行政院國家科學委員會專題研究計畫成果報告

鑭系稀土離子之多氨基酸配位化學及生物醫學之應用

計畫類別： 個別計畫 整合計畫

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個別型計畫: 計畫主持人： 張 正
共同主持人： N/A

整合型計畫： 總計畫主持人： N/A
子計畫主持人： N/A

註： 整合型計畫總報告與子計畫報告請分開編印各成一冊, 彙整一起繳送國科會。

處理方式： 可立即對外提供參考
一年候可對外提供參考
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(必要時, 本會得展延發表時限)

執行單位：交通大學生物科技研究所

中華民國 91 年 03 月 30 日

This is a research program originally started seven years ago (since 1995). Because the Institute of Biological Science and Technology of National Chiao Tung University was a new research institute, we spent most of the first two years to establish our laboratories. This includes renovating laboratory, recruiting postdoctoral research fellows (Dr. Liu, Yuh-Liang, 劉育良-目前任職永光化學公司; Dr. Chen, Chang-Yuh, 陳成裕-目前任職永勞工委員會勞工安全衛生研究所), research assistants and graduate students (陳煥源，陳玉衡，陳桂添，謝發坤，郭永斌，萬磊，潘美蓉，李亮緯，許呈安，林孟嘉，管佈雲，鄭昇沛，謝明宏，許地利，張志杰，陳家翎，宋婉貞，謝瑞偉，曾繼鋒，羅千婷，吳柏宏，林志誠，王文宏，管燕芸，藍佩菁，林俊傑，戴金華，康名慰，黃淑敏，李盈慧), and undergraduate students (陳彥璋，黃崇道，陳伯翰，王文卿) purchasing chemicals and research equipment (automatic titroprocessor, stopped-flow uv-vis spectrophotometer, diode-array uv-vis spectrophotometer, PCR, capillary and gel electrophoresis apparatus), and setting up softwares and hardwares. Over the years, we have made considerable research progress as proposed and described previously. Recent progress shall be briefly discussed as follows:

A. Ligand Preorganization in Metal Ion Complexation: Molecular Mechanics/Dynamics, Kinetics and Laser-Excited Luminescence Studies of Trivalent Lanthanide Complex Formation with Macrocyclic Ligands TETA and DOTA

The molecular mechanics and dynamics calculations, kinetics, and laser-excited luminescence studies were carried out for trivalent lanthanide (Ln^{3+}) complexes of macrocyclic polyaminopolycarboxylate ligands TETA and DOTA (where TETA is 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid and DOTA is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) to further understand the observed thermodynamic, kinetic and structural properties and to examine how ligand preorganization affects metal ion complexation. Excitation spectroscopy (emission monitored at 614.0 nm) of the $^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} was used to study the aqueous properties of the Eu^{3+}-TETA system. A stopped-flow spectrophotometric method was used to study the formation kinetics of the aqueous Ce^{3+}-TETA/DOTA systems in the pH range 6.1 – 6.7. Molecular mechanics calculation results are consistent with the proposed mechanism of $Ln(DOTA)^-$ formation, i.e. formation of a carboxylate O-bonded precursor, followed by metal ion moving into the pre-formed macrocyclic cavity. For $Ln(TETA)^-$ formation, at least two carboxylate O-bonded intermediates have been predicted and Ln^{3+} ion assisted reorganization of the TETA ligand is present. The calculated bond-distances and overall structures of $Ln(DOTA)^-$ and $Ln(TETA)^-$ were in agreement with the single crystal and solution NMR structural data. The origin of the difference in thermodynamic stability of $Ln(DOTA)^-$ and $Ln(TETA)^-$ complexes and the corresponding formation intermediates is mainly due to the differences in water-occupancy energy (i.e. whether there is an apical coordinated water molecule), the ligand strain energy and the cation-ligand interaction energy. Kinetic studies revealed that the formation rates of the $Ce(TETA)^-$ complex are smaller at lower pH and temperature but become greater at higher pH and temperature, as compared to those of the $Ce(DOTA)^-$ complex. This is attributed to

the lanthanide ion and both mono- and di-hydroxide ion assisted TETA conformational reorganization and higher kinetic activation parameters. The presence of di-hydroxide ion assisted intermediate rearrangement pathway could make $Ce(TETA)^-$ complex formation rate to be faster at higher pH and the higher activation barrier makes $Ce(TETA)^-$ complex formation rate slower at lower pH, as compared to those of the $Ce(DOTA)^-$ complex.

B. Dissociation Kinetics of Lanthanide(III) Complexes of Macrocyclic Polyaza Polycarboxylate Ligand DO2A

The acid-catalyzed dissociation rate constants of several lanthanide(III) complexes of 1,4,7,10-tetraazacyclododecane- 1,7-diacetic acid ($LnDO2A^+$, Ln = La, Ce, Pr, Nd, Sm, Eu, Gd, and Lu) have been determined at four different temperatures (i.e. 25.0°C, 31.0°C, 37.0°C, 45.0°C) in aqueous media (μ = 0.10 M, HC1/$LiClO_4$). In the presence of 0.005 – 0.046 M HCl, the dissociation reactions follow the simple rate law: $-d[ML]T/dt = k_H[H^+][ML]_T$, where k_H is the dissociation rate constant for the pathway involving monoprotonated species. The absence of an acid-independent pathway indicates that $LnDO2A^+$ complexes are kinetically rather inert. The obtained k_H values follow the order: $LaDO2A^+ > CeDO2A^+ > PrDO2A^+ > NdDO2A^+ > SmDO2A^+ > EuDO2A^+ > GdDO2A^+ > LuDO2A^+$, consistent with their thermodynamic stability order, i.e. the more thermodynamically stable the more kinetically inert. The rate activation parameters, $\Delta H^‡$, $\Delta S^‡$ and $\Delta G^‡$, for the acid- catalyzed dissociation pathway have also been obtained. In particular, an isokinetic behavior is found, i.e. a linear relationship between $\Delta H^‡$ and $\Delta S^‡$ values, which supports a common reaction mechanism. The data are compared with those obtained previously for other lanthanide(III) complexes and the dissociation rate order is: $LnK21DA^+ > LnK22DA^+ > LnDO2A^+ > LnDO3A > LnDOTA^-$, where K21DA is 1,7-diaza-4,10,13- trioxacyclo- pentadecane-N,N'-diacetic acid, K22DA is 1,10-diaza-4,7,13,16- tetraoxacyclooctadecane-N,N' -diacetic acid, DO3A is 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, and DOTA is 1,4,7,10-tetraazacyclododecane-1,4,7,10- tetraacetic acid. This is also consistent with the order of thermodynamic stability.

C. Macrocyclic Lanthanide Complexes as Artificial Nucleases：Hydrolysis of Phosphodiester Bonds by LnDO2A and LnK21DA

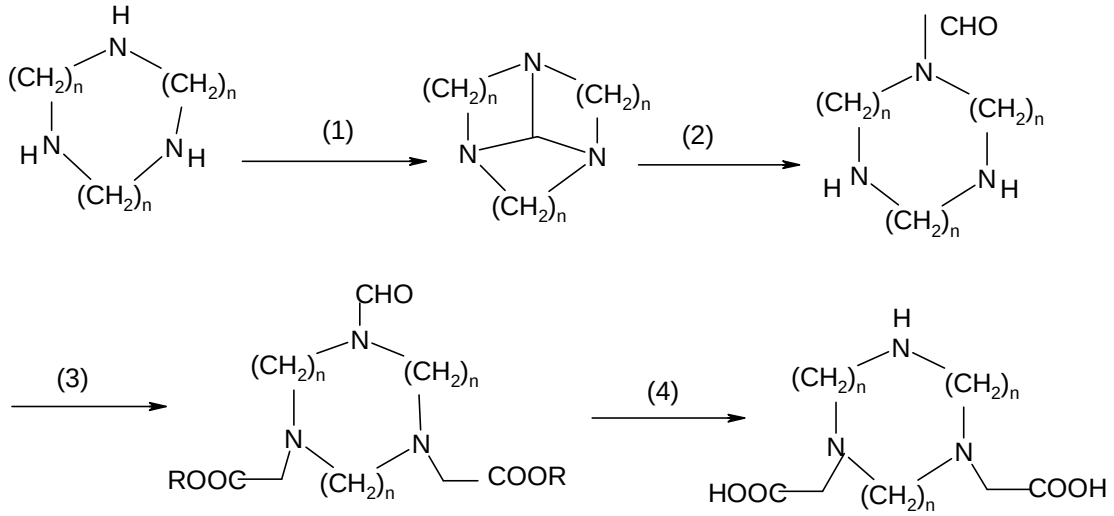
The phosphodiester bond in DNA is very stable with an estimated $t_{1/2}$ to be 130,000 years and nature has evolved a number of enzymes such as restriction endonucleases and topoisomerases to efficiently catalyze DNA hydrolysis. Recently considerable effort has been expended to develop small molecules as artificial nucleases to hydrolyze DNA, and some trivalent lanthanide complexes are known to promote phosphodiester bond quite efficiently. We have studied the hydrolysis of phosphodiester bond of a model compound BNPP（sodium bis(4-nitrophenyl) -phosphate）, a plasmid DNA（pUC19）, and a model of 5'-cap of mRNA (GpppG) by the use of $LnDO2A^+$（Ln = Ce and Eu）and $LnK21DA^+$（Ln = La and Eu）complexes, where DO2A is 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid and K21DA is 1,7-diaza-4,10,13-

trioxacyclopentadecane-N,N'-diacetic acid. In general, $LnDO2A^+$ is a better cleavage agent than $LnK21DA^+$, presumably due to the fact that $LnDO2A^+$ has at least 3 inner-sphere coordinated water molecules, one more than that of $LnK21DA^+$. The pH-rate profile for the hydrolysis of BNPP by both $LnDO2A^+$ and $LnK21DA^+$ shows interesting titration-like curves which is tentatively explained by the stepwise formation of coordinated hydroxide species, perhaps through forming hydroxo-bridged di- or more complex multinuclear clusters, and which act as the active catalysts. The rates for the hydrolysis of plasmid DNA and mRNA model have also been measured which indicates that our lanthanide(III) complexes are roughly 10-times faster than those of other transition or lanthanide complexes reported previously.

D. Synthesis and Characterization of Macrocyclic Ligands to be Used for Lanthanide Artificial Nucleases and MRI Contrast Agents

Lanthanide complexes as artificial nucleases and magnetic resonance imaging（MRI）contrast agents require high thermodynamic stability and low kinetic lability. We have designed a number of macrocyclic ligands for such purposes and the synthetic details and characterizations are reported in this paper.

NO2A and1,5-di-(carbomethyl)-1,5,9-triazacyclododecane：



n=2 or 3,

(1) Me2NCH(OMe)2, benzene, reflux, 2-4 h (~100%)

(2)EtOH-H2O, room temp., 2 h (70~85%)

(3)BrCH2COOR', iPr2NEt, MeCN (~90%)

(4)H+

E. Publications (refereed, 1996-present) Total publications = 97

1. **C.A. Chang**. "Selectivity of Macrocyclic Aminocarboxylates for Alkaline Earth Metal Ions and Stability of Their Complexes", *J. C. S. Dalton Trans.*, 1996, 2347-2350.
2. **C.A. Chang**. "Dissociation Kinetics of Nickel(II), Zinc(II), and Cadmium(II) Complexes of 1,7-Diaza-4,10,13-trioxacyclopentadecane-N,N'-diacetic Acid and 1,10-Diaza-4,7,13,16-tetraoxacyclooctadecane-N,N'-diacetic Acid", *J. Chin. Chem. Soc. (Taipei)*, 1996, **43**, 419-426.
3. L. Wan, M.-Y. Luo, **C.A. Chang**, Y.-L. Lin, and E.-R. Chan. "*Helicobacter pylori* Induced Genes Expression in Human Gastric Cells Identified by mRNA differential Display", *Biochem. Biophys. Res. Commun.* 1996, **228**, 484-488.
4. **C.A. Chang**. "Macrocyclic Lanthanide Coordination Chemistry", *Proc. Natl. Sci. Counc. ROC(A)*, 1997, **21**, 1-13.
5. J.L. Laing, R.W. Taylor, and **C.A. Chang**. "The Acid-Catalyzed Dissociation of the Copper(II) and Lead(II) Complexes of Macrocyclic Diazapolyoxa-N,N'- diacetic Acid", *J. C. S. Dalton Trans.*, 1997, 1195-1200.
6. **C.A. Chang**, F.-K. Shieh, Y.-L. Liu, Y.-H. Chen, H.-Y. Chen, and C.-Y. Chen. "Capillary Electrophoresis, Potentiometric and Luminescence Studies of Lanthanide(III) Complexes of 1,7-Dicarboxymethyl-1,4,7,10-tetraaza -cyclododecane (DO2A)", *J. C. S. Dalton Trans.*, 1998, 3243-3248.

7. **C.A. Chang**, F.-K. Shieh, Y.-L. Liu, and C.-S. Chung. “Effects of Chain Length and Terminal N-alkylation on the Protonation Constants and Stability Constants of Some Transition Metal Complexes of Linear Tetraaza and Pentaaza Ligands”, *J. Chin. Chem. Soc. (Taipei)*, 1998, **45**, 753-759.
8. E.-R. Chan, **C.A. Chang**, T.-Z. Wu, and Y.-L. Lin, “Effects of Recombinant Lysostaphin on Cytotoxicity and Interleukin-8 Level in Normal Human Epidermal Keratinocites Cell Lines”, *Biotech. Lett.,* 1998, **20**, 1053-1056.
9. E.-R. Chan, M.-R. Pan, **C.A. Chang**, T.-Z. Wu, and Y.-B. Kuo, “A Synthetic Complement C1q-like Peptide Selectively Interacts with Immune Complexes”, *Biotech. Lett.,* 1998, **20**, 1119-1123.
10. **C.A. Chang,** H.-Y. Chen, and C.-Y. Chen**. “**Determination of Stability Constants of Metal Ion Complexes by Capillary Electrophoresis.” *J. Chin. Chem. Soc. (Taipei)*, 1999, **46**, 519-528.
11. E.R. Chan, C.C. Chang, and **C.A. Chang**. “Purification and Characterization of Neutral Sphingomyelinase from *Helicobacter pylori*”, *Biochemistry,* 2000, **39**, 4838-4845.
12. **C.A. Chang** and Y.-L. Liu. “Dissociation Kinetics of $Ce(TETA)^{-}$ and $Ce(DOTA)^{-}$”, *J. Chin. Chem. Soc. (Taipei),* 2000, **47**, 1001-1006.
13. K.-T. Chen, J.-D. Lin, T.-C. Chao, **C.A. Chang**, H.-F. Weng, and E.-C. Chan. “Quantitative Monitoring of Gene Expression Patterns in Metastatic and Follicolar Human Thyroid Carcinoma Using a Complementary DNA Array”, *Thyroid,* 2001, **11**, 41-46.
14. **C.A. Chang**, Y.-L. Liu, C.-Y. Chen, X.-M. Chou, and J.-S. Ho. “Ligand Preorganization in Metal Ion Complexation: Molecular Mechanics/Dynamics, Kinetics and the Laser-Excited Luminescence Studies of Trivalent Lanthanide Complex Formation with Macrocyclic Ligands DOTA and TETA”, *Inorg. Chem*, 2001, **40**, 3448-3455.
15. D.-L. Sheu, H.-A. Fan, K.-C. Hsu, **C.A. Chang**, Y.-S. Li, C.-C. Chiou, and E.-C. Chan. “Down-Regulation of Matrix Gla Protein Messenger RNA in Human Colorectal Adenocarcinomas”, 2002, *Disease of the Colon and Rectum,* submitted.
16. **C.A. Chang** and P.-Y. Kuan. “Effects of pH on the Rates of Phosphate Diester Hydrolysis by Macrocyclic lanthanide Complexes as Artificial Nucleases”. 2002, to be submitted.
17. **C.A. Chang,** M.C. Lin, and C.F. Tseng. “Expression and Optimization of recombinant Human Placental Lactogen in *E. coli.*” 2002, to be submitted.
18. **C.A. Chang** and C.-L. Chen. “Dissociation Kinetics of $Ln(DO2A)^{+}$.” 2002, to be submitted.
19. **C.A. Chang** and C.-L. Chen. “Kinetics of DNA/RNA Hydrolytic Scission by Macrocyclic Lanthanide Complexes $Ln(DO2A)^{+}$.” 2002, to be submitted.

Other Articles

1. 張正，"生化醫藥造福人群-生物科技簡介"、交大友聲雜誌，347期，48-49頁，民國83年12月31日出刊。
2. 張正，"生物科技蓄勢待發-交大生物科技研究所簡介"、交大友聲雜誌，348期，51-52頁，民國84年2月28日出刊。
3. 張正，"交大生物科技學系增設計畫"、交大友聲雜誌，356期，8-11頁，民國85年6月1日出刊。
4. 張正，"從恐龍基因及基因專利談起：淺論生物科技與交大"、交大世紀之慶學術研討論文集，交大理學院百年校慶特刊，167-174頁，民國85年6月31日出刊。
5. 張正、張永立、“生物科技產業之人力資源培育”、中華亞太經濟與管理學會「國家競爭力與人力資源再造策略」研討會論文，中華民國 86 年 12 月 5 日，台灣高雄，中山

大學。

6. 李秀眉、沈燕士、張正*，“生物技術產業”，勞委會職訓局「行職業展望」第十三輯，中華民國 89 年 11 月。

F. Recent Abstracts and Papers Presented at Scientific Meetings (1996 - 2002)

1. **C.A. Chang**. "Macrocyclic Lanthanide Complexes as Magnetic Resonance Imaging (MRI) Contrast Agents." *1996 Symposium on Isotope and Radiation Application,* Lungtan, Taiwan, April 26-27, 1996.
2. **C.A. Chang**. "Metal Complexes as Magnetic Resonance Imaging (MRI) Contrast Agents." *Second APSB International Symposium & Workshop,* Hong Kong, July 8-11, 1996.
3. **C.A. Chang**. "Metal Chelate Nuclear Medicine and Magnetic Resonance Imaging Contrast Agents“, *1996 Research Conference, Atomic Energy Council, R.O.C.*, Lung-Tan, Taiwan, November 20-21, 1996.
4. **C.A. Chang**. “Capillary Electrophoresis, Potentiometric, Luminescence, and Molecular Mechanics Studies of Lanthanide(III) Complexes of Some Macrocyclic Tetraazapolycarboxylate Ligands”, *1996 Annual Meeting of the Chinese Chemical Society (Taipei)*, Kaohsiung, Taiwan, December 6-8, 1996. **[Invited Lecture]**.
5. Y.-H. Chen and **C.A. Chang**. “Aqueous Solution Properties of Eu^{3+}-DO2A System: A Laser-Excited Luminescence Study.” *1996 Annual Meeting of the Chinese Chemical Society (Taipei)*, Kaohsiung, Taiwan, December 6-8, 1996.
6. F.-K. Shieh, Y.-L. Liu and **C.A. Chang**. “Effects of Chain Length and Terminal N-alkylation on the Protonation Constants and Stability Constants of Some Transition Metal Complexes of Linear Tetraaza and Pentaaza Ligands.” *1996 Annual Meeting of the Chinese Chemical Society (Taipei)*, Kaohsiung, Taiwan, December 6-8, 1996.
7. Huan-Yuan Chen, Chang-Yuh Chen and **C.A. Chang. “**Determination of Stability Constants of Metal Ion Complexes by Capillary Electrophoresis.” *1996 Annual Meeting of the Chinese Chemical Society (Taipei)*, Kaohsiung, Taiwan, December 6-8, 1996.
8. Xiu-Mei Chou, Jyh-Shyong Ho and **C.A. Chang**. “Preorganization in Metal Ion Complexation: A Molecular Mechanics Study of Lanthanide Complex Formation with Macrocyclic Ligands DOTA and TETA.” *1996 Annual Meeting of the Chinese Chemical Society (Taipei)*, Kaohsiung, Taiwan, December 6-8, 1996.
9. **C.A. Chang** and Y.-H. Chen. “Laser Luminescence Study of Aqueous Solution Properties of Eu^{3+}-DO2A System.” 213th ACS National Meeting at San Francisco, April 13-17, 1997.
10. M.-R. Pan, C.-H. Lin, **C.A. Chang**, and E.-C. Chan. “Development of C1q Like Peptide for Using as an Universal Tracer in Immunoassay.” *The Twelfth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, April 19-20, 1997.
11. L. Wan, M.-Y. Lue, **C.A. Chang**, Y.-L. Lin, and E.-C. Chan. “*Helicobacter Pylori* Induced Genes Expression in Human Gastric Cells Identified by mRNA Differential Display.” *The Twelfth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, April 19-20, 1997.
12. **C.A. Chang**. “Capillary Electrophoresis and Laser-Excitation Fluorescence Studies of Lanthanide Complex Equilibria”. *6th International Seminars on Electroanalytical Chemistry* (6th ISEC), Changchun, China, Oct. 10-12, 1997 (invited speaker).

13. **C.A. Chang**." The Physicoanalytical Chemistry of Lanthanide and Transition Metal Magnetic Resonance Imaging Contrast Agents". *7th International Beijing Conference and Exhibition on Instrumental Analysis* (7th BCEIA), Shanghai, China, Oct. 14-17, 1997 (invited speaker).
14. Y.M. Wang and **C.A. Chang**. "Metal Chelate Nuclear Medicine and Magnetic Resonance Imaging Contrast Agents", *1997 Research Conference, Atomic Energy Council, R.O.C.*, Lung-Tan, Taiwan, November 26-27, 1997.
15. S.-P. Cheng, E.-C. Chan and **C.A. Chang**. "The Use of Piezoelectric Crystal Sensor for the Diagnosis of Human *Helicobacter Pylori". 1997 Annual Meeting of the Chinese Chemical Society (Taipei)*, Hsinchu, Taiwan, December 5-7, 1997.
16. M.-R. Pan, Y.-B. Kuo, C.-H. Lin, E.-C. Chan and **C.A. Chang**. "Isolation of Porcine Complement C1q and the Development of a C1q-like Peptide for Immunoassay".. *1997 Annual Meeting of the Chinese Chemical Society (Taipei)*, Hsinchu, Taiwan, December 5-7, 1997
17. Chang-Yuh Chen and **C.A. Chang. "**Determination of Stability Constants of Metal Ion Complexes by Capillary Electrophoresis. II. The Method of Metal-Ligand Double Exchange." *1997 Annual Meeting of the Chinese Chemical Society (Taipei)*, Hsinchu, Taiwan, December 5-7, 1997.
18. S.-P. Cheng, E.-C. Chan and **C.A. Chang**. "Development of a Piezoelectric Immunosensor for the Detection of Anti-*Helicobacter Pylori* Antibodies *". 1998 The Thirteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, April 18-19, 1998.
19. **C.A. Chang**, Y.-L. Liu and C.-Y. Chen. "Kinetics and the Laser-Excited Luminescence Study of Solution Properties of the Ce^{3+}/Eu^{3+}-TETA/DOTA Systems". *1998 Annual Meeting of the Chinese Chemical Society (Taipei)*, Chia-Yi, Taiwan, November 27-29, 1998.
20. D.-L. Shi, E.-C. Chan and **C.A. Chang**. "Expression of the Matrix Gla Protein in Colorectal Tumor Cells *". 1999 The Fourteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, April 10-11, 1999.
21. Y.-L. Yen, C.-C. Chiou, **C.A. Chang**, and E.-C. Chan. "The Study of Melanoma Antigen Gene Expression in Colorectal Cancer by RT-PCR *". 1999 The Fourteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, April 10-11, 1999.
22. **C.A. Chang**. "Biotechnology in Taiwan". Taiwan-Australia Collaboration Research Symposium, Taipei, Taiwan, April 8-9, 1999.
23. **C.A. Chang**. "Recent Advances of Biotechnology R&D in National Chiao Tung University". 高科技產業發展趨勢暨技術合作與投資研討會, Taipei, Taiwan, May 1, 1999.
24. **C.A. Chang**. "Strategies for Biotechnology Industry and Academic Institutions in Taiwan." Annual Conference of Midwest Chinese American Science & Technology Association, St. Louis, MO, U.S.A. June 11-13, 1999.
25. **C.A. Chang**. "Metal Complexes as Magnetic Resonance Imaging Contrast Agents and DNA/RNA Cleavage Agents", Annual Conference of Midwest Chinese American Science & Technology Association, St. Louis, MO, U.S.A. June 11-13, 1999.
26. D.L. Sheu, **C.A. Chang,** and E.-C. Chan. "Expression of Cell Cycle Regulator Genes CDC25, Wee1HU, and Proto-Oncogen C-MYC in Human Colonrectal Cancer". *2000 The Fifthteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 25-26, 2000.
27. K.-T. Chen, **C.A. Chang,** and E.-C. Chan. "Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Array in Metastatic and Human Thyroid Follicular

Tissues". *2000 The Fifthteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 25-26, 2000.

28. K.-T. Chen, J.D. Lin, M.J. Liou, **C.A. Chang,** C.C. Chiou, and E.-C. Chan. "Detection of Distinctive Gene Expression in Thyroid Follicular Carcinoma Cell Line by Using cDNA Array Technology". *The Sixteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 24-25, 2001.
29. J.W. Hsieh, J.D. Lin, M.J. Liou, **C.A. Chang,** and E.-C. Chan. "A Rapid Modified Method of Polymerase Chain Reaction for Detection of Point Mutated-Iodide Transport Gene". *The Siteenth Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 24-25, 2001.
30. Chih-Huai Chen, Wen-Joan Chiang, Pei-Lin Kang, Lie-Fen Shyur, Ning-Sun Yang, **C.A. Chang**, Chi-Meng Tzeng. "Establishment of Platform Technology of Pharmacogenomics for Herbal Medicine Validation. (Molecular pharmaco-mechanism of Chinese Herbal Medicine (CHM))". *IBC's Annual International Microtechnology Event*, San Diego, CA, U.S.A., Oct. 28-Nov. 1, 2001.
31. Chih-Huai Chen, Wen-Joan Chiang, Pei-Lin Kang, Lie-Fen Shyur, Ning-Sun Yang, **C.A. Chang**, Chi-Meng Tzeng. "Establishment of Platform Technology of Pharmacogenomics for Herbal Medicine Validation. (Molecular pharmaco-mechanism of Chinese Herbal Medicine (CHM))". *IBC's Annual International Microtechnology Event*, San Diego, CA, U.S.A., Oct. 28-Nov. 1, 2001.
32. **C.A. Chang**; Chia-Ling Chen. "Dissociation Kinetics of Lanthanide(III) Complexes of Macrocyclic Polyaza Polycarboxylate Ligand DO2A". *2001 Annual Meeting of the Chinese Chemical Society (Taipei)*, Tainan, Taiwan, December 29-30.
33. **C.A. Chang**, Pu-Yun Kuan, Chia-Ling Chen, Po-Hong Wu. "Macrocyclic Lanthanide Complexes as Artificial Nucleases：Hydrolysis of Phosphodiester Bonds by LnDO2A and LnK21DA". *2001 Annual Meeting of the Chinese Chemical Society (Taipei)*, Tainan, Taiwan, December 29-30.
34. **C.A. Chang**, Wen-Hung Wang, Chun-Chieh Lin, Bo-Hung Wu, Yen-Yun Kwan, and Chih-Cheng Lin. "Synthesis and Characterization of Macrocyclic Ligands to be Used for Lanthanide Artificial Nucleases and MRI Contrast Agents". *2001 Annual Meeting of the Chinese Chemical Society (Taipei)*, Tainan, Taiwan, December 29-30.
35. P.C. Lan and **C.A.Chang**. "Cloning of Recombinant Porcine Placental Lactogen in E. coli." *The Seventh Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 23-24, 2002.
36. C.F. Teng, M.C. Lin, and **C.A. Chang**. "Expression and Renaturation of Recombinant Human Placental Lactogen in E. coli." *The Seventh Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 23-24, 2002.
37. K.T. Chen, J.D. Lin, M.J. Liou, **C.A. Chang**, and E.C. Chan. "Characterization of Distinctive Gene Expression in Thyroid Follicular Carcinoma Cells." *The Seventh Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 23-24, 2002.
38. J.W. Hsieh, F.S. Lo, **C.A. Chang**, and E.C. Chan. "Development of a Method for a High Throughput Screening Gene Variation." *The Seventh Joint Annual Conference of Biomedical Sciences*, Taipei, Taiwan, March 23-24, 2002.

Conference/Symposium Organized

1. The 3rd International Symposium of Worldwide Chinese Scholar on Analytical Chemistry (第三屆國際華裔學者分析化學研討會). Hong Kong, December 16-19, 1998.
2. Taiwan-Australia Collaboration Research Symposium (台澳科技合作研討會).

Bio-Medical Technologies Session, Taipei, Taiwan, April 8-9, 1999.

3. 高科技產業發展趨勢暨技術合作與投資研討會,生化組. 台北, 台灣, May 1, 1999.
4. 二十一世紀知識經濟研討會,生物科技發展趨勢及策略分析,交大思源金會,台北,台灣, May 12, 2001.

Thesis Supervised (Since 1980: total 45+ Ph.D. and M.S. students, 10+ postdoctoral fellows)

	學生姓名	論文題目
1. M.S.	謝發坤 F.K. Shieh	多胺與多乙酸基配位子與金屬離子錯合物穩定常數研究 The Stability Constants Studies of Metal Ion Complexes of Polyamines & Polyamino Polycarboxylates
2. M.S.	郭永斌 Y.B. Kuo	豬源補體蛋白 C1q 的純化及定性與定量之研究 The Purification and Characterization of Complement C1q From Swine
3. M.S.	萬磊 L. Wan	幽門螺旋桿菌感染為組織細胞所引發基因差異表現之研究 Study of the Differential Gene Expression in Human Gastric Cells Infected with *Helicobacter pylori*
4. M.S.	潘美蓉 M.R. Pan	豬源補體蛋白 C1q 之製備與其類 peptide 應用之研究 The Preparation and Applications of Porcine C1q and The C1q-like Peptide
5. M.S.	李亮緯 L.W. Lee	結腸直腸癌基因差別表現之研究 Study of Genes Expression in Colorectal Adenocarcinoma by DDRT-PCR Method
6. M.S.	許呈安 T.A. Hsu	甲狀腺腫瘤標記之研究 The Search of Tumor Markers for Thyroid Carcinoma
7. M.S.	鄭昇沛 S.P. Cheng	壓電晶體免疫感測器於醫療檢驗之應用 The Application of Piezoelectric Crystal Immunosensor in Clinical Diagnosis
8. M.S.	謝明宏 M.H. Shieh	固定細胞生產和回收溶葡萄球菌素之研究 Study of the *in situ* Recovery of Lysostaphin by Immobilized Recombinant Cells
9. M.S.	管佈雲 P.Y. Kuan	鑭系金屬與大環配位子錯合物切割磷酸二酯與去氧核醣核酸動力學及催化效力之研究 Kinetic & Catalytic Activity Study of Lanthanide Complex of DO2A & K21DA in Phosphate Diester & DNA Hydrolysis
10. M.S.	張永立 Y.L. Chang	台灣醫療儀器產業環境與市場競爭策略 The Environment of Medical Instrument Industry and Its Competitive Strategies in Taiwan
11. M.S.	林孟嘉 M.G. Lin	以大腸桿菌表現人類胎盤促乳激素之研究 The Expression of Human Placental Lactogen by *E. coli* Expression System
12. M.S.	許地利 D.L. Hsu	大腸直腸分子標幟之研究 Study of the Bio-molecular Markers in Colorectal Cancers
13. M.S.	張至杰 C.G. Chang	幽門螺旋桿菌鞘磷脂脢的純化及其特性之研究 Purification and Characterization of the Sphingomyelinase of *Helicobacter pylor*
14. M.S.	陳家翎 G.L. Chen	鑭系金屬與大環多胺多酸基配位錯合物的動力學研究及其在DNA/RNA 水解上的應用 Kinetics Studies of Lanthanide Polyaza Polycarboxylate Macrocyclic Complexes Ln(DO2A) and Their Applications in DNA/RNA Hydrolytic Scission
15. M.S.	宋婉貞 W.C. Song	以大腸桿菌表現人類表皮生長因子接受體-2 之研究 Expression of Recombinant Human Epidermal Growth Factor Receptor-2 in *E*

		coli
16. Ph.D.	陳桂添 K.T. Chen	甲狀腺腫瘤分子標螺旋桿菌之膜蛋白 硝磷酸酵素之定性定量研究 Studies of Potential Protein Markers of Thyroid Carcinoma
17. M.S.	曾繼鋒 G.F. Tseng	Optimization of the expression of human placental lactogen by *E. coli* expressior system
18. Ph.D.	羅千婷 C.T. Lo	多醣真菌菌絲體之篩選與基因比對 Polysarcharide and DNA Ringer Printing of Microorganisms
19. M.S.	管燕芸 Y.Y. Kuan	稀土配位化合物之 DNA/RNA 切割劑 Artificial Lanthanide DNA/RNA Nucleases
20. M.S.	謝瑞偉	A Rapid Modified Method of Polymerase Chain Reaction for the Dection of Point Mutation in Sodium Iodide Symporter Gene
21. M.S.	王文宏 W.H. Wang	稀土配位化合物之 DNA/RNA 切割劑 Artificial Lanthanide DNA/RNA Nucleases
22. Ph.D.	林志誠 C.C. Lin	雙體磁振造影劑 Dimeric MRI Contrast Enhancement Agents
23. Ph.D.	藍佩菁 P.G. Lan	The Functional Study of Human Placental Lactogen
24 M.S.	吳柏宏 B.H. Wu	稀土配位化合物之 DNA/RNA 切割劑 Artificial Lanthanide DNA/RNA Nucleases
25 Ph.D.	陳治淮	Fast Screening and Pharmacological Studies of Chinese Herbal Medicines Using Gene Chips
26 M.S.	林俊傑	稀土配位化合物之 DNA/RNA 切割劑 Artificial Lanthanide DNA/RNA Nucleases
27 Ph.D.	戴金華	Microorganisms Functional Genenomics, Proteomics, Glycomics, and Polysaccharides Functional Studies

In addition to the above-mentioned publications, abstracts and meetings, the PI has been invited by various institutions to present more than 60 seminars for the last 7 years.

C.A. Chang; Chia-Ling Chen. "Dissociation Kinetics of Lanthanide(III) Complexes of Macrocyclic Polyaza Polycarboxylate Ligand DO2A". *2001 Annual Meeting of the Chinese Chemical Society (Taipei)*, Tainan, Taiwan, December 29-30.

Dissociation Kinetics of Lanthanide(III) Complexes of Macrocyclic Polyaza Polycarboxylate Ligand DO2A

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The acid-catalyzed dissociation rate constants of several lanthanide(III) complexes of 1,4,7,10-tetraazacyclododecane- 1,7-diacetic acid ($LnDO2A^+$, Ln = La, Ce, Pr, Nd, Sm, Eu, Gd, and Lu) have been determined at four different temperatures (i.e. 25.0°C, 31.0°C, 37.0°C, 45.0°C) in aqueous media (μ = 0.10 M, HC1/$LiClO_4$). In the presence of 0.005 – 0.046 M HCl, the dissociation reactions follow the simple rate law: $-d[ML]T/dt = k_H[H^+][ML]_T$, where k_H is the dissociation rate constant for the pathway involving monoprotonated species. The absence of an acid-independent pathway indicates that $LnDO2A^+$ complexes are kinetically rather inert. The obtained k_H values follow the order: $LaDO2A^+ > CeDO2A^+ > PrDO2A^+ > NdDO2A^+ > SmDO2A^+ > EuDO2A^+ > GdDO2A^+ > LuDO2A^+$, consistent with their thermodynamic stability order, i.e. the more thermodynamically stable the more kinetically inert. The rate activation parameters, $\Delta H^‡$, $\Delta S^‡$ and $\Delta G^‡$, for the acid- catalyzed dissociation pathway have also been obtained. In particular, an isokinetic behavior is found, i.e. a linear relationship between $\Delta H^‡$ and $\Delta S^‡$ values, which supports a common reaction mechanism. The data are compared with those obtained previously for other lanthanide(III) complexes and the dissociation rate order is: $LnK21DA^+ > LnK22DA^+ > LnDO2A^+ > LnDO3A > LnDOTA^-$, where K21DA is 1,7-diaza-4,10,13- trioxacyclo-pentadecane-N,N'-diacetic acid, K22DA is 1,10-diaza- 4,7,13,16-tetraoxacyclooctadecane-N,N' -diacetic acid, DO3A is 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, and DOTA is 1,4,7,10-tetraazacyclododecane-1,4,7,10- tetraacetic acid. This is also consistent with the order of thermodynamic stability.

Experimental

- All reagents are of analytical reagent grade.
- Solutions were prepared and standardized by described methods.
- A HP8453 UV-Visible spectrophotometer equipped with a constant temperature circulating bath (FIRSTEK SCIENTIFIC B403) was used
- for the kinetic rate measurements using dye indicators（25℃、31℃、37℃、45℃，250 nm）。

$$A_t = A_\infty + (A_0 - A_\infty)\exp(-k_{obs}\,t)$$

Scheme 1 Possible Reaction Mechanisms

$$ML + nH^+ \xrightarrow{k_{obs}} M^{2+} + H_nL^{(n-4)+}$$

$$\begin{array}{ccccc} Ln + L & \xleftarrow{k_d} & \boldsymbol{LnL} & \xrightarrow{+H^+} & [LnHL] \xrightarrow{k_{H1}} Ln + HL \\ & & \downarrow {\scriptstyle +2H^+} & & \downarrow {\scriptstyle k_{\lim}} \\ & & LnH_2L & & [LnHL]^* \xrightarrow{fast} Ln + HL \\ & & \downarrow k_{H2} & & \\ & & Ln + H_2L & & \end{array}$$

$k_{obs} = k_d + k_H[\mathrm{H}^+]$

k_d = acid-independent rate constant (~0, for the present cases)

k_H = acid-catalyzed rate constant。

Activated Parameter Calculations and Plots (Eyring equation)

$$\ln\left(k/T\right) = \ln\left(k_B/h\right) - \left(\Delta H^*/RT\right) + \left(\Delta S^*/R\right)$$

R = 1.98716 cal・mol^{-1}K^{-1}，k_B = Boltzmann's constant (1.381× 10^{-23} JK^{-1})，h = Planck's constant (6.626× 10^{-34} J・s)。

Plots of ln(k_H/T) vs.1/T give slopes (-ΔH*/R) and intercepts (ΔS*/R + ln(k_B/h)) and activation enthalpy (ΔH*) and activation entropy (ΔS*) could be obtained。ΔG* could then be obtained by

$$\Delta G^* = \Delta H^* - T\Delta S^*$$

Results and Discussion

1. 由每一個鑭系金屬錯合物($Ln(DO2A)^+$)觀察到的解離反應速率常數（k_{obs}）與酸濃度作圖之後，所得到的圖形呈現一個線性的關係，即為一級反應(first-order)，這表示了 $Ln(DO2A)^+$與酸作用了之後所形成的中間體(LnHDO2A)並不是很穩定，因此反應進行中當一形成反應中間體會很快速的繼續進一步的解離反應。
2. 而以我們所做的八種金屬和 DO2A 所形成的金屬錯合物之解離常數(k_{obs})值來比較，會發現其解離速率快慢的順序為：$La(DO2A)^+ > Ce(DO2A)^+ > Pr(DO2A)^+ > Nd(DO2A)^+ > Sm(DO2A)^+ > Eu(DO2A)^+ > Gd(DO2A)^+ > Lu(DO2A)^+$，而此順序和金屬錯合物的穩定常數成反比，亦和金屬的離子半徑(ionic size)的減小及電荷密度的(charge density)的增加有絕對的關係。
3. 由所求得的自行解離常數(k_d)與酸催化解離常數(k_H)來看，我們發現所有 $Ln(DO2A)^+$的自行解離常數值非常的低，幾乎趨近於零因此可推知 $Ln(DO2A)^+$的解離反應可能極少經由自行解離途徑的，必須一定要在加入酸時才會進行解離的反應，由此，我們也可印證 $Ln(DO2A)^+$的穩定性是相當高的。
4. 由所求得的活化參數(activated parameter)來看，$Ln(DO2A)^+$在酸解離反應進行時所需的焓(activation enthalpy、ΔH^*)其順序為：$Lu(DO2A)^+ > Gd(DO2A)^+ > Eu(DO2A)^+ > Sm(DO2A)^+ > Nd(DO2A)^+ > Pr(DO2A)^+ > Ce(DO2A)^+ > La(DO2A)^+$，此順序也是與金屬錯合物的穩定性呈正比的關係，即金屬和配位子之間結合能力越強，穩定性越高的錯合物，相

對地要使其金屬和配位子之間的離子鍵結斷裂所需要的能量也要越多，因此，由此可再次證實：擁有高穩定常數的鑭系金屬錯合物，相對地其會擁有較緩慢的解離速率常數。

5. 由於熱力學穩定常數高的金屬錯合物，其解離的速度會相對地變慢，而根據以往的文獻整理所得到的鑭系金屬與大環配位子之穩定常數來看，可得知鑭系金屬錯合物的穩定常數之順序為 $Ln(DOTA)^-$ > $Ln(DO3A)$ > $Ln(DO2A)^+$，且 12、15、18 圓環的配位子與金屬形成錯合物後，其穩定常數的順序為 $Ln(DO2A)^+$ > $Ln(K22DA)^+$ > $Ln(K21DA)^+$，這是因為 K22DA 與 K21DA 比起 12 圓環的 DO2A 來有著較高的結構柔軟性（more flexible），並且 DO2A 的鹼性較這兩個配位子高，因此我們可推測 $Ln(DO2A)^+$之酸解離常數應符合以下的順序 $Ln(DOTA)^-$ > $Ln(DO3A)$ > $Ln(DO2A)^+$ > $Ln(K22DA)^+$ > $Ln(K21DA)^+$，而最後從結果來看，吾人的推測順序是十分正確的。

Table1. Kinetic Data (k_{obs}, s^{-1}) for the H^+-catalyzed Dissociation of Lanthanide DO2A Complexes.

Metal	T (℃)	$10^2[H^+]$ (M)			
		0.5769	1.1538	2.3077	3.4615
La	25	0.00503	0.00840	0.00802	0.0137
	31	0.00694	0.01100	0.01760	0.0265
	37	0.00802	0.01740	0.02990	0.0448
	45	0.01370	0.02480	0.04810	0.0683
Ce	25	0.00448	0.00800	0.01370	0.0220
	31	0.00692	0.01400	0.02330	0.0386
	37	0.01070	0.02230	0.03400	0.0551
	45	0.01340	0.03020	0.04530	0.0706
Pr	25		0.00672	0.00970	0.0167
	31		0.00840	0.01440	0.0255
	37		0.01360	0.02420	0.0398
	45		0.02400	0.04350	0.0680
Nd	25		0.004566	0.00553	0.0066
	31		0.006588	0.00811	0.0101
	37		0.007394	0.01030	0.0138
	45		0.008040	0.01410	0.0184
Sm	25		0.000394	0.00044	0.0005
	31		0.000485	0.00088	0.0020
	37		0.000751	0.00144	0.0028
	45		0.00151	0.00348	0.0060
Eu	25	0.00004	0.00017	0.00044	0.0006
	31	0.00038	0.00104	0.00197	0.0028
	37	0.00072	0.00146	0.00375	0.0057
	45	0.00106	0.00436	0.00748	0.0103
Gd	25	0.00002	0.00033	0.00047	0.0006
	31	0.00032	0.00057	0.00096	0.0015
	37	0.00041	0.00076	0.00174	0.0025
	45	0.00115	0.00236	0.00479	0.0071
Lu	25	0.00008	0.00015	0.00021	0.0002
	31	0.00010	0.00034	0.00053	0.0008
	37	0.00024	0.00044	0.00079	0.0012

45	0.00039	0.00109	0.00232	0.0037

[LaDO2A]=[CeDO2A]=[EuDO2A]=[GdDO2A]=0.538462 × 10^{-3}M，

[PrDO2A]=[NdDO2A]=[SmDO2A]=1.07692 × 10^{-3}M，μ=0.10M($LiClO_4$)

Table.2

	T(℃)	k_H
LaDO2A	25	0.5872 (± 0.0271)
	31	0.7520 (± 0.0160)
	37	1.3735 (± 0.0388)
	45	1.9738 (± 0.0741)
CeDO2A	25	0.6133 (± 0.0543)
	31	1.0641 (± 0.0730)
	37	1.5488 (± 0.1080)
	45	2.0517 (± 0.1078)
PrDO2A	25	0.4975 (± 0.0534)
	31	0.7068 (± 0.0579)
	37	1.2042 (± 0.0620)
	45	1.9650 (± 0.0645)
NdDO2A	25	0.4566 (± 0.0023)
	31	0.7744 (± 0.0104)
	37	1.3049 (± 0.0081)
	45	1.8220 (± 0.0124)
SmDO2A	25	0.0181 (± 0.0035)
	31	0.0572 (± 0.0092)
	37	0.0854 (± 0.0120)
	45	0.1823 (± 0.0145)
EuDO2A	25	0.0176 (± 0.0014)
	31	0.0840 (± 0.0042)
	37	0.1606 (± 0.0069)
	45	0.3694 (± 0.0137)

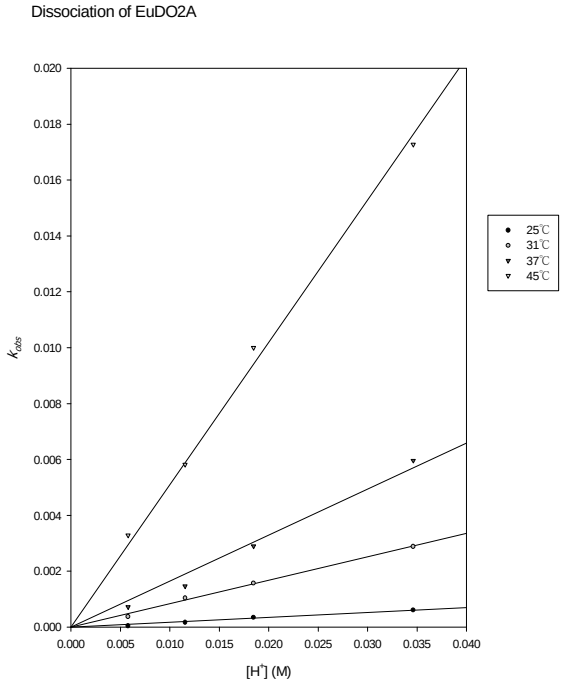
GdDO2A	25	0.0128 (± 0.0005)
	31	0.0446 (± 0.0028)
	37	0.0734 (± 0.0027)
	45	0.2662 (± 0.0012)
LuDO2A	25	0.0062 (± 0.0009)
	31	0.0249 (± 0.0012)
	37	0.0348 (± 0.0008)
	45	0.1165 (± 0.0013)

Table 3.

	ΔH^*(kcal/mol)	ΔS^*(e.u.)
LaDO2A	10.2340 (± 0.129)	-21.3270 (± 0.388)
CeDO2A	11.5722 (± 0.151)	-20.5909 (± 0.392)
PrDO2A	13.2942 (± 0.642)	-15.4699 (± 0.108)
NdDO2A	15.4574 (± 0.373)	-8.2263 (± 0.321)
SmDO2A	20.1876 (± 0.306)	1.6122 (± 0.033)
EuDO2A	27.0747 (± 0.455)	24.8598 (± 0.511)
GdDO2A	28.0271 (± 0.338)	26.8541 (± 0.453)
LuDO2A	28.1734 (± 0.555)	25.7963 (± 0.637)

Dissociation Kinetics of EuDO2A

Plots of k_{obs} vs [H+]



Eyring plot