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**

計畫編號：NSC 89-2113-M-009-010

執行期間：88 **年** 08 **月** 01 **日 至** 89 **年** 12 **月** 31 **日**

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**
  兩年候可對外提供參考
  (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 90 **年** 03 **月** 30 **日**

中文摘要

本研究計畫為一多年整合基礎與應用研究之長期計畫(1995-2000+)**，其主要目的是對鑭系金屬離子與多氨基酸配位子所形成錯合物之配位化學（熱力學、動力學、光譜學、及結構）作進一步的探討，進而找出影響鑭系稀土離子多氨基酸錯合物之物理化學性質（如穩定性、選擇性、反應速率、核磁共振、**relaxation **及螢光性質等）之重要因素，以便能在磁振造影劑、生物分子之螢光附著劑及切斷** RNA **及** DNA **反應之催化劑等有所應用。計劃執行成果要點包括：**

（1） **實驗室之建立、儀器之購買、及研究人員之招覽、訓練。**

（2） **大環多氨基酸配位子**(R-DO3A)**的合成、特性研究（**NMR**，**IR**，電化學）、及鹼土金屬離子**R-DO3A**錯化物的穩定常數及選擇性之研究。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.

（3） **過渡金屬離子與大環多氨基酸配位子**(K21DA**、**K22DA)**錯化物形成及解離反應之速率及反應機理之研究。C.A. Chang**. "Dissociation Kinetics of Nickel(II), Zinc(II), and Cadmium(II) Complexes of 1,7-Diaza-4,10,13-trioxa -cyclopentadecane-N,N'-diacetic Acid and 1,10-Diaza-4,7,13,16- tetraoxa - cyclooctadecane-N,N'-diacetic Acid", *J. Chin. Chem. Soc.(Taipei)*, 1996, **43**, 419-426; 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, 1195-120

（4） **鑭系金屬離子多氨基酸配位子錯化物之熱力學與動力學因素與多氨基酸配位子之**preorganization**或**ring strain**之能量關係之初步研究。C.A. Chang** and Y.-L. Liu. "Dissociation Kinetics of $Ce(TETA)^-$ and $Ce(DOTA)^-$", *J. Chin. Chem. Soc.*, 2000, **47**, 1001-1006; **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, accepted for publication.

(5) **毛細管電泳測定金屬離子多氨基酸配位子錯化物穩定常數方法之開發。** **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.

(6) **鑭系稀土離子與多氨基酸配位子**(DO2A)**錯化物之**NMR**及螢光光譜的特性研究。** **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-Dicarboxy - methyl-1,4,7,10-tetraazacyclododecane (DO2A)", *J. C. S. Dalton Trans.*, 1998, 3243-3248.

(7) **過渡金屬離子與線性多氨基配位子錯化物穩定常數及選擇性之研究。** **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) **其他**: 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; **C.A. Chang**. "Macrocyclic Lanthanide Coordination Chemistry", *Proc. Natl. Sci. Counc. ROC(A)*, 1997, **21**, 1-13; *Biotech. Lett.,* 1998, **20**, 1053-1056; *Biotech. Lett.,* 1998, **20**, 1119-1123; E.R. Chan, C.C. Chang, and **C.A. Chang**. "Purification and Characterization of Neutral Sphingomyelinase from *Helicobacter pylori*", *Biochemistry,* 2000, **39**, 4838-4845; 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.

關鍵詞：　多氨基酸分子配位基、大環錯化物、鑭系稀土離子、穩定常數、選擇性、形成動力學、解離動力學、螢光光譜、結構、磁振顯影劑、冠醚。

Abstract

This research project is a long-term one integrating both fundamental and applied aspects of lanthanide coordination chemistry (1995-2000+). The primary objective of the proposed research is to develop fundamental understanding of the key thermodynamic, kinetic, and structural factors that influence the desired physico-chemical properties of lanthanide complexes of macrocyclic and linear polyaminocarboxylate ligands (e.g. complex formation stability and selectivity, reaction kinetics, NMR relaxation, luminescence, and structure) for applications in magnetic resonance imaging (MRI), luminescence labeling for biomolecules and catalysis for DNA and RNA phosphate diester bond cleavage. The research results include the following:

(1) Construction of research laboratories, purchasing equipment, and personnel recruitment.

(2) Selectivity of Macrocyclic Aminocarboxylates for Alkaline Earth Metal Ions and Stability of Their Complexes. J.C.S. Dalton Trans. 1996, 2347-2350.

(3) Dissociation Kinetics of Nickel(II), Zinc(II), and Cadmium(II) Complexes of 1,7–Diaza- 4,10,13-trioxacyclopentadecane-N,N'-diacetic Acid (K21DA) and 1,10-Diaza-4,7,13,16- tetraoxacyclooctadecane-N,N'-diacetic Acid (K22DA). J. Chin. Chem. Soc. (Taipei), 1996, 43, 419-426; J.C.S. Dalton Trans. 1997, 1195-1200.

(4) Preorganization in Metal Ion Complexation: **C.A. Chang** and Y.-L. Liu. "Dissociation Kinetics of $Ce(TETA)^-$ and $Ce(DOTA)^-$", *J. Chin. Chem. Soc.*, 2000, **47**, 1001-1006; **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, accepted for publication.

(5) Determination of Stability Constants of Metal Ion Complexes by Capillary Electrophoresis. *J. Chin. Chem. Soc.(Taipei)*, 1999, **46**, 519-528.

(6) Aqueous Solution Properties of Eu^{3+}-DO2A System: A Laser-Excited Luminescence

Study. J. C. S. Dalton Trans., 1998, 3243-3248.

(7) 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) Other Studies: (1) "*Helicobacter pylori* Induced Genes Expression in Human Gastric Cells Identified by mRNA differential Display", *Biochem. Biophys. Res. Commun.* 1996, 228, 484-488; (2) "Macrocyclic Lanthanide Coordination Chemistry", *Proc. Natl. Sci. Counc. ROC(A)*, 1997, 21**,** 1-13; (3) "Effects of Recombinant Lysostaphin on Cytotoxicity and Interleukin-8 Level in Normal Human Epidermal Keratinocites Cell Lines", *Biotech. Lett.,* 1998, **20**, 1053-1056; (4) "A Synthetic Complement C1q-like Peptide Selectively Interacts with Immune Complexes", *Biotech. Lett.,* 1998, **20**, 1119-1123; (5) C.C. Chang, **C.A. Chang**, and E.R. Chan. "Purification and Characterization of Neutral Sphingomyelinase from *Helicobacter pylori*", *Biochemistry,* 2000, **39**, 4838-4845; (6) 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.

Keywords: Polyaminocarboxylates, Lanthanides Complexes, Macrocyclic and Linear Ligands, Stability Constants, Selectivity, Formation and Dissociation Kinetics, Structure, Luminescence, NMR, Molecular Mechanics.

A. Introduction

This is a research program that has been performed for more than five years (from February 1, 1995 to December 31, 2000). Because the Institute of Biological Science and Technology of National Chiao Tung University is a new research institute, we spent most of the first year to establish our laboratories. This includes renovating laboratory, recruiting postdoctoral research fellows (Dr. Liu, Yuh-Liang, **劉育良**; Dr. Chen, Chang-Yuh, **陳成裕**; Dr. Huang, Chiung-Hua, **黃瓊華**), research assistants and graduate students (**陳煥源，陳玉衡，林禎桓，謝發坤，萬磊，潘美蓉，李亮緯，許呈安，林孟嘉，管佈雲，許地利，張志杰，宋婉真，陳家翎，陳桂添，郭珍佑，曾繼鋒，羅千婷，吳柏宏，管燕芸，藍珮菁，林志誠，王文宏**), 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. On the other hand, we have made considerable research progress as originally proposed. The progress in terms of ligand/complex synthesis, physical characterizations, thermodynamics, kinetics, and molecular mechanics calculations shall be briefly discussed as follows:

B. Ligand/Complex Synthesis and Physical Characterizations, Thermodynamics, Kinetics, and Molecular Mechanics Calculations

We have synthesized the 12-membered and 14-membered tetraaza macrocycles, cyclen and cyclam - the precursors of DOTA and TETA, according to published methods. Carboxymethylation reactions have been carried out with the tetraaza macrocycles and the resulting DOTA and TETA compounds were purified and recrystallized. DOTA and Cube-like TETA crystals have been obtained. The rates of formation and dissociation reactions of Ce(DOTA)- and Ce(TETA)- have been repeatedly determined at various temperatures and pH. The detailed analysis of these rate data has been completed. The ligand, K21DA, has also been synthesized according to published method.

The ligand, 1,7-dicarboxymethyl-1,4,7,10-tetraazacyclododecane (DO2A), has been synthesized and the macrocycle ring protonation sites have been determined by NMR techniques to be the secondary amine nitrogen atoms. The protonation constants (log K: 10.48, 10.01, 3.85, 2.55) and the stability constants of trivalent lanthanide (Ln^{3+}) metal complexes of DO2A (log KML: 10.94-13.31) were determined by the potentiometric pH titration and capillary electrophoresis methods, respectively. In general, the stabilities of the $Ln(DO2A)^{+}$ complexes increase with increasing atomic number for the lighter lanthanides (La^{3+} - Sm^{3+}) and remain relatively unchanged for the heavier lanthanides (Eu^{3+} - Lu^{3+}). Laser-excited spectroscopy of the $^{7}F_o$ -> $^{5}D_o$ transition of Eu^{3+} is used to study the aqueous Eu^{3+} - DO2A complex system. At low pH (e.g. pH 5 ~ 6), Eu^{3+} forms a 1:1 species with the ligand DO2A, presumably $Eu(DO2A)(H_2O)q^{+}$, where q is the number of inner-sphere coordinated water molecules. As the solution pH increases, the hydrolysis product, Eu(DO2A)(OH)(H_2O)q-1, is formed. Lifetime measurements of each species in H_2O and D_2O allow the determination of the corresponding number of the inner-sphere coordinated water molecules to be 2.96 and 2.64, consistent with the proposed structures (i.e. q = 3). The hydrolysis constant (pK_h) is estimated to be 8.1 ± 0.3. These results were reproducibly obtained by different researchers.

The use of $Eu(DO2A)^{+}$ and $Eu(K21DA)^{+}$ complexes to catalyze the hydrolysis of phosphate ester bond has been tested. Initial results indicated that both complexes are active in this regard. Detailed studies are underway.

The determination of ligand protonation constants and stability constants of metal complexes of several linear polyamine ligands has been of interest because of the continued desire of making metal-selective reagents and use of data as reference to understand the thermodynamic origin of “macrocyclic effect”. A number of terminal N-alkylated linear tetraaza and pentaaza ligands have been prepared, e.g. (2,2,2), (2,3,2), (3,2,3), etc. Their ligand protonation constants and some transition and post-transition

metal (Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}) complex stability constants have been determined by potentiometric titration methods. In general, methylation and ethylation at the terminal nitrogen atoms cause the corresponding ligand nitrogen basicity to increase; however, the corresponding metal complex stabilities are decreased as compared to the non-alkylated structural analogs, presumably due to the steric effect.

The linear polyamines, the precursors for the 15-membered pentaaza macrocycles, 1,4,7,10,13-pentaazapentadecane (2.2.2.2) and its structural analogue (2.2.3.2.) have been synthesized according to methods established by Dr. Liu, Yuh-Liang. The two linear polyamines will be used to carry out cyclization and carboxymethylation reactions as proposed.

To design ligands with fast complex formation rates for a number of applications such as solvent extraction and diagnostic imaging agent manufacturing, we have decided to synthesize linear aminopolycarboxylate ligands selective toward lanthanide metals ions. Thus, polyamines (2.2.2.), (2.3.2.), (3.2.3.), (3.3.3.), (2.2.2.2.) and (2.2.3.2) with different cumulative backbone ring strain have been prepared with methylation or ethylation to replace one proton of each terminal amine functional group. We are currently carrying out the carboxymethylation reactions to prepare the corresponding aminopolycarboxylic acids. (PI Note: Due to the unexpected resignation of my postdoctoral fellows, Dr. Chin, **秦建譜** and Dr. Huang, Chiung-Hua, **黃瓊華**, the synthetic and other characterization work has been delayed.) For comparison purpose, 4,8-dicarboxymethyl-1,4,8,11-tetraazaundecane has also been prepared.

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 $^7F_0$ ì $^5D_0$ 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.

The acid-catalyzed dissociation rate constants of the cerium(III) complexes of 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) have been determined at four different temperatures (i.e. 25.0°C, 32.0°C, 39.0°C, 45.0°C) in aqueous media (« =

0.10 M, HC1/KCl) to obtain additional kinetic data and to evaluate possible effects of ligand pre-organization for metal ion complexation. The rates are much faster for Ce(TETA)⁻ than for Ce(DOTA)⁻, indicating the lower thermodynamic stability of the former. In the presence of excess strong acid, 0.1 - 1.0 M HCl, the dissociation reactions follow the rate law: $-d[ML]_T/dt = (k_d + k_H[H^+])[ML]_T$ and $-d[ML]_T/dt = (k_H[H^+] + k_{H2}[H^+]^2)[ML]_T$, respectively, where k_d is acid-independent dissociation reaction rate constant and k_H and k_{H2} are the respective dissociation rate constants for the pathways involving monoprotonated and diprotonated species. The rate activation parameters, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$, for each dissociation pathway have been obtained and their values are consistent with the proposed mechanisms. In particular, the rate difference between Ce(TETA)⁻ and Ce(DOTA)⁻ for the monoprotonated complex dissociation pathway is mainly due to difference in $\Delta H_H^{\ddagger}$. It has been concluded that ligand pre-organization results in more stable complexes and slower complex dissociation rates.

A new method for the determination of stability constants by capillary electrophoresis has been developed, particularly for kinetically inert metal complexes. Different approaches, i.e., direct formation, ligand exchange, metal exchange and metal-ligand double exchange were studied. Stability constants of lanthanide (La^{3+}, Ce^{3+}, Eu^{3+} and Yb^{3+}) and transition metal (Ni^{2+}, Cu^{2+} and Zn^{2+}) ions with DO2A (1,7-dicarboxymethyl-1,4,7,10-tetraazacyclododecane) were measured under direct complex formation. In ligand exchange approach, stability constants were measured by complex formation of Ni^{2+} with DO2A and Et_2-2,3,2-tet (3,6,10,13-tetraazaundecane). Stability constants of DO2A with Cu^{2+} and Zn^{2+} complexes were measured in metal exchange approach. Complex formation competitions of Ni^{2+} and Cu^{2+} between DO2A and Et_2-2,3,2-tet were studied in metal-ligand double exchange.

To verify the capillary electrophoresis method, we have also determined the stability constant of a known metal complex system, i.e. Cu^{2+}/Zn^{2+} - EDTA/HEDTA (N-hydroxyethyl-ethylenediaminetriacetic acid), using the metal-ligand double exchange

approach. Assuming that the stability constants of $CuEDTA^{2-}$, $ZnEDTA^{2-}$, and $ZnHEDTA^{-}$ are known, the stability constant of $CuHEDTA^{-}$ has been redetermined to be logKf = 17.47 $\pm$ 0.20, consistent with the literature reported value 17.50. The criteria for proper selection of this metal-ligand double exchange approach and experimental conditions as well as the feasibility for future applications of this method are noted.

The dissociation kinetics of the complexes of nickel(II), zinc(II), and cadmium(II) of 1,7-diaza-4,10,13-trioxacyclopentadecane-N,N'-diacetic acid (K21DA) and 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane-N,N'-diacetic Acid (K22DA) were studied in constant ionic strength aqueous medium with various $[H^+]$-range, i.e., (0.88-53.9) x 10^{-5} M and (0.5-7.5) x 10^{-3} M. Copper(II) was used as the scavenger of free ligand and the rates of dissociation of these complexes have been found to be independent of $[Cu^{2+}]$. All the complexes exhibit acid-independent and acid-dependent pathways. For NiK21DA, CdK21DA and CdK22DA complexes, the acid-dependent rates are linear functions of $[H^+]$. For NiK22DA and ZnK21DA complexes, a saturation kinetics is observed, i.e., $[H^+]$ dependence at low $[H^+]$ and $[H^+]$-independent at high $[H^+]$. The rationalization of such different observations is proposed to be due to difference in complex solution structures rather than the thermodynamic stabilities. Influence of acetate content in the buffer, temperature, and total electrolyte concentration on the rate of dissociation has also been investigated and discussed. For detailed description of each paper and new research progress, please see the presentations listed below.

Preliminary studies using lanthanide DO2A complexes for the hydrolytic cleavage of phosphor diester bonds, DNA, and RNA molecules have also been performed. We have found that our systems are more effective artificial nucleases than all other reported systems. Detailed results will be reported when reproduction of the data is completed.

Additional studies have been on the following topics which are related to biological chemistry, molecular biology and biotechnology: (1) The Preparation and Applications of

Porcine C1q and the C1q-like Peptides, (2) Study of the Differential Gene Expression in Human Gastric Cells Infected with Helicobacter pylori, (3) Study of Genes Expression in Colorectal Adenocarcinoma by DDRT-PCR Method, (4) The Use of Piezoelectric Crystal Sensor for the Diagnosis of Human Helicobacter Pylori, and (5) Purification and Characterization of Neutral Sphingomyelinase from *Helicobacter pylori.* A total of fifteen master theses have also been completed. Papers related to the above studies are now been written for publication.

C. Thesis Supervised

	學生姓名	論文題目	畢 業 時間
1. M.S.	謝發坤	**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	1996.06
2. M.S.	郭永斌	**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	1996.06
3. M.S.	萬磊	**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*	1997.06
4. M.S.	潘美蓉	**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	1997.06
5. M.S.	李亮緯	**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	1997.06
6. M.S.	許呈安	**甲狀腺腫瘤標記之研究**	1998.06
7. M.S.	鄭昇沛	**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	1998.06

8. M.S.	**謝明宏**	**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	1998.06
9. M.S.	**管佈雲**	**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	1998.06
10. M.S.	**張永立**	**台灣醫療儀器產業環境與市場競爭策略**	1998.06
11. M.S.	**林孟嘉**	The expression of human placental lactogen by *E. coli* expression system	1999.06
12. M.S.	**許地利**	**大腸直腸分子標幟之研究** Study of the bio-molecular markers in colorectal camcers	1999.06
13. M.S.	**張至杰**	Purification and Characterization of the Sphingomyelinase of *Helicobacter pylori*	1999.06
14. M.S.	**陳家翎**	**稀土配位化合物之** DNA/RNA **切割劑**	2000.07
15. M.S.	**宋婉貞**	**自體免疫試劑開發**	2000.07
16. Ph.D	**陳桂添**	**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**	N/A
17. M.S	**曾繼鋒**	Optimization of the expression of human placental lactogen by *E. coli* expression system	N/A
18. M.S	**羅千婷**	**多醣真菌菌絲體之篩選與基因比對**	N/A
19. M.S	**管燕芸**	**稀土配位化合物之** DNA/RNA **切割劑**	N/A
20. Ph.D	**藍佩菁**	The functional study of human placental lactogen	N/A
21. M.S	**吳柏宏**	**稀土配位化合物之** DNA/RNA **切割劑**	N/A
22. Ph.D	**林志誠**	**雙體磁振造影劑**	N/A
23.	**王文宏**	**稀土配位化合物之** DNA/RNA **切割劑**	N/A

M.S			

D. Recent Publications (1996-2001) Total Refreed 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-tetraazacyclododecane (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.*, 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, accepted for publication.
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", 2001, *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". 2001, submitted.
17. **C.A. Chang,** M.C. Lin, and C.F. Tseng. "Expression and Optimization of recombinant Human Placental Lactogen in *E. coli.*" 2001, in preparation.
18. **C.A. Chang** and C.-L. Chen. "Dissociation Kinetics of $Ln(DO2A)^+$." 2001, in preparation.

19. **C.A. Chang** and C.-L. Chen. “Kinetics of DNA/RNA Hydrolitic Scission by Macrocyclic Lanthanide Complexes Ln(DO2A)$^{+}$.” 2001, in preparation.

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。**

E. Recent Abstracts and Papers Presented at Scientific Meetings (1996 - 2001)

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.

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.

In addition to the above mentioned publications, abstracts and meetings, the PI has been invited by various institutions to present seminars for more than 70 times for the last 5 years.

G. 鑭系金屬與大環多胺多乙酸基配位子錯合物之解離動力學研究

G-1 藥品與儀器

Lanthanum(III) nitrate hydrate, Cerium(III) nitrate hydrate, Praseodymium(III) nitrate hydrate, Neodymium(III) nitrate hydrate, Europium(III) nitrate hydrate, Gadolinium(III) nitrate hydrate 皆為 Aldrich G.R 級試藥，Disodium Ethylenediamine Tetraacetic Acid (Na_2H_2EDTA) 為 Merck G.R 級試藥，Calcium Carbonate ($CaCO_3$) 為 Merck G.R 級試藥，NH_3-NH_4Cl，pH9.5-10 為 Merck G.R 級試藥，Lithium perchlorate 為 Aldrich A.C.S 級試藥，Perchloric acid 為 Riedel-deHaen A.C.S 級試藥，Cresol red 為 Merck G.R 級試藥，Xylenol orange Tetrasodium Salt 為 Merck G.R 級試藥，CH_3COOH-NaOH 為 Merck G.R 級試藥，Eriochrome Black T 為 Merck G.R 級試藥，Tris(hydroxymethyl)aminomethane (THAM)為 Merck G.R 級試藥，DO2A 則參見第三章合成部分所述，UV-Visible (HP 8453 型)，恆溫循環水槽（FIRSTEK SCIENTIFIC B403 型）。

G-2 溶液濃度的標定

G-2-1 Na_2H_2EDTA 的標定

將置於 100 烘箱內 2-3 小時的 $CaCO_3$ 1.01g 置入 100ml 的定量瓶內，再利用滴管取數滴 HCl(12N)將 $CaCO_3$ 緩慢溶解，此時會產生 CO_2 氣體，直到溶液澄清後稀釋至 100ml 即得到 0.1M 的 $CaCO_3$。

取 50ml 的燒杯數個，依序分別將 0.1M 的 Na_2H_2EDTA Stock solution 2-3ml 加入，在加入指示劑 Eriochrome Black T 1-2 滴，並稀釋至 10-15ml。

以配製好的 $CaCO_3$ 來滴定，滴定開始時顏色為藍色，滴定終點時顏色則轉為

粉紅色，重複數次後求濃度及標準偏差。

G-2-2 金屬溶液濃度的標定

取 50ml 的燒杯數個，依序分別加入配製好的 0.01M Ln^{3+} Stock solution 2-3ml，指示劑 Xylenol orange 1-2 滴，並稀釋至 10-15ml。
以 Na_2H_2EDTA 來進行滴定，滴定開始時顏色為藍色，而滴定終點時顏色則轉為橘紅色，重複數次之後即可求出濃度及標準偏差。

G-2-3 酸溶液濃度的標定

取 50ml 的燒杯數個，依序分別加入配製好的 0.05M 過氯酸(perchloric acid)溶液 2-3ml，指示劑 Bromothymol blue 1-2 滴，並稀釋至 10-15ml。
以一級標準溶液（primary standard）0.1M 的 THAM 來進行滴定，滴定開始時溶液顏色為紫色，而滴定終點時顏色則轉為藍色，重複數次之後即可求出濃度及標準偏差。

G-3 金屬錯合物水溶液的配製

將標定好的鑭系金屬離子水溶液和配位子水溶液以莫耳數 1：1.01 的比例混合，置於小樣本瓶中，調整 pH 值，使 pH 值在 6-7 之間，再將 0.1M 離子強度劑（$LiClO_4$）加入，使之攪拌超過 24 小時以確定金屬離子與配位子皆以完全形成錯合物。

G-4 動力學計算原理

在化學反應中，能用最簡單數學式分析的動力學反應，就是 A **物質發生自發且不可逆的分解反應。**

$$A \xrightarrow{k} P$$

對 A **而言此反應為一級反應。**

$$\text{Rate} = -d[A]/dt = k[A]$$

一個一級分裂反應對分析化學而言沒有什麼用處，因為分析至少要包含兩個物質 Analyte **和** Reagent**。然而，包含兩個物質的反應，在速率定律中又常常因為太複雜而使得無法達到分析的目的。所以，在動力學終將複雜的速率定律簡化成類似一級反應的方式，是被化學家所普遍採用的。將高級數（**Order**）的反應簡化成擬似一級反應，以便於分析的反應即稱為** psuedo-first order**。**

在一典型的反應中，1 mol **的** A **物質和** 1mol **的** B **物質反應產生單一產物** p**，假設反應為不可逆，則反應式如下：**

$$A + B \xrightarrow{k} P$$

如果反應是一步的反應，則反應速率會和個別物種的濃度成正比，即寫成

$$-d[A]/dt = k[A][B]$$

這個反應對個別物質是一級的反應，對整個反應而言則是二級的反應。假設將 B **的濃度放大使**[B] >> [A]**，則在反應中** B **物質的濃度改變非常微小幾乎不變，我們就能將** k[B] =**常數=** k **帶入上式而將上式改寫成**

$-d[A]/dt = k_{obs}[A]$

如此便將反應用一級的方式呈現出來，對 A **而言此即為** pseudo-first order **反應。**

在我們設計的實驗中，我們將酸的濃度訂為鑭系金屬錯合物溶液濃度的十倍以上，再進行酸解的反應，這樣所得的結果也就能符合擬一級反應的條件了。

G-5 **實驗步驟**

將八種鑭系金屬錯合物水溶液分別和不同濃度的過氯酸混合之後，在 25**℃、**31**℃、**37**℃、**45**℃下反應，在** 250nm **下觀察其反應的變化，以** UV-visible **儀求出其反應速率常數(**k_{obs}**)。**

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

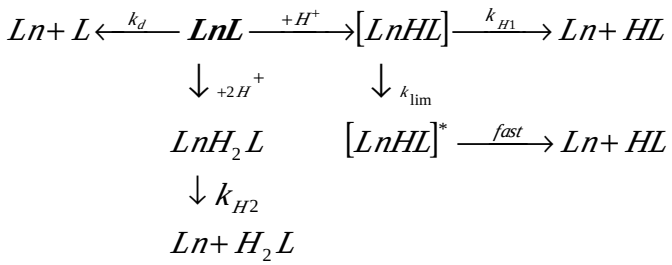
G-6 **反應結果與速率常數的計算**

酸(H^+**)在整個金屬錯合物(**ML**)解離的反應中可視為** scavenger**，整個反應式如下** (n=1~4)**：**

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

每一個反應最後所求出的速率常數(k_{obs}**)以表一** (Table I) **所示。**

由以往的文獻中我們可得知，鑭系金屬與大環配位子的解離會經由幾種途徑，如下圖示（scheme 1**）所示：**



Scheme 1

而上式所表示的速率可導出下列的速率公式：

$$-d[ML]_T/dt = (k_d + k_{H1}[H^+] + k_{H2}[H^+]^2)[ML]_T$$

而根據我們測得 k_{obs} **值看來** DO2A **這個大環配位子和鑭系金屬的反應系統為一級反應，且** k_{obs} **值與酸濃度（**H^+**）作圖呈線性，因此我們可推測其反應速率的公式如下：**

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

其式子中的 k_d **為** acid-independent rate constant**，即不加入酸時，其錯合物自行解離（**self-dissociation**）的速率常數；而** k_H **則為經由酸催化解離的速率常數** (acid-catalyzed rate constant)**。**

並且經由上述式子運算之後, 我們即可得到在不同溫度下鑭系金屬與 DO2A **的** k_d **和** k_H **值，但是求出來的** k_d **值非常小，所以在誤差的範圍內，我們可將其值視為零，而最後求得的** k_H **值見表二所示。**

G-7 Activated Parameter **的計算**

由於活化參數（Activated parameter**）可得知更多在反應進行當中的資訊，包括可能的反應機制，再經由** G-6 **中所求出變溫條件下的** k_H **值，我們可根據艾苓方程式**(Eyring equation)

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

式中 R **為氣體常數（**1.98716 cal • $mol^{-1}K^{-1}$**），**k_B **為波茲曼常數**(Boltzmann's constant, 1.381×10^{-23} JK^{-1})**，而** h **為蒲朗克常數**(Planck's constant, 6.626×10^{-34} J • s)**。由** ln(k_H/T)**對** 1/T **作圖之後，由所得到的斜率**(-**Δ**H^*/R)**及截距**(**Δ**S^*/R + ln(k_B/h))**即可求出焓**(activation enthalpy) **Δ**H^***和亂度**(entropy) **Δ**S^***，最後再由以下的公式**

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

又可求出於室溫（25**℃）下的自由能Δ**G^***，所求出的這些活化參數值整理列於表三。**

G-8 **結果討論**

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	4.6152
La	25	0.00503	0.00840	0.00802	0.01370	
	31	0.00694	0.01100	0.01760	0.02650	
	37	0.00802	0.01740	0.02990	0.04480	
	45	0.01370	0.02480	0.04810	0.06830	
Ce	25	0.00448	0.00800	0.01370	0.02200	
	31	0.00692	0.01400	0.02330	0.03860	
	37	0.01070	0.02230	0.03400	0.05510	
	45	0.01340	0.03020	0.04530	0.07060	
Pr	25		0.00672	0.00970	0.01670	0.02400
	31		0.00840	0.01440	0.02550	0.03270
	37		0.01360	0.02420	0.03980	0.05890
	45		0.02400	0.04350	0.06800	0.09130
Nd	25		0.004566	0.00553	0.00664	0.07762
	31		0.006588	0.00811	0.01010	0.01240
	37		0.007394	0.01030	0.01380	0.01720
	45		0.008040	0.01410	0.01840	0.02280
Sm	25		0.000394	0.00044	0.00055	0.00084
	31		0.000485	0.00088	0.00209	0.00282
	37		0.000751	0.00144	0.00283	0.00436
	45		0.00151	0.00348	0.00608	0.00910
Eu	25	0.00004	0.00017	0.00044	0.00060	
	31	0.00038	0.00104	0.00197	0.00288	
	37	0.00072	0.00146	0.00375	0.00570	
	45	0.00106	0.00436	0.00748	0.01030	
Gd	25	0.00002	0.00033	0.00047	0.00061	
	31	0.00032	0.00057	0.00096	0.00156	
	37	0.00041	0.00076	0.00174	0.00254	
	45	0.00115	0.00236	0.00479	0.00713	
Lu	25	0.00008	0.00015	0.00021	0.00027	
	31	0.00010	0.00034	0.00053	0.00088	

37	0.00024	0.00044	0.00079	0.00120
45	0.00039	0.00109	0.00232	0.00378

[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)

LuDO2A	31	0.0446 (±0.0028)
	37	0.0734 (±0.0027)
	45	0.2662 (±0.0012)
	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.)	ΔG^*(kcal/mol)
LaDO2A	10.2340 (±0.129)	-21.3270 (**±0.388**)	16.5890 (±0.302)
CeDO2A	11.5722 (**±0.151**)	-20.5909 (**±0.392**)	17.7080 (**±0.338**)
PrDO2A	13.2942 (**±0.642**)	-15.4699 (**±0.108**)	17.9032 (**±0.178**)
NdDO2A	15.4574 (**±0.373**)	-8.2263 (**±0.321**)	17.9088 (**±0.699**)
SmDO2A	20.1876 (**±0.306**)	1.6122 (**±0.033**)	19.0718 (**±0.391**)
EuDO2A	27.0747 (**±0.455**)	24.8598 (**±0.511**)	19.6666 (**±0.404**)
GdDO2A	28.0271 (**±0.338**)	26.8541 (**±0.453**)	20.0246 (**±0.297**)
LuDO2A	28.1734 (**±0.555**)	25.7963 (**±0.637**)	20.4861 (**±0.506**)