (pH 7.0), 5 mM $NaNO_3$.

人工DNAを用いた金属イオンのヘテロ集積化

○竹沢 悠典[1]、田中 健太郎[1,2]、加藤 立久[3]、塩谷 光彦[1]

[1]東京大学 大学院理学系研究科 化学専攻、[2]JST さきがけ、[3]分子科学研究所

Poster 08 Syntheses of cyclometalated ruthenium(II) complexes and application for electron transfer mediators

○Masahiro KINOSHITA , Yasuo NAKABAYASHI , Osamu YAMAUCHI

Unit of Chemistry, Faculty of Engineering, Kansai University, Osaka, Japan

The self-exchange rate is known to be higher for complexes with a rigid ligand shell, which minimizes the size difference between the oxidized and reduced states of complexes. A cyclometalated fragment with a metal-carbon σ bond is a crucial element of an electron transfer mediator to achieve rigidity. In addition, the metal-carbon bond largely lowers the redox potentials of ruthenium(III/II) complexes. Thus, cyclometalated ruthenium complexes may lead to good electron transfer mediators for glucose oxidation. In this study, 2-phenylpyridine- or azobenzene-containing ruthenium(II) complexes $[RuL(bpy)_2]^+$, where L is 2-phenylpyridine (2-phpy) or azobenzene (azben) and bpy is 2,2'-bipyridine, were synthesized from $RuCl_3$ and characterized by spectroscopic (UV-vis, ^{1}H-NMR) and elecrochemical (CV) methods. The interactions between glucose oxidase (GOx) and these ruthenium(II) complexes were further investigated in order to evaluate them as electron transfer mediators for amperometric glucose sensors. The absorption spectral and electrochemical data for $[RuL(bpy)_2]^+$ in acetonitrile are summarized in Table 1. When both glucose and GOx were added together to a solution of a ruthenium(II) complex, the so-called catalytic current wave was observed for all the ruthenium(II) complexes, indicating that these complexes may serve as electron transfer mediators for amperometric glucose sensors. In addition, $[Ru(2\text{-}phpy)(terpy)(S)]^+$, where terpy is 2,2':6',2"-terpyridine and S is solvent, was synthesized, and the characterization of this ruthenium(II) complex is in progress.

Table 1. Absorption spectral and electrochemical data of ruthenium(II) complexes in acetonitrile

Complex	λ_{max} / nm (ε / $M^{-1}cm^{-1}$)		$E_{1/2}$ / V vs.Ag/Ag^+ (ΔE_p / mV)[1]
$[Ru(2\text{-}phpy)(bpy)_2]^+$	542 (6080)	482 (5300)	0.154 (71)
	400 (6830)	364 (7330)	
$[Ru(azben)(bpy)_2]^+$	496 (11900)	334 (13290)	0.413 (69)

1) Values for the Ru(III/II) couple.

金属－炭素結合を有するルテニウム(II)錯体の合成と電子移動メディエータへの適用

○木下 晶広、中林 安雄、山内 脩

関西大学 工学部 教養化学教室

Poster 09 Effects of metals on post-embryonic development and reproduction in *C. elegans*

Koji ARIZONO[1,4] , ○Yukari KOGA[1,4] , Fumiyo MORITA[1] , Takashi NAKAMOTO[1] , Hirokazu KIMURA[1] , Kazuhiro URA[2] , Taisen IGUCHI[3,4]

[1]Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto, Japan , [2]Graduate School of Fisheries Sciences & Faculty of Fisheries, Hokkaido University, Hokkaido, Japan , [3]Center for Integrative Bioscience, Okazaki National Research Institutes, Aichi, Japan , [4]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama, Japan

Since metals have been released and remained in soil and water over the long term, we are anxious about soil and water pollution. Metal could be taken in inside of the body through the contaminated food and water and accumulated in living things. Therefore, it is quite possible that some metals could have the potential to interfere with embryonic development, sexual development and reproduction of human and wildlife.

Caenorhabditis elegans (*C. elegans*) is free-living bacteriovorous soil nematode with a compact body and a short life cycle. This organism grows easily on agar plates or in liquid medium containing bacteria as food, and they dose not require large space to culture. In addition, the genetic study results in the approximately 40% of the similarity compared to that of human.

In this study, we investigated the effects of metals on embryonic development and reproduction using *C. elegans*.

Wild-type hermaphrodite *C. elegans* was used in this study. The following metals were tested: $AlCl_3$; $NiCl_2$; $CuCl_2$; CrO_3; $CdCl_2$; $Pb(CH_3COO)_2$; Na_2HAsO_4; and $NaAsO_2$. L1 larvae of *C. elegans* were dispensed into 24-well tissue culture plate containing various concentrations of the metals. After 24 h exposure, the number of dead and/or live worms was measured under dissecting microscope. The 24-h median lethal concentration values for the larvae were calculated by probit analysis. Furthermore, the body length and the percentage of gravid worms after 60 h exposure, and the number of offspring were measured. *E. coli.* was given during this test as feed.

Among metals tested, $CuCl_2$ and $CdCl_2$ showed highest acute toxicity, and they inhibited growth, maturity and reproduction by lower concentration. Although $AlCl_3$ and $NiCl_2$ showed lower acute toxicity, they delayed growth, maturity and reproduction by lower concentration as well. $Pb(CH_3COO)_2$ effected growth, maturity and reproduction by the close concentration. Although Na_2HAsO_4 and $NaAsO_2$ delayed growth and maturity, they affected reproduction by relatively higher concentration. These results indicate that each metal affects *C. elegans* in different concentration and mode of action. Further research is to be planned to clarify the target organ and genes of each metal.

金属類がセンチュウ(*C. elegans*)の発生や繁殖に与える影響

有薗 幸司[1,4]、○古賀 由香里[1,4]、守田 文代[1]、中本 貴士[1]、木村 宏和[1]、浦 和寛[2]、井口 泰泉[3,4]

[1]熊本県立大学 環境共生学部、[2]北海道大学大学院 水産科学研究科、[3]岡崎国立共同研究機構 統合バイオサイエンスセンター、[4]科学技術振興機構 戦略的創造研究推進事業(CREST)

Poster 10 Research on purification of soil and water pollution by the heavy metals using *Lotus japonicus*

○Rieko HIRUNUMA[1] , Kaori IGARASHI[1] , Hiroyuki SAITO[1] , Yutaka MIYAZAWA[2] , Hiromitsu HABA[1] , Hiroshige TAKEICHI[1] , Tomoko ABE[1] , Shuichi ENOMOTO[1]

[1]RIKEN , [2]Graduate School of Life Sciences, Tohoku University, Miyagi, Japan

Lotus japonicus has been proposed as a model leguminous plant for molecular genetic study[1]. We expect that it is a toxic-metal hyperaccumulator. In the present study, the multitracer technique was applied to studies on uptake, distribution, and accumulation of various elements in *Lotus japonicus* (Miyakojima MG-20 and Gifu B-129).

Forty seedlings of each variety (Miyakojima MG-20 and Gifu B-129) were grown on soil in a growth chamber for about two months. After blooming and the start of seed maturation, the plants were cultivated in a nutrient solution containing the multitracer prepared from a Ag target. Five plants each variety were transplanted to a 1000 mL plastic pot containing the multitracer solution. The plant samples were in contact with the solution via their roots. After one week, the plants were removed from the solution and divided into roots, primary shoots (shoot apex; upper part; middle part; lower part), secondary shoots, flowers, pods, and seeds. The radioactivities in these samples were assayed by γ-ray spectrometry using Ge detectors.

The behavior of Be, Na, Sc, V, Cr, Mn, Fe, Co, Zn, As, Se, Rb, Sr, Y, Zr, Tc, Ru, and Rh was determined. In the whole plant, uptakes of all elements except Na were high. The uptakes of trace elements of Miyakojima MG-20 were the same as those of Gifu B-129. In the aboveground parts, Cr, Ru, Sc, and Zr were not detected; Be, Fe, Rh, V, and Y were slightly detected (0.1-0.6%). Although Fe is an essential element, the amount of Fe absorbed by plants was hardly detectable. On the other hand, uptakes of Tc, Sr, Rb, and Zn were high: Tc (22-33%), Sr (19-29%), Rb (46-52%), and Zn (47-53%). The uptakes of Tc, Sr, Rb, and Zn in Miyakojima MG-20 were higher than those in Gifu B-129. Among these elements were found to be widely distributed to the aboveground parts except that Rb was absent in flowers. High Tc concentration was found in upper part of primary shoots and secondary shoots. Strontium was found mainly in the primary shoots. High Rb concentration was found in seeds. High Zn concentration was found in seeds and the lower part of primary shoots. The accumulation of Zn into seeds of Miyakojima MG-20 was the highest. These results suggest usefulness of *Lotus japonicus* for phytoremediation of pollution by Tc, Sr, Rb, and Zn.

1) Hayashi M. et al., DNA Res., 31, 301-310 (2001).

ミヤコグサを用いた重金属元素による土壌汚染及び水質汚染の浄化に関する研究

○蛭沼 利江子[1]、五十嵐 香織[1]、斉藤 宏之[1]、宮沢 豊[2]、羽場 宏光[1]、竹市 博臣[1]、阿部 知子[1]、榎本 秀一[1]

[1]理化学研究所、[2]東北大学大学院生命科学研究科

Poster 11 Design and Synthesis of Zinc Selective Chelators

○Eri KAWABATA[1] , Kazuya KIKUCHI[1,2] , Akira ODANI[3] , Tetsuo NAGANO[1]

[1]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan , [2]Presto, JST Corporation, Kawaguchi, Saitama, Japan , [3]Department of Chemistry, Graduate School of Science, Nagoya University, Nagoya, Aichi, Japan

Zinc (Zn^{2+}) is found in every cell in our bodies. Most is tightly bound to proteins, but certain neurons in our brain contain a large pool of free Zn^{2+}, that can be sequestered in vesicles in their terminals. These Zn^{2+} containing neurons, which use glutamate as a transmitter, are not uniformly distributed, but are concentrated in certain forebrain regions including the hippocampus, amygdala and neocortex. In brain, a few millimolar of free Zn^{2+} exists in the vesicles of presynaptic neurons, and is released by synaptic activity or depolarization, modulating the function of certain ion channels and receptors. Although many reports describe the significance of Zn^{2+} in biological systems, its mechanisms of action are poorly understood.

Research on Zn^{2+} signals in the brain has traditionally employed several chelators, but they have some problems. Use of TPEN (*N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine), a classical membrane-permeable Zn^{2+} chelator, fails to manipulate extracellular or intracellular Zn^{2+} selectively. On the other hand, CaEDTA(calcium-ethylenediaminetetraacetic acid), an extracellular (membrane-impermeable) Zn^{2+} chelator, has disadvantage of the slow kinetics of Zn^{2+} binding to chelator. A higher concentration of CaEDTA markedly reduces the concentration of Ca^{2+}. The development of new, more rapid Zn^{2+} chelators which have low affinity to Ca^{2+} are required for the study of synaptically released Zn^{2+}. We will present the design, synthesis and propaties of new Zn^{2+} chelators and describe biological applications in hippocampal slices.

新規亜鉛イオン選択的キレーターの開発

○川端 絵里[1]、菊地 和也[1,2]、小谷 明[3]、長野 哲雄[1]

[1]東京大学大学院 薬学系研究科、[2]科学技術振興機構さきがけ、[3]名古屋大学大学院 理学研究科

Poster 12 Susceptibility to excitotoxity of glutamate under zinc deficiency and effect of saiko-ka-ryukotsu-borei-to, a herbal medicine, on the excitotoxity

○Haruna TAMANO , Atsushi TAKEDA , Naoto OKU

Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Glutamate is the major excitatory neurotransmitter in the brain and plays its signaling role by acting on glutamate receptors. Excessive activation of glutamate receptors is harmful and excess of glutamate concentration in the synaptic cleft induces neurotoxicity associated with numerous pathological processes such as stroke/ischemia and epilepsy. Thus, it is important to find out a drug to suppress excitotoxicity of glutamate. In the hippocampus of rats injected with kainate, the concentrations of glutamate and zinc in the extracellular fluid were increased in association with seizure activity[1]. Zinc acts against glutamate release suppressively and vesicular zinc is decreased by zinc deficiency. It is possible that extracellular glutamate concentration during seizures is increased by zinc deficiency. In point of fact, glutamate concentration in hippocampal extracellular fluid was excessively increased in zinc-deficient rats after treatment with kainate or during perfusion with 100 mM KCl[2,3]. The excess of extracellular glutamate concentration by the increase of extracellular potassium concentration has been reported in neurological disorders such as ischemia and epilepsy. These results demonstrate that zinc-deficient rat is a possible model for neurological diseases associated with excitotoxicity of glutamate and is susceptible to excitotoxicity of glutamate. To find out a drug suppressing the excess of extracellular glutamate concentration, the effect of *Saiko-ka-ryukotsu-borei-to*, a herbal medicine, was examined in normal and zinc-deficient rats by using in vivo microdialysis. The excessive increase of extracellular glutamate in the hippocampus was induced by perfusion with 100 mM KCl for 40 min. The administration of Saiko-ka-ryukotsu-borei-to did not influence the increase in body weight of normal and zinc-deficient rats. However, the administration of *Saiko-ka-ryukotsu-borei-to* to normal rats completely suppressed the excessive increase in glutamate concentration induced with high K^+. The suppressive effect of *Saiko-ka-ryukotsu-borei-to* was also observed in zinc-deficient rats. These results suggest that *Saiko-ka-ryukotsu-borei-to* is a useful drug for prevention or cure of excitotoxicity of glutamate. References: 1) Epilepsy Res., 54, 123-129 (2003). 2) J. Neurosci. Res., 72, 537-542 (2003). 3) J. Neurochem., 85, 1575-1580 (2003).

亜鉛不足によるグルタミン神経毒性の増大とそれに対する柴胡加竜骨牡蛎湯の抑制効果

○玉野 春南、武田 厚司、奥 直人

静岡県立大学 薬学部 医薬生命化学教室

Poster 13 Bioavailability of Zinc Yeast in Rats: Stimulatory Effect on Bone Calcification in Vivo

○Satoshi UCHIYAMA , Aki IGARASHI , Masayoshi YAMAGUCHI

Laboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka, Shizuoka, Japan.

Osteoporosis is widely recognized as a major public health problem. Bone loss with increasing age induces osteoporosis. Bone loss may be due to increased bone resorption and decreased bone formation. A decrease in bone mass leads to bone fracture. Pharmacologic and nutritional factors may help to prevent bone loss with increasing age. Zinc has been demonstrated to have a wide variety of roles in the mammalian system. Bone growth retardation is a common finding in various conditions associated with zinc deficiency. Zinc has been demonstrated to stimulate osteoblastic bone formation and to inhibit osteoclastic bone resorption. The supplemental intake of dietary zinc may have a role in the prevention of osteoporosis with increasing age. Zinc yeast may be useful as a functional food ingredient. The bioavailability of zinc yeast as a functional food ingredient in rats was investigated. Zinc yeast, zinc sulfate, or zinc oxide was used in this experiment. Serum zinc concentrations were significantly increased by a single oral administration of zinc yeast, zinc sulfate or zinc oxide at a dose of 10 mg Zn/100 g body weight. A significant increase was observed 1 hr after administration and it was also seen with the lowest dose of Zn 0.5 mg/100 g of zinc yeast or zinc oxide. A single oral administration of zinc yeast, zinc sulfate, or zinc oxide (10 mg Zn/100 g) caused a significant increase in liver zinc content. A significant increase in femoral-diaphyseal and -metaphyseal zinc contents was observed with the administration of zinc yeast or zinc sulfate. When, zinc yeast or zinc oxide (10 mg Zn/100 g) was orally administered once daily for 7 days to rats, a significant increase in zinc levels in the serum, liver, and femoral-metaphyseal tissues was seen. Femoral-diaphyseal zinc content was significantly increased with the administration of zinc yeast. A significant increase in calcium content and alkaline phosphatase activity in the femoral-diaphyseal and -metaphyseal tissues occurred with the administration of zinc yeast or zinc oxide. The effect of zinc yeast was greater than that of zinc oxide. This study demonstrated that zinc yeast has high bioavailability in rats, and that its administration induces an anabolic effect on bone calcification in vivo [Journal of Health Science 50:75-81(2004)]. Zinc yeast may be useful as a functional food ingredient.

ラットにおける亜鉛酵母の有用性と骨量増加効果について

○内山 聡志、五十嵐 亜紀、山口 正義

静岡県立大学 大学院 生活健康科学研究科 代謝調節学

Poster 14 The role of glutathione in the stability of biliary arsenic metabolites, arsenic triglutathione [$As(GS)_3$] and methylarsenic diglutathione [$CH_3As(GS)_2$]

○Yayoi KOBAYASHI[1,2] , Seishiro HIRANO[1]

[1]Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan , [2]Japan Foundation for Aging and Health, Chita, Aishi, Japan

Inorganic arsenicals such as arsenite (iAs^{III}) and arsenate (iAs^{V}) are well-known human carcinogens. Arsenic is known to be metabolized by reduction and oxidative methylation, and excreted mainly in urine as monomethylated arsenic (MMA) and dimethylated arsenic (DMA). It has been reported that arsenic is also excreted into bile and biliary excretion of glutathione (GSH) increases following administration of arsenic. It has been suggested that hepatic GSH plays an important role in arsenic biotransformation as a reducing agent. Recently, it has been shown that arsenic was excreted into bile as arsenic-GSH (As-GSH) complexes such as arsenic triglutathione [$As(GS)_3$] and methyarsenic diglutathione [$CH_3As(GS)_2$] and these complexes are transported by multidrug resistance associated protein 2 (MRP2/cMOAT). In order to carry out the speciation of As-GSH complexes, it is important to understand the stability of these arsenic complexes. However, very little is known about their stability in bile. The present study was designed to clarify the stability of As-GSH complexes in rat bile, and the role of GSH in stabilizing these complexes. We studied the stability of $As(GS)_3$ and $CH_3As(GS)_2$ in the presence or absence of GSH. A sample of 10 μg/ml $As(GS)_3$ or $CH_3As(GS)_2$ was incubated at 37 °C for 10 min with various concentrations of GSH (at final concentrations of 0, 1, 5 and 10 mM). Arsenicals were analyzed on an anion exchange column by high-performance liquid chromatography-inductively coupled argon plasma mass spectrometry (HPLC-ICP MS). In the present report we show that $As(GS)_3$ and $CH_3As(GS)_2$ were unstable in bile and hydrolyzed to give iAs^{III} and monomethylarsonic acid (MMA^{III}) in the absence of GSH, and the stability of these As-GSH complexes depends on the concentration of GSH. It has been suggested that trivalent arsenicals, especially MMA^{III}, are more toxic than corresponding pentavalent ones. These results suggest that GSH plays an important role in preventing hydrolysis of As-GSH complexes and the generation of well-known toxic trivalent arsenicals.

胆汁中ヒ素代謝物 $As(GS)_3$ と $CH_3As(GS)_2$ の安定性におけるグルタチオンの役割

○小林 弥生[1,2]、平野 靖史郎[1]

[1]独立行政法人 国立環境研究所 環境健康研究領域 健康指標研究室、[2]長寿科学振興財団

Poster 15 Distributions of ^{67}Cu in hepatoma rats and Cu chaperone knock-down HepG2 cells

○Akihiro NAKAYAMA , Hiromitsu HABA , Shuichi ENOMOTO

Cyclotron Center, RIKEN, Saitama, Japan

Copper (Cu) is an essential metal ion in various proteins and enzymes, and plays an important role in living systems. An excess of Cu, however, exhibits cytotoxity for cells and leads to development of cancer. We previously reported that Cu was accumulated in the livers of human hepatocellular carcinoma and the accumulation of Cu deeply related to the development of cancer through generation of hydroxyl radicals. However, little is known about the Cu accumulation mechanism. One of the reasons for this, radioactive Cu isotope, which can be available to biological experiment, is difficult to prepare, because the half-lives of radioactive Cu isotopes are too short and cost for such isotope production is expensive. Thus, we produced radioactive ^{67}Cu, which has the longest half-life ($t_{1/2}$=61.7 h) among the radioactive Cu isotopes, *via* $^{70}Zn(p,\alpha)$ reaction by using RIKEN AVF cyclotron and applied to living systems. First, we administrated a mixture solution of ^{67}Cu and multitracer to N-nitrosodimethylamine (DMN)-induced hepatoma rats and evaluated the distributions of 10 elements (Cu, Co, Mn, Rb, Ru, Se, Sr, Zn, Zr, and Y) to the livers, kidney, bone, muscle, and blood. The result showed that Cu is the only elements that accumulated in the livers of hepatoma rats. Principal component analysis of distribution ratio (distribution of DMN rats / distribution of control rats) showed that Cu and Mn accumulated and Y and Sr decreased to the body of DMN-induced hepatoma rats. Second, Cu chaperone, Hah1p (human antioxidant protein 1 homologue 1 protein) and CCS (copper chaperone for superoxide dismutase), knock-down HepG2 cells were prepared by RNAi technique. Then, the knock-down and control cells were cultured in ^{67}Cu supplemented medium and measured ^{67}Cu radioactivity incorporated in Cu containing proteins after SDS-PAGE. Knock-down of CCS resulted to decrease in ^{67}Cu distribution for SOD and increase in that for metallothionein. Furthermore, the CCS knock-down cells has tendency to be decreased in ^{67}Cu distribution for Hah1p (not significant, p=0.06). Above results suggested that knock-down of CCS leads to Cu accumulation for the cell and elevates cellular oxidative stress, which in turn develops cellular injury involving cancer.

---

^{67}Cu を用いた肝癌ラットにおける Cu の体内動態と細胞内における Cu 代謝経路の評価

○中山 明弘、羽場 宏光、榎本 秀一

理化学研究所 加速器基盤研究部

Poster 16 Elution behavior of elements from insoluble minerals as food additives in the manufacturing process of liquid food

○Takashi AMAGASA[1] , Rie ITO[1] , Koichi INOUE[1] , Yoshihiro YOSHIMURA[1] , Makiko YAMADA[2] , Teruhisa FUJIMAKI[3] , Masakazu HORIE[4] , Hiroyuki NAKAZAWA[1]

[1]Faculty of Pharmaceutical Science, Hoshi University, Japan , [2]SaneigenFFI, Tokyo, Japan , [3]Kanagawa Institute of Health, Kanagawa, Japan , [4]Saitama Institute of Health, Saitama, Japan

[Introduction]Various insoluble minerals have been used for filter aids as food additives in the manufacture process of liquid food. However, there are no studies regarding the identification and migration of inorganic substances from the filter aids in the food manufacture, so far. It is therefore important to know the elution behavior of the corresponding elements in respect with food hygiene. In the present study, the analytical methods for the precise measurement of residual inorganic substances in filter aids such as perlite, diatomite, talc, bentonite, activated clay, acid clay, granite, and green tuff by fluorescence X-rays analyzer and ICP-AES. Approximately 20 elements, such as aluminum(Al), silicon(Si), iron(Fe), calcium(Ca) and barium(Ba) were measured in insoluble minerals by fluorescence X-rays analyzer and about 30 elements such as Al, Si, Fe, Ca, Ba, arsenic (As) and lead (Pb) were measured in insoluble minerals by ICP-AES.[Methods](1)Identification of inorganic substances in filters aidsThe elements in the tablets, made from the insoluble minerals by the tableting machine were analyzed with the fluorescence X-rays analyzer.(2) Migration test of inorganic substances in filters aids and the case study of migration behaviorThe solvents selected for the migration of inorganic substances from filters aids were 5% nitric acid, distilled water, 4% acetic acid and 20% ethanol. The migration into these solvents was investigated for 30 min, or 24 hrs at 60 degrees by the analysis with ICP-AES. [Results and discussion]Ytterbium and tellurium, which could not be detected by these methods in inorganic substances, were used as internal standards.Based on the results of investigation using X-rays analyzer, Al and Si were identified in all samples examined. In addition, trace amount levels of cadmium, mercury, chromium, As, and Pb were detected in some of insoluble minerals. When 4% acetic acid was used as solvent of migration test, As and Pb were detected in some filter aids.

液状食品製造過程における不溶性鉱物由来金属の溶出挙動

○天笠 高志[1]、伊藤 里恵[1]、井之上 浩一[1]、吉村 吉博[1]、山田 真記子[2]、藤巻 照久[3]、堀江 正一[4]、中澤 裕之[1]

[1]星薬科大学大学院 薬学研究科、[2]三栄源FFI、[3]神奈川衛研、[4]埼玉衛研

Poster 17 NMR analysis of mouse urine: application to toxicity evaluation of trace elements

○Hideki MIYATAKA , Toshiyuki OZAKI , Seiichiro HIMENO

Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

[Introduction]
Several trace elements were used as "supplementation for health promotion", but the evaluation of chronic toxicity of trace elements such as selenium (Se) has not yet been established. Recent years, several research groups have developed a NMR-based metabonomics using rat urine for the evaluation of chemical toxicity by measuring multiple urinary metabolites simultaneously. The purpose of this study was to evaluate the feasibility of mouse urine for NMR analysis. In addition, the effects of chronic Se exposure on urine NMR spectra in mice were examined.

[Materials and Methods]
Urine samples were collected from Wistar rats, and various strains of mice such as ICR, C57Bl/6, C3H/He, Balb/c, DBA2, A/J, and kk. Metabolic cages for mice or rats were used for urine collection. After the filtration, the urine samples were added with TSP (sodium 3-(trimethylsilyl)-[2,2',3,3'-$^2H_4$]-1-propionate) as a chemical shift reference, and applied to 200 MHz or 400 MHz NMR. ICR mice were given 0, 1, 3, and 10 ppm Se as sodium selenite from drinking water. Urine samples of these mice were collected every two weeks.

[Results and Discussion]
After the suppression of the peaks of water, various peaks of low-molecular-weight materials in urine appeared in NMR spectra. The volume of urine necessary for NMR analysis was 0.5 mL, which can be easily obtained from mice. We have identified various metabolites in urine samples from rats and mice; creatinine, taurine, dimethylamine, trimethylamine, trimethylamine N-oxide, citrate, lactate, acetate, succinate, hippuric acid, dimethyl glycine, and phenylalanine. It was possible to identify the above-mentioned metabolites even by using 200 MHz NMR. The NMR spectra showed a clear species difference between mice and rats. Generally, the peaks of creatinine in mice were lower than those in rats. The ratios of trimethylamine to trimethylamine N-oxide were very low in rats but rather higher in mice, suggesting the lower ability of trimethylamine oxidation in mice. Strain difference in the ratios was also recognized. Although human studies in Se-polluted area in China showed changes in urine NMR spectra, it was difficult to reproduce the same changes in mice at least during the period of Se exposure in our experiment.

マウスの尿の NMR 解析ー微量元素の毒性評価への応用

○宮高 透喜、尾崎 俊幸、姫野 誠一郎
徳島文理大学 薬学部 衛生化学

Poster 18 Radical-Scavenging Reactions of 4-Hydroxycinnamic Acid Derivatives in the Presence of Metal Ions

Ikuo NAKANISHI[1,2], Yoshihiro UTO[3], ○Tomonori KAWASHIMA[1,4], Haruko YAKUMARU[1], Hideko KANAZAWA[4], Toshihiko OZAWA[1], Hideko NAGASAWA[3], Hitoshi HORI[3], Nobuo IKOTA[1]

[1]Redox Regulation Research Group, National Institute of Radiological Sciences, Chiba, Japan, [2]Department of Material and Life Science, Graduate School of Engineering, Osaka University, CREST, Japan Science and Technology Agency, Osaka, Japan, [3]Department of Biological Science & Technology, Faculty of Engineering,The University of Tokushima, Tokushima, Japan, [4]Department of Physical Chemistry, Kyoritsu College of Pharmacy, Tokyo, Japan

Artepillin C (**1**), a major component of Brazilian propolis, is a member of a class of 4-hydroxycinnamic acids that has recently been reported to show important biological activities, such as antitumor, apoptosis-inducing, immunomodulating, and antioxidative activities. It is known that hydrogen transfer from the phenolic hydroxyl group to reactive oxygen species, such as hydroxyl radical and lipid peroxyl radical, is responsible for the antioxidative activities of the phenolic compounds. There are two possibilities in the mechanism of hydrogen-transfer reactions from phenolic compounds to radical species, *i.e.*, a one-step hydrogen atom transfer or electron transfer followed by proton transfer. Recently, we have reported that the hydrogen transfer from (+)-catechin to cumylperoxyl radical proceeds *via* an electron transfer from (+)-catechin to cumylperoxyl radical, which is accelerated by the presence of scandium ion (Sc^{3+}), followed by proton transfer in propionitrile. On the other hand, no acceleration of the scavenging reaction of galvinoxyl radical by a vitamin E model compound in the presence of magnesium ion (Mg^{2+}) has been observed, indicating that the hydrogen transfer from the vitamin E model to galvinoxyl radical proceeds *via* one-step hydrogen atom transfer. We herein report the effect of metal ions on the rates of hydrogen transfer from **1** to radical species, such as galvinoxyl and DPPH raidcals determined by the stopped-flow technique in acetonitrile (MeCN) to distinguish between one-step hydrogen- or electron-transfer mechanisms for the radical-scavenging reactions of **1**.

Addition of galvinoxyl radical to a deaerated MeCN solution of **1** resulted in an instant decay of the absorption band at 428 nm due to galvinoxyl radical. This indicates that a hydrogen transfer from **1** to galvinoxyl radical takes place rapidly. From the time course change of the decay of the absorbance at 428 nm was determined the hydrogen transfer rate constant (k_{HT}) from **1** to galvinoxyl radical. No acceleration effect of Mg^{2+} on the hydrogen-transfer reaction has been observed as in the case of the vitamin E model, indicating that the hydrogen-transfer reaction of **1** proceeds *via* the one-step hydrogen atom transfer mechanism.

金属イオン存在下における4-ヒドロキシケイ皮酸誘導体のラジカル消去反応

中西 郁夫[1,2]、宇都 義浩[3]、○川島 知憲[1,4]、薬丸 晴子[1]、金澤 秀子[4]、小澤 俊彦[1]、長沢 秀子[3]、堀 均[3]、伊古田 暢夫[1]

[1]放射線医学総合研究所 レドックス制御研究グループ、[2]大阪大学大学院 工学研究科 物質・生命工学専攻 科学技術振興機構 CREST、[3]徳島大学 工学部 生物工学科、[4]共立薬科大学 創薬物理化学講座

Poster 19 Effects of Metal Ions on the Stability of Oxygen and Nitrogen Radicals

○Ikuo NAKANISHI[1,2] , Tomonori KAWASHIMA[1,3] , Hideko KANAZAWA[3] , Kei OHKUBO[2] , Kiyoshi FUKUHARA[4] , Toshihiko OZAWA[1] , Shunichi FUKUZUMI[2] , Nobuo IKOTA[1]

[1]Redox Regulation Research Group, National Institute of Radiological Sciences, Chiba, Japan , [2]Department of Material and Life Science, Graduate School of Engineering, Osaka University, CREST, Japan Science and Technology Agency , [3]Department of Physical Chemistry, Kyoritsu College of Pharmacy , [4]Division of Organic Chemistry, National Institute of Health Sciences

The stability of oxygen and nitrogen radicals in biological redox systems has attracted considerable interest with regard to diseases associated with reactive oxygen and nitrogen species. Recently, metal ions have been reported to act as a catalyst in the electron-transfer redox reactions. We report herein the effects of metal ions such as Mg^{2+} and Sc^{3+} on the stability of bio-related oxygen and nitrogen radicals in acetonitrile (MeCN).

Addition of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) to a deaerated MeCN solution of a vitamin E model, 2,2,5,7,8-pentamethylchroman-6-ol (**1H**), resulted in the formation of the corresponding phenoxyl radical (**1**·) (Chart 1). The phenoxyl radical **1**· thus generated gradually decomposed via a bimolecular electron-transfer disproportionation to give the corresponding cation $\mathbf{1}^+$ and anion $\mathbf{1}^-$ (Chart 1). However, in the presence of $Mg(ClO_4)_2$ (0.1 M), the disproportionation reaction has been significantly retarded due to a complexation between Mg^{2+} and **1**· (Chart 1).

DPPH· is stable in the presence or absence of Mg^{2+} in MeCN, while an addition of $Sc(OSO_2CF_3)_3$ to a deaerated MeCN solution of DPPH· resulted in an instant decay of DPPH·. The decay rate of DPPH· obeys the second-order kinetics, indicating that a bimolecular electron-transfer disproportionation of DPPH· occurs in the presence of Sc^{3+} and that Sc^{3+} may act as a catalyst which binds to the resulting DPPH⁻ (Chart 2).

The detailed kinetic data obtained in this study provide valuable information about the reactivities of oxygen and nitrogen radicals in the presence of metal ions.

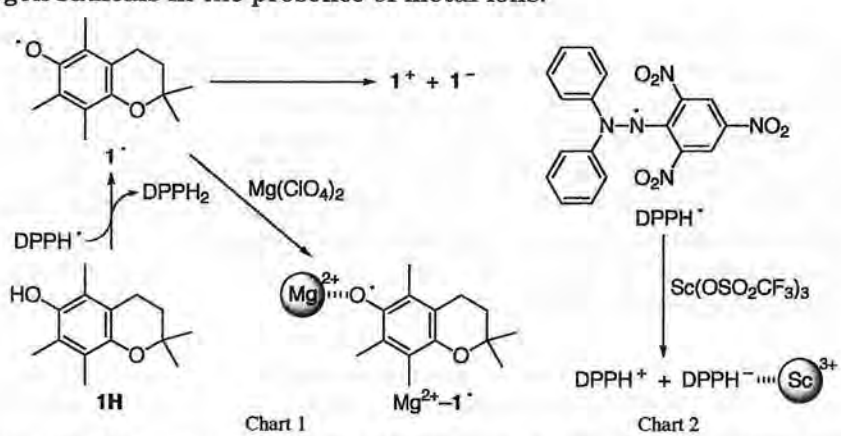


Chart 1 Chart 2

酸素および窒素ラジカルの安定性に及ぼす金属イオンの影響

○中西 郁夫[1,2]、川島 知憲[1,3]、金澤 秀子[3]、大久保 敬[2]、福原 潔[4]、小澤 俊彦[1]、福住 俊一[2]、伊古田 暢夫[1]

[1]放射線医学総合研究所 レドックス制御研究グループ、[2]大阪大学大学院 工学研究科 物質・生命工学専攻 科学技術振興機構 CREST、[3]共立薬科大学創薬物理化学講座、[4]国立医薬品食品衛生研究所 有機化学部

Poster 20 Control of geometric stability and spin state of the iron(III) atom by isomeric porphyrins

○Saburo NEYA , Tyuji HOSHINO , Masayuki HATA

Department of Physical Chemistry, Graduate School of Phramaceutical Sciences, Chiba University

Introduction

Iron is one of the essential metals for living organisms. Cytochrome and hemoglobin contain iron atom in their prosthetic group called heme. The iron atom in hemoprotein adopts out-of-plane or in-plane according to the coordination environment.[1] Iron displacement from the porphyrin ring is suggested to be one of crucial factors to regulate the function of hemoglobin. Position of heme iron depends on the shape of the metallocavity as well as the nature of the axial ligands. Modification of the metallocore shape, which is square in porphyrin (P), is now achieved with isomeric porphyrins like porphycene (Pc: rectangular), corrphycene (Cn: trapezoidal), and hemiporphycene (Hpc, distorted quadrilateral) in Figure 1. Despite pronounced importance of the metallocavity geometry to the axial ligation behavior of the central iron, the systematic investigation has not been pursued. We accordingly examined the geometric stability and spin state of the Fe(III) atom by the isomeric porphyrins.

Materials and Methods

The macrocycles depicted in Figure 1 were synthesized according to the literature.[2] Iron was inserted by refluxing the macrocycles with ferric chloride in dimethylformamide, and the crude material was purified by silica-gel column chromatography to afford the ferric chloride complex. The five-coordinate iodo and azide derivatives were prepared by acid cleavage of the μ-oxo-dimer in the presence of a saturating amount of aqueous sodium iodide or sodium azide. Visible absorption spectra were recorded on Shimadzu MPS-2000 spectrophotometer. Infrared (IR) absorption was measured at 2 cm^{-1} resolution with a JASCO FT-IR-610 spectrometer.[3]

Results

Ligation of 1-Methylimidazole. Binding of 1-methylimidazole (1-MeIm) was monitored in dry benzene at room temperature with Soret band. We noted that the isosbestic points initially observed at low lower concentration of 1-MeIm disappeared at higher concentrations. The results suggest that the 1-MeIm ligation to the ferric iodo complexes in Figure 1 proceeded in two steps, reflecting formation of the mono- and bis-1-MeIm adducts. The first processes were analyzed with the plots of $1/(A_0 - A)$ *vs* 1/[1-MeIm], and the second changes were examined with the equation to analyze the overlapping first and second equilibria.The first and second ligation constants K_1 and K_2 in M^{-1} were determined to be (K_1, K_2) = (1700, 1000), (13000, 5500), (79000, 800), and (19000, 300) for P, Pc, Cr, and Hpc, respectively.[4]

Infrared Spectra of the Iron-Bound Azide. We monitored the infrared (IR) stretching band of the azide in the 6-coordinate Fe(III) complex bearing 1-MeIm and N_3^-. The mixed-ligand complex is expected to exhibit thermal equilibrium between the high- (S = 5/2) and low-spin (S = 1/2) states, and the equilibrium position is susceptible to the molecular shape of the porphyrinoids. The high- and low-spin bands of IR spectra for the macrocycles in Figure 1 appeared at 2048 cm^{-1} and 2018 cm^{-1}, respectively. The position of the spin-state equilibrium was found to be largely dependent on the molecular shape of the macrocycles. The intensity ratios of the two IR bands $I_{high\text{-}spin}/I_{low\ spin}$ were found to be 10/90, 0, 0, and 52/48 for the P, Pc, Hpc, and Cn macrocycles, respectively.

Discussion

The above results demonstrates that the affinity of 1-MeIm to the Fe(III) is much dependent on the tetrapyrrole array of the macrocycles. The significantly larger K_1 for Cn macrocycle indicates that the out-of-plane displacement of the Fe(III) atom is favored in the trapezoidal metallocore. Large K_2 for Pc reflects weaker Fe-I bond due to shorter Fe-N(pyrrole) bonds. The shape effect of the macrocycle is also demonstrated by the spin equilibrium analyses of the azide complexes. The Pc and Hpc compounds are pure low-spin while the high-spin state is dominant in the Cn derivative. Variation of the spin-state equilibrium position is consistently explained in terms of geometric stability of the Fe(III) atom that reflects different length of the Fe-N(pyrrole) bond and deviation of the N(pyrrole)-Fe-N(pyrrole) bond angle from the orthogonality. We therefore conclude that the rearrangement of the tetrapyrrole array over the porphyrin macrocycle is a novel and promising approach to control the geometric stability of the Fe(III) atom.

References

1. Neya, S.; Tsubaki, M.; Hori, H.; Yonetani, T.; Funasaki, N. *Inorg. Chem.* **2001**, *40*, 1220-1225.
2. Ohgo, Y.; Neya, S.; Ikeue, T.; Takahashi, M.; Takeda, M.; Funasaki, N.; Nakamura, M. *Inorg. Chem.* **2002**, *41*, 4652-4629.
3. Neya, S.; Imai, K.; Hori, H.; Ishikawa, H.; Ishimori, K.; Okuno, D.; Nagatomo, S.; Hoshino, T.; Hata, M.; Funasaki, N. *Inorg. Chem.* **2003**, *42*, 1456-1461.
4. Neya, S; Hoshino, T.; Hata, M.; Funasaki, N. *Chem. Lett.* **2004**, *33*, 114-115.

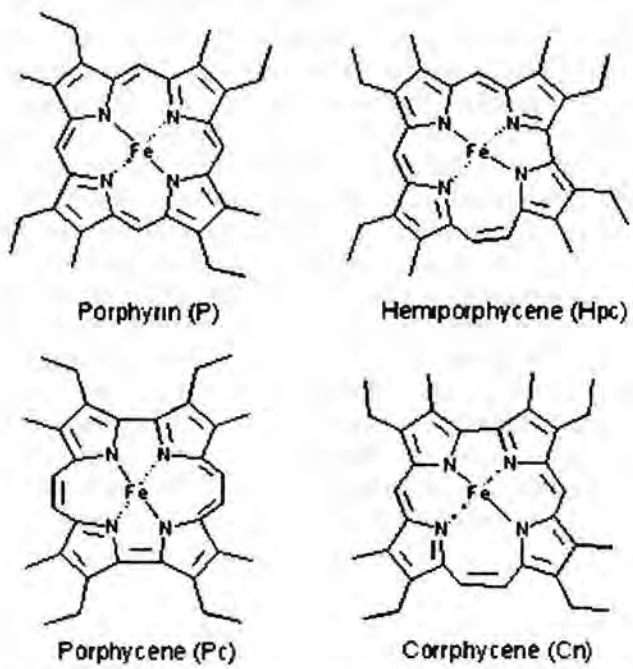


Figure 1 Structure of porphyrin and isomeric porphyrinoids

ポルフィリン異性体による鉄(III)原子の幾何学的安定性およびスピン状態の制御

○根矢 三郎、星野 忠次、畑 晶之
千葉大学大学院 薬学研究院 薬品物理化学研究室

Poster 21 Increased binding affinity of aluminum in the presence of oxalate for asialo-transferrin compared with native-transferrin studied by HPLC/HR-ICP-MS

○Megumi HAMANO NAGAOKA , Hiroshi AKIYAMA , Tamio MAITANI

Division of Foods, National Institute of Health Sciences

SUMMARY

Transferrins (Tfs) are N-glycosylated proteins generally containing four molecules of sialic acids in the C-lobe site. Carbohydrate-deficient Tfs (CDTs) and sialic acid-deficient Tfs, are observed in various diseases. In this study, focusing on the fact that Tfs are glycoproteins, the binding affinity of both Al and Fe for asialo-Tf was compared with its binding affinity for native-Tf by on-line high-performance liquid chromatography/high-resolution inductively coupled plasma mass spectrometry (HPLC/HR-ICP-MS). The results suggested that the affinity of Al for asialo-Tf is much higher than that for native (sialo)-Tf in the presence of oxalate, and thus the possibility of adverse effects of Al may be increased in various diseases.

INTRODUCTION

Aluminum (Al) is a ubiquitous metal. Although originally considered non-toxic, Al neurotoxicity has had a long and controversial history in neuroscience since the discovery of the dialysis encephalopathy. The Al in the blood is bound to transferrin (Tf), a glycoprotein of about 80 kDa with the characteristic property of requiring a synergistic anion. Although the principal role of Tf in the blood is to transfer iron (Fe) to cells, it can bind a large number of other metals. The Tf molecule contains two homologous lobes for metal-binding, the N-lobe site and the C-lobe site, and they are connected by a single short peptide. The preferential site depends on the kind of Tf, the metal, and the synergistic anion. For example, we have reported preferential binding of Fe and Al to the N-lobe site in the presence of bicarbonate as the synergistic anion[1].

A striking difference between n two lobes is the presence of a carbohydrate chain with sialic acid residues in the C-lobe. The carbohydrate chain can differ in the degree of branching, and the difference is known as microheterogeneity. Tfs are present in disialo-, trisialo-, tetrasialo-, pentasialo-, and asialo-forms. The most common Tf contains two biantennary complex-type glycans with a total of four terminal sialic acid residues. Carbohydrate-deficient Tfs (CDTs) have fewer sialic acids and are increased in several diseases. In this study, the affinity of two metals, Al and Fe, to native-Tf and asialo-Tf was compared by HPLC/high resolution (HR)-ICP-MS to determine whether the presence of sialic acids in Tf influences the metal binding (the principal role) of Tf.

MATERIALS AND METHODS

Reagents

Apo-human serum Tf (hTf) (>97%) was purchased from Sigma (St. Louis, MO, USA) and used without further purification. Aluminum chloride, ferric chloride hexahydrate, sodium bicarbonate, and sodium oxalate were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka). Other chemicals were of reagent grade or of the highest grade commercially available. The ultra-pure water was bubbled with nitrogen gas for 30 min to completely remove dissolved oxygen and used throughout the experiment.

Sialidase treatment

Apo-hTf was incubated at 37°C with sialidase (*Clostridium perfringens* neuraminidase (sialidase) type V) in 0.1 M sodium acetate buffer (pH 5.0) for 24 h at a ratio of 40 mU enzyme per mg hTf. Asialo-hTf was dialyzed against 0.1 M citric acid, and then dialyzed against water bubbled with nitrogen gas for 30 min to completely remove the dissolved carbon dioxide.

Sample preparation for HPLC/HR-ICP-MS

Blood was collected from healthy volunteers in accordance with the ethics guidelines of our institute, and serum was obtained by centrifugation at 3000xg. Apo-hTf was dissolved in 10 mM Tris-HCl (pH 7.4) containing 25 mM $NaHCO_3$ as the synergistic anion. Each of the metal solutions was added to the apo-hTf solution (2 mg apo-hTf ml^{-1}), and after preparing each of the sample solutions, all tubes and bottles were sealed under a nitrogen-gas atmosphere.

HPLC/HR-ICP-MS

An HPLC apparatus (LC-10Ai, Shimadzu, Kyoto) equipped with an anion-exchange column (MonoQ HR 5/5 (5 mm i.d. x 50 mm, Amersham Biosciences, Uppsala, Sweden) was connected directly with an HR-ICP-MS instrument (ELEMENT, Finnigan MAT, Bremen, Germany), and a 100 µl sample (serum or 0.2 mg hTf) was applied to column. Two solvents were used for gradient elution: solvent A (50 mM Tris-HCl, pH 7.4) and solvent B (A + 0.25 M ammonium acetate). The eluate was transferred to a UV detector (at 280 nm, SPD-10AVi, Shimadzu) and then introduced into the Meinhard nebulizer of the HR-ICP-MS. The levels of ^{27}Al, ^{56}Fe, and ^{32}S, which are interfered with by polyatomic ions such as $^{13}C^{14}N^+$, $^{12}C^{15}N^+$, and $^{12}C^{14}N^1H^+$, $^{40}Ar^{16}O^+$ and $^{40}Ca^{16}O^+$, and $^{16}O_2^+$, respectively, when quadrupole ICP-MS is used, were monitored simultaneously by HR-ICP-MS in the medium resolution mode. After each analysis, the column was washed to eliminate the Al ions adsorbed by the column according to the clean-up procedures reported previously[2]. The detection limit (S/N = 3) estimated for ^{27}Al in the medium resolution mode was 0.1 ng ml^{-1}.

RESULTS AND DISCUSSION

Three major Fe-binding hTfs and apo-hTf were detected in Fe-supplemented apo-hTf and assigned to Fe_C-hTf (23 min), $metal_2$-hTf (28 min), apo-hTf (34 min), and Fe_N-hTf (38 min), respectively, as reported previously[3]. In addition, a small Fe peak was observed at 10 min. The peak was ascribed to Fe bound to hTf with smaller numbers of sialic acid molecules, because sialidase treatment removed sialic acid molecules bearing anionic charges and the treated-hTf eluted more quickly than the native-hTf. The ^{32}S level was useful for monitoring the protein levels in the HPLC eluate.

Fe added as Fe-citrate in the presence of bicarbonate preferred the N-lobe site, and binding affinity to native- and asialo-hTfs was similar. Fe-NTA, on the other hand, preferentially bound to the C-lobe site of native-Tf and asialo-hTfs.

Al in the serum of a healthy person without any in vitro Al spike was present as both Al_N-Tf and Al_N,Fe_C-Tf[1]. The chemical states were reproduced in the apo-hTf solution supplemented with Fe (Fe/hTf ratio = 0.6) and Al (Al/hTf ratio = 1) successively.

Al added to apo-hTf as both Al-citrate and Al-NTA at Al/hTf = 1 almost selectively bound to the N-lobe site, and its binding affinity to asialo-hTf was higher than to native-hTf. After the addition of Al-oxalate, the affinity to the N-lobe site of asialo-hTf increased further. Al_2-hTf was the largest peak of asialo-hTf even at Al/hTf = 1. In the absence of bicarbonate, Al-oxalate showed preference for the C-lobe site in native-hTf and comparable affinity to both lobes of asialo-hTf. Thus, the lack of sialic acid in the glycans and the presence of oxalate enhanced the binding affinity of Al to Tf. CDTs have been reported as sensitive biological markers for alcohol abuse, and it has recently been reported that both CDTs and sialidase activity increase in coronary heart disease. Therefore, the findings in this study may suggest that the binding affinity of Al to Tf is enhanced in patients with CDTs.

REFERENCES

1) Megumi Hamano Nagaoka, Tamio Maitani: Analyst., 125, 1962-1965 (2000)
2) Megumi Hamano Nagaoka, Tamio Maitani: Biochim Biophys Acta., 1526, 175-182 (2001)
3) Megumi Hamano Nagaoka, Tamio Maitani: Biochim Biophys Acta., 1523, 182-188 (2000)

トランスフェリンの糖鎖改変による金属結合能への影響

○長岡 浜野 恵、穐山 浩、米谷 民雄

国立医薬品食品衛生研究所 食品部

Poster 22 The Metal Binding Motif of Dipeptidyl Peptidase III Directly Influences the Enzyme Activity in the Copper Derivative of Dipeptidyl Peptidase III

○Junzo HIROSE[1] , Hiroshi KAMIGAKIUTI[1] , Hiroyuki IWAMOTO[1] , Satoru YAMAMOTO[2] , Kayoko FUKASAWA[3]

[1]Department of Applied Biological Science, Fukuyama University, Fukuyama, Japan , [2]Department of Biotechnology, Fukuyama University , [3]Department of Oral Biochemistry, School of Dentistry, Matsumoto Dental University

Recently we proposed that dipeptidyl peptidase III (DPP III) (EC 3.4.14.4), which has a HELLGH...E (residues 450-455, 508) motif as the zinc binding site, be classified as a zinc metallopeptidase. The zinc binding motif (HELLGH) of dipeptidyl peptidase III is different from the common zinc binding motif (HExxH) of metallopeptidases. In a previous study we replaced the zinc ions in DPP III with Co(II) and Cu(II) in order to characterize the coordination geometry of the metal ions. The visible absorption spectrum of Co(II)-DPP III and the EPR spectrum of Cu(II)-DPP III clearly indicated that the coordination geometry was distorted tetrahedral and the donor atom set around the metal ions in DPP III were similar to those of thermolysin and carboxypeptidase A. The copper derivative of carboxypeptidse A or thermolysin, a well known zinc-peptidase, has no or little peptidase activity, but the copper derivative of DPP III (Cu(II)-DPP III) showed high peptidase activity for Arg-Arg-β-NA, the substrate (90% of the native enzyme). What is the important factor of the copper derivative of DPP III (Cu(II)-DPP III) to have peptidase activity? This is a question we very much want to answer. To clarify the importance of the zinc binding motif part (HELLGH) of DPP III for the enzyme activity, we measured the recovery of the enzyme activity of apo-Leu453 deleted dipeptidyl peptidase III (apo-Leu453-del-DPP III), which has a motif (HELGH) like that of the common peptidase (HExxH), in the presence of various metal ions. The enzyme activity of apo-Leu453-del-DPP III could not be recovered by the addition of cupric ions, while apo-DPP III could be easily reactivated by the addition of cupric ions. The metal derivatives of Leu453-del-DPP III were isolated and their kinetic parameters were determined. Cu(II)-Leu453-del-DPP III had very low specific activity. The visible and EPR spectra of the isolated Cu(II)-Leu453-del-DPP III clearly show that the cupric ions of Cu(II)-Leu453-del-DPP III bound to the motif part (HELGH) but didn't exhibit any enzyme activity. The motif part of DPP III directly influences the expression of the enzyme activity in the copper derivative of DPP III.

Dipeptidyl Peptidase III 中の亜鉛結合モチーフ構造の変化が酵素活性に直接影響を与える

○廣瀬 順造[1]、上垣内 宏司[1]、岩本 博行[1]、山本 覚[2]、深沢 加与子[3]

[1]福山大学 生命工学部 応用生物科学科、[2]福山大学 生命工学部 生物工学科、[3]松本歯科大学 口腔生化学

Poster 23 Roles of Heme Axial Ligands in the Regulation of CO Binding to CooA

○Taku YAMASHITA , Youhei HOASHI , Kimiko WATANABE , Yoshikazu TOMISUGI , Yoshinobu ISHIKAWA , Tadayuki UNO

The Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

[Introduction]

A new class of heme proteins is emerging that senses small gaseous molecules such as NO, O_2, and CO. Binding of these diatomic ligands to the heme triggers the biological response. The heme moieties in sensor proteins are directly involved in binding their respective effector molecules and thereby regulating the activity of the proteins. CooA is a member of these sensor proteins and is a homodimeric (221 amino acids per monomer) transcriptional activator from the photosynthetic bacterium *Rhodospirillum rubrum*. This protein is activated by CO binding, leading to the transcription of a series of genes whose products are associated with CO metabolism. CooA consists of two domains; the N-terminal CO sensing domain, and the C-terminal DNA binding domain. These two domains are linked by a long α-helix (C-helix) which shapes an interface between two subunits, and are analogous to those found in the catabolite gene activator protein CAP (or CRP). CooA contains a six-coordinate and low-spin heme in both ferrous and ferric states, and the crystal structure of ferrous CooA revealed His77 and Pro2 are the axial ligands. The six-coordination in CooA, however, is not suitable for the binding of exogenous ligands, because the internal protein ligands must be a barrier for the incoming ligand. In this paper, roles of Pro2 and His77 ligands are studied in the light of CO recognition and redox-linked ligand switching mechanisms in CooA.

[Experimental Procedures]

In order to achieve high expression, a gene encoding CooA was synthesized using a modified recursive PCR strategy. Based on the known amino acid sequence of *R. rubrum* CooA, six forward and six reverse primers were designed. The full length DNA was recovered and amplified by the second PCR. The product was inserted at XhoI/BamHI site of the plasmid pBluescriptII SK(+) (Stratagene), and was used to transform *E. coli* strain XL-1 Blue MRF' (Stratagene). The DNA sequence of the plasmid product was confirmed with a Li-Cor Model 4200S2 DNA sequencer. The full length CooA coding sequence was excised from the pBluescript derivative and ligated into a pET3a-based expression vector pBEX. The ligation product was introduced into *E. coli* strain BL21Gold(DE3) (Stratagene). The cells were cultured in LB broth, harvested, and sonicated. The soluble fraction containing CooA was applied to Q-Sepharose column, and was further purified by Sephacryl S-100. The CooA fraction was homogenous as judged by SDS polyacrylamide gel electrophoresis criteria. CooA concentrations were evaluated from the heme content which was measured by a pyridine hemochrome assay. The QuikChange system (Stratagene) was used to introduce site-directed mutations into the CooA coding sequence, and the mutants were prepared similarly. Absorption and resonance Raman spectroscopic methods are employed in order to examine structural changes of CooA mutants. The CO binding affinity was evaluated by CO titration method. The CO titration of ferrous CooA were performed in 0.1 M Tris-HCl (pH 8.0). CooA solution was purged extensively with nitrogen gas and stoppered by a rubber septum, through which CO solution was injected by a syringe.

[Results and Discussion]

In this study, the over expression system of CooA was constructed and various mutants are prepared using modified recursive PCR strategy. With this system, more than 10~20 mg of wild-type CooA was purified per liter of culture medium. Absorption and resonance Raman spectra of wild-type CooA were identicqusly reported. We first measured the CO affinity of CooA by CO titration method. The spectral changes in the Q-band region clearly showed one set of isosbestic points, indicating that CO binding is a one-step equilibrium process. Thus, the absorbance change

was analyzed as a simple equilibrium between the ferrous and CO-bound forms, and was normalized and plotted against the [CO]/[CooA] ratio. A theoretical curve provides a nice fit to the titration data, and an apparent Kd value of 11 μM was calculated for CO binding to wild-type CooA. This value is similar to those for other heme proteins which sense gaseous ligands, and seems to be unique in this class of proteins. We further prepared axial ligand mutants, P2H and H77A, and physiological significance of the Pro2/His77 coordination in CooA was studied. P2H mutation greatly disturbed the absorption and resonance Raman spectra of the ferric protein, although the ferrous spectra were less affected. This indicates that the substituted histidine residue or N-terminus amino group coordinates the ferrous heme in P2H mutant. In the resonance Raman spectrum of P2H mutant in ferrous CO-bound form, a ν(Fe-CO) stretching Raman line was detected at 490 cm^{-1}, which was similar to that observed in wild-type CooA. In the CO titration analysis, the K_d value for P2H, however, was greatly decreased, indicating ~200-fold higher affinity than wild-type protein. Therefore, Pro2 coordination fine-tunes CO sensing level in CooA, and Pro2 is the ligand that is replaced by incoming CO. On the other hand, the proximal His77 ligand has been revealed to be crucial for the activation of CooA. In the H77A mutant, equilibrium CO binding is biphasic unexpectedly. This indicates that two distinguishable heme pockets are constructed by His77 substitution and two CO molecules occupy both axial sites at high CO pressures. The ν(Fe-CO) stretching Raman line for the first CO molecule was observed at 497 cm^{-1} and a minor component was detected as a shoulder around 525 cm^{-1}. In order to correlate the biphasic CO binding with the two ν(Fe-CO) stretch, we further prepared various His77 mutants. All His77 mutants showed the ν(Fe-CO) stretching Raman line commonly at 497 cm^{-1}. This frequency is higher than that of wild-type CooA by 7 cm^{-1}, indicating a unique heme pocket created by the His77 mutation. Some His77 mutants clearly showed an additional ν(Fe-CO) line at 525 cm^{-1}. The intensity of this line and the binding affinity of the second CO molecule were found to correlate with the five-coordinated component in the ferrous His77 mutants and also with the acidity of the side chain at position 77. Thus, we propose that the Cys75-His77 ligand switch is controlled by His77 acting as a proton reservoir, and His77 coordination prevents the binding of excess CO to the ferrous heme.

[Reference]
Taku Yamashita, Yohei Hoashi, Kimiko Watanabe, Yoshikazu Tomisugi, Yoshinobu Ishikawa, and Tadayuki Uno
J. Biol. Chem. 279, in press (2004)

CooA の CO 結合性制御に対する軸配位子の役割

○山下 沢、帆足 洋平、渡辺 季美子、富杉 佳計、石川 吉伸、宇野 公之
熊本大学大学院 薬学教育部

Poster 24 Study on Structures and Characterizations of Linear Au(I) Thiolato Complexes

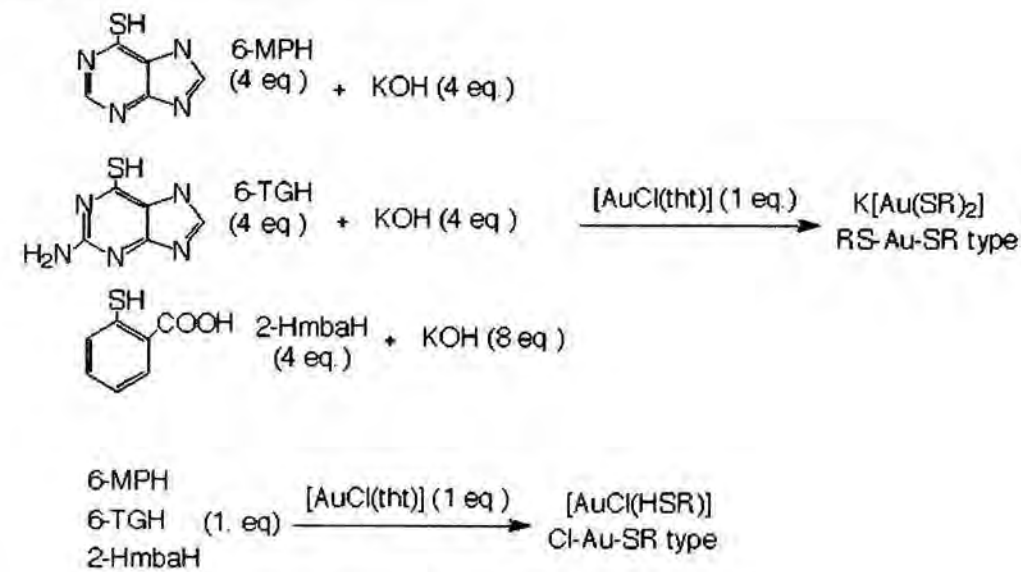
○Yuki MATSUNAGA , AMIR NAGINA , Yoshitaro MIYASHITA , Kiyoshi FUJISAWA , Ken-ichi OKAMOTO

Department of Chemistry, University of Tsukuba, Ibaraki, Japan

The metal-sulfur bonds have been found in many metalloproteins containing Mn, Fe, Cu, Zn, Mo, Cd and Hg. These are expected to affect the activity or stability of structures. Thus the model complex study has given important information together with the biological study. Recently Cu(I) site has been found in CueR, which is a member of the MerR family of transcriptional activators. In this complex, two sulfur atoms from Cys coordinate to Cu(I) ion. And metal substituted CueR from Cu(I) to Ag(I) and Au(I) were studied for their crystal structure and it was shown that these are also two coordinated linear structure.

In this context, we are interested in the linear Au(I) thiolato complex. We show here two types of complexes, the RS-Au-SR and Cl-Au-HSR type with three thiolato ligands, 6-mercaptopurine (6-MPH), 6-thioguanine (6-TGH) and thiosalicylic acid (2-HmbaH). 6-MPH and 6-TGH are consistent with nucleic acids, nucleotide and nucleoside in biological system. 2-HmbaH is used as their structural analogous. The RS-Au-SR complexes were formed by the reaction of [AuCl(tht)] (tht = tetrahydrothiophene) (1 eq.) with 6-MPH or 6-TGH (4 eq.) and KOH (4 eq.). When the HSR = 2-HmbaH was used, 8 eq. of KOH was added. The Cl-Au-HSR complexes formed by the reaction of [AuCl(tht)] (1 eq.) with each ligand (1 eq.). The formations of complexes were confirmed by UV-Vis absorption spectra and elemental analysis. The structure of $(Et_4N)[Au(6\text{-}TG)_2]$ which is the RS-Au-SR type complex was determined by X-ray crystal analysis.

Scheme



直線型 Au(I)チオレート錯体の構造と性質

○松永 有樹、AMIR NAGINA、宮下 芳太郎、藤澤 清史、岡本 健一

筑波大 化

Poster 25 Syntheses of dinuclear ruthenium(II)-2,2'-bipyridine complexes and characterization of their DNA-binding activities

○Nobuaki IWAMOTO , Yasuo NKABAYASHI , Osamu YAMAUCHI

Faculty of Engineering, Kansai University, Osaka, Japan

There has been considerable recent interest in the DNA binding of dinuclear ruthenium(II) complexes with polypyridyl ligands. These dinuclear complexes can bind DNA by bisintercalation or associate as positively charged species within the grooves of DNA. Dinuclear ruthenium(II) complexes have some advantages such as increased variations in shape and size over mononucler complexes as photoprobes and stereochemical probes of DNA. In this study, diamine-bridged dinuclear ruthenium(II)-2,2'-bipyridine complexes $[\{RuCl(bpy)_2\}_2(\mu\text{-}BL)]^{2+}$ (bpy=2,2'-bipyridine; $BL=H_2N(CH_2)_nNH_2$(n = 4,8,12)) were synthesized and characterized by spectroscopic (UV-vis, ^1H NMR) and electrochemical (CV) methods. In addition, to investigate the specifity of the binding to nucleobases, we have studied reactions of $[\{RuCl(bpy)_2\}_2(\mu\text{-on})]^{2+}$ (on = $H_2N(CH_2)_8NH_2$) with various nucleosides (guanosine (Guo), adenosine (Ado)) and nucleotides (guanosine 5'-monophosphate (5'-GMP), adenosine 5'-monophosphate (5'-AMP)) in aqueous solution at 37 °C by UV-vis and ^1H NMR spectroscopies. UV-vis spectra for all the dinuclear ruthenium(II) complexes were recorded in acetonitrile, where they exhibited 10 to 20 nm red shift of the two intense $d\pi(Ru^{II})$-π^*(bpy) metal-to-ligand charge transfer (MLCT) bands to *ca.* 350 and 510 nm relative to $[Ru(en)(bpy)_2]^{2+}$ (en:ethylenediamine). The CVs of all the dinuclear complexes in acetonitrile showed one reversible redox reaction of the Ru(III/II) couple at *ca.* 0.23 V *vs.* Fc/Fc+ which is 320 mV less positive than the redox potential of $[Ru(en)(bpy)_2]^{2+}$. At the Guo (or 5'-GMP) : ruthenium(II) complex ratio of 10, the hyperchromisms and blue shifts of the MLCT bands increased with time, indicating that Guo and 5'-GMP can react with $[\{RuCl(bpy)_2\}_2(\mu\text{-on})]^{2+}$. On the other hand, no UV-vis spectral charge was observed for ruthenium(II) complex-Ado (or 5'-AMP) systems. At the Guo (or 5'-GMP) : ruthenium(II) complex ratio of 2, ^{1} H NMR data showed that N7 of Guo (or N7 and O(PO_3) of 5'-GMP) can coordinate to $[\{RuCl(bpy)_2\}_2(\mu\text{-on})]^{2+}$ to form Ru-*N*7Guo or Ru*N*7GMP and Ru-*O*(PO_3)GMP bonds.

複核ルテニウム(II)-2,2'-ビピリジン錯体の合成とDNAへの結合活性

○岩本 展明、中林 安雄、山内 脩

関西大学大学院 工学研究科 応用化学専攻

The 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04
Thursday, June 10 – Friday, June 11, 2004

List of Contributors

登録者リスト

127-155ページ 浦田

御　　礼

第14回金属の関与する生体関連反応シンポジウムの開催に際して、その趣旨にご賛同いただき、多大のご支援を賜りました下記の施設ならびに諸企業・団体に、謹んで感謝の意を表します。

助　　成

21 世紀 COE プログラム推進拠点、学術集会助成

静岡県立大学学長特別研究　学術集会助成

静岡総合研究機構学術教育研究推進　学術集会助成

賛助企業・団体一覧

浜理薬品工業株式会社

ゼリア新薬工業株式会社

株式会社　ミツカングループ本社　中央研究所

日本クリニック株式会社

フナコシ株式会社

太陽堂書店

スズ化合物環境技術協議会（TETA）

昭島化学工業株式会社

旭電化工業株式会社

勝田化工株式会社

共同薬品株式会社

郡山化成株式会社

堺化学工業株式会社

三共有機合成株式会社　　大協化成工業株式会社

大日本インキ化学工業株式会社　東京ファインケミカル株式会社

日東化成株式会社　　北興化学工業株式会社

株式会社エーピーアイコーポレーション

機材・商品提供

財団法人　静岡コンベンションビューロー

NECグループ　日本アビオニクス販売株式会社

Message from Oyster

糖質、タンパク質、アミノ酸、ビタミン、ミネラル。
豊富な栄養価から「海のミルク」と称される牡蠣、
その深化したチカラを、さらなる健康生活へ
ぜひどうぞ。

世界で初めて牡蠣のエキス抽出に成功した日本クリニックの「かき肉エキス」は製法特許に加え、その後の品質向上に伴う種々の特許に基づいて製造されています。また、素晴らしい環境を誇る「天橋立」臨海自社工場での一貫生産も品質への自信を示すひとつの例。独立した研究開発部門や、毎月5,000件以上ものご相談を承る「食養相談室」など、日本クリニックはそのすべてを通して、現代人が求める健康生活を支え続けます。
かき肉エキスの「旬」は通年。
加工食品の日常的な摂取をはじめ、健康を支えるはずの食生活に深刻なシグナルが発せられている現代社会。そんな今、健康を得るに"生牡蠣以上"の真価を発揮する「かき肉エキス」が、栄養補助食品として脚光を浴びるのは当然なのかもしれません。しかも、嬉しいことに「かき肉エキス」の旬は通年。確かな健康へ、毎日の摂取をお勧めいたします。





製法特許:第1770901号　物質特許:第1813693号
W抽出製法特許:第3429726号　FC物質特許:第3267962号

W抽出製法かき肉エキス
バランスター®WZ

●120粒瓶入／12,600円　●260粒瓶入／25,200円
●480粒瓶入／42,000円　●携帯用(4粒×30袋)12,600円
※価格はすべて「税込」です。

バランスターWZはJCOE研究会専売品です。
全国のJCOE研究会会員薬局・薬店でお求めください。

牡蠣の神秘を科学で届ける——かき肉エキスのパイオニア

日本クリニック株式会社

●本社／〒616-8555 京都市右京区太秦開日町10番地1
●食養相談室／TEL.075-871-2299
●営業所／札幌・仙台・東京・名古屋・関西・広島・福岡　●工場／京都府宮津市

http://www.japanclinic.co.jp/

VistaLab TECHNOLOGIES
Dispensing With Tradition.

研究用

手が疲れない！新型ピペット

Ovation

BioNatural Pipette

人間工学に基づいたユニークな形状と
快適な操作性を備えたピペットです。

INDUSTRIAL DESIGNERS SOCIETY OF AMERICA 2001 GOLD



手になじむ心地よさ！
快適な操作性！

デモ承ります！
手に取ってお試し下さい。

少ない力で動くプランジャー
2 μl でも 1,000 μl でも指で押す
力はほとんど変わりません！

※写真は容量可変タイプです。

容量可変タイプ



モーターまかせの容量変更
上下ボタンで容量を変更するだけで，あとはモーターまかせ。手でダイヤルを回す必要はありません。しかも，5種類までの容量をプリセットできます。



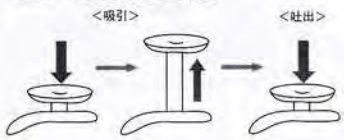
キャリブレーション機能搭載
ピペット容量の補正にも，面倒な調整は不要です。実測値を入力するだけで自動補正されます。

容量固定タイプ

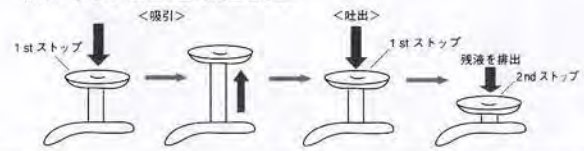


2 種類のストローク
吐出方法の異なる2種類のタイプから各種容量のピペットが選択可能です。

Single Stroke タイプ（一段階吐出）
精度：吐出された量を保証



Two Stroke タイプ（二段階吐出）
精度：チップの中に蓄えた量を保証



※詳細は，当社テクニカルサポート（機器担当：下記参照）までお問い合わせ下さい。

フナコシのライフサイエンス研究用試薬と機器
日本総代理店
フナコシ株式会社
〒113 - 0033　東京都文京区本郷２丁目９番７号
http://www.funakoshi.co.jp/　e-mail：info@funakoshi.co.jp
試薬に関して：Tel. 03-5684-1620 Fax 03-5684-1775　e - mail ：reagent@funakoshi.co.jp
機器に関して：Tel. 03-5684-1619 Fax 03-5684-5643　e - mail ：kiki@funakoshi.co.jp

ささやかながら世界の人々の健康を支えています

医薬品原薬

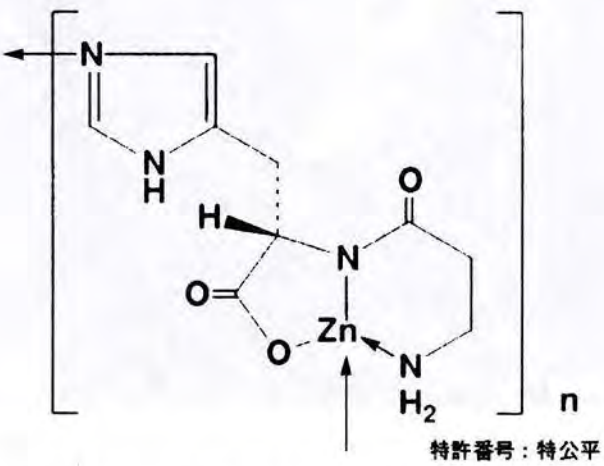
医薬品中間体

アミノ酸原料
ペプチド等

健康食品素材

食品添加物

化粧品原料



特許番号：特公平 7-116160

含亜鉛 抗潰瘍薬ポラプレジンク*

＊ 製剤名 プロマック（ゼリア新薬）

浜理薬品工業株式会社

〒553-0024 大阪市東淀川区柴島 1 丁目 4 番 29 号
TEL：06-6322-0191　FAX：06-6323-2115
http://www.hamarichemicals.com

亜鉛とL-カルノシンから誕生したプロマック

Direct Healing

潰瘍部に直接作用し創傷治癒促進



［効能・効果］胃潰瘍

［用法・用量］

通常、成人にはポラプレジンクとして1回75mgを1日2回朝食後及び就寝前に経口投与する。なお、年齢、症状により適宜増減する。

［使用上の注意］

1.相互作用

併用注意（併用に注意すること）

薬剤名等	臨床症状・措置方法	機序・危険因子
ペニシラミン製剤	同時に服用することにより、併用薬剤の効果を減弱するおそれがあるので、やむを得ず投与する場合には、同時に服用させないなど注意して投与すること。	同時投与した場合、本剤が併用薬剤とキレートを形成し、吸収を低下させる可能性がある。
レボチロキシンナトリウム		

2.副作用

総症例4,126例中、121例に臨床検査値の変動を含む副作用が報告された。その主なものは、発疹等の過敏症状0.12%、便秘0.22%、嘔気0.12%であった。臨床検査値では、AST(GOT) 上昇0.44%、ALT(GPT) 上昇0.68%、Al-P上昇0.44%、LDH上昇0.27%、好酸球増多0.27%、トリグリセライド上昇0.17%等がみられた（1997年8月安全性定期報告時）。

(1) 重大な副作用

肝機能障害、黄疸（頻度不明）：AST（GOT）、ALT（GPT）、γ-GTP、Al-Pの上昇等の肝機能障害、黄疸があらわれることがあるので、異常が認められた場合には、直ちに投与を中止し、適切な処置を行うこと。

(2) その他の副作用

以下の副作用が認められた場合には、症状に応じて適切な処置を行うこと。

	0.1%～1%未満	0.1%未満	頻度不明
過敏症※1		発疹、瘙痒感	蕁麻疹
血　液	好酸球増多、白血球減少、血小板減少		
肝　臓	AST(GOT)上昇、ALT(GPT)上昇、Al-P上昇、LDH上昇、γ-GTP上昇		
消化器	便秘、嘔気、腹部膨満感	嘔吐、胸やけ、下痢	

※1このような場合には投与を中止すること。

3.高齢者への投与

一般に高齢者では消化器機能が低下していることがあるので、減量（1日100mg）するなど患者の状態を観察しながら投与することが望ましい。

4.妊婦、産婦、授乳婦等への投与

(1) 妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上まわると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。］

(2) 授乳中の婦人には本剤投与中は授乳させないよう注意すること。［動物実験（ラット）で乳汁中への移行がみられたとの報告がある。］

5.小児等への投与

小児等に対する安全性は確立していない（使用経験がない）。

※詳細については、製品添付文書をご参照ください。

亜鉛含有胃潰瘍治療剤　Promac granules 15%

指定医薬品

プロマック®顆粒15%

Promac® ポラプレジンク製剤

薬価基準収載

（製造発売元）〒103-8351 東京都中央区日本橋小舟町10-11

ゼリア新薬工業株式会社

（資料請求先）医薬部　☎03(3661)0277

2002年2月改訂（第4版）

YAKUGAKU ZASSHI 第 124 巻 増刊号 Suppl. 1

第 14 回金属の関与する生体関連反応シンポジウム

プログラム・アブストラクト集

The 14th Symposium on Roles of Metals in Biological Reaction, Biology and Medicine '04 — Program and Abstracts —

2004年(平成16年)6月1日 発行

発行: 社団法人 日本薬学会
東京都渋谷区渋谷 2 丁目 12 番 15 号(〒150-0002)

編集: 日本薬学会物理系薬学部会
第 14 回金属の関与する生体関連反応シンポジウム
実行委員会
静岡市谷田 52-1(〒422-8526)
静岡県立大学・公衆衛生学・生体衛生学研究室内
TEL&FAX: 054-264-5563
E-mail: arakawa@u-shizuoka-ken.ac.jp
学術大会専用 E-mail: srm2004-office@umin.ac.jp
学術大会専用ホームページ URL: http://srm2004.umin.ne.jp

編集責任者: 荒川 泰昭

印刷: 黒船印刷株式会社
静岡市登呂 2-4-25(〒422-8033)
TEL: 054-286-0236(代) FAX: 054-281-3881
E-mail: kurofune@basil.ocn.ne.jp

YAKUGAKU ZASSHI 第124巻別冊号　平成16年6月1日発行　第3種郵便物認可　発行　東京都渋谷区渋谷2の12の15 社団法人 日本薬学会　編集 第14回SRMシンポジウム実行委員会（TEL&FAX 054-264-5563）

http://yakushi.pharm.or.jp

PHARMACEUTICAL SOCIETY OF JAPAN

12-15 Shibuya 2-Chome, Shibuya-ku, Tokyo 150-0002, Japan
TEL: 81-3-3406-3321　FAX: 81-3-3498-1835
http://www.pharm.or.jp/index_e.html