計劃類別：個別型計畫  
計畫編號：NSC92-2113-M-009-021-  
執行期間：92年08月01日至93年07月31日  
執行單位：國立交通大學生物科技研究所  

計畫主持人：張正  

報告類型：精簡報告  
報告附件：出席國際會議研究心得報告及發表論文  
處理方式：本計畫可公開查詢  

中華民國93年05月26日
行政院國家科學委員會專題研究計畫進度報告

雙體鐳系稀土離子之大環多氨基酸配位化學
及 DNA/RNA 切割劑之應用

計畫類別：■ 個別計畫 整合計畫

計畫編號：NSC 92-2113-M-009-007 (2nd Year)

執行期間：92 年 08 月 01 日 至 93 年 07 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
起缴送國科會。

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 93 年 05 月 25 日
Dimeric Macrocyclic Polyaminocarboxylate Lanthanide Coordination Chemistry and DNA/RNA Artificial Nucleases

Keywords: Dimeric and Monomeric Macrocyclic Polyaminocarboxylate Ligands, Lanthanides Complexes, Stability Constants, Selectivity, Formation and Dissociation Kinetics, Structure, Luminescence, NMR, Molecular Mechanics, DNA/RNA Cleavage.

Abstract:

The primary objective of this multi-year proposed research is to develop fundamental understanding of the major thermodynamic, kinetic, and structural factors that influence the desired physico-chemical properties of lanthanide complexes of macrocyclic polyaminocarboxylate ligands (e.g. complex formation stability and selectivity, reaction kinetics, NMR relaxation, luminescence, and structure) for applications in magnetic resonance imaging (MRI), solvent extraction, photodynamic therapy, luminescence labeling for biomolecules and catalysis for DNA and RNA phosphate diester bond cleavage. Recently, we have found that the rate constants measured for the Eu(DO2A)⁺ reaction with BNPP (a model compound with phosphate diester bond) had a titration-curve-like dependence with pH. Our initial hypothesis was that at high pH, Eu(DO2A)⁺ could form the more reactive hydroxo-bridged [EuL(OH)]₂ dimer. To verify this point, the research targets of this present project are primarily on the dimeric lanthanide macrocyclic complexes. The specific aims include the following: (1) Synthesize and characterize (NMR, IR, X-ray structural determination and potentiometry) new monomeric and dimeric macrocyclic polyaminocarboxylate ligands (e.g. DO2A and NO2A) with variable cumulative ring strains and pendent arms (e.g. carboxymethyl, amidemethyl and hydroxyethyl groups). (2) Determine the thermodynamic and conditional complex formation constants of these macrocyclic ligands with various metal ions including all trivalent lanthanide ions, alkaline earth metal ions, selected first and second row divalent transition metal ions (e.g. Ni²⁺, Cu²⁺, Zn²⁺ and Cd²⁺) and post transition metal ions (e.g. Pb²⁺) in aqueous solution. Evaluate ligand selectivity toward lanthanide complex formation. (3) Determine the macrocyclic lanthanide complex formation, dissociation, and metal/ligand exchange reaction rates in aqueous solution at various conditions (i.e. changing pH, metal ion/ligand concentration, temperature and ionic strength) and evaluate possible reaction mechanisms. (4) Determine if the thermodynamic and kinetic parameters, i.e. stability and selectivity constants, formation and dissociation reaction rates, activation enthalpy and entropy, of lanthanide DO2A/NO2A and dimeric complexes correlate with the ligand conformation, whether preorganized or not. (5) Synthesize and characterize (while possible, by single-crystal X-ray analyses, solution NMR, laser-excitation luminescence spectroscopy, NMR relaxation and molecular mechanics calculations) the macrocyclic complexes of La³⁺, Eu³⁺, Gd³⁺, and Yb³⁺. Correlate structural features (e.g. number of inner-sphere coordinated water molecules, number of ligand coordinated donor atoms, NMR chemical shifts, ligand cavity size, ligand cumulative ring strain, and metal ionic radii) with previously found thermodynamic and kinetic properties. (6) Use cationic lanthanide complexes such as Ce(DO2A)⁺, Eu(NO2A)⁺ and dimeric structural analogues to promote the cleavage of phosphate ester bonds of model compounds (e.g. disodium 4-nitrophenyl phosphate and diphenyl 4-nitrophenyl phosphate) and
DNA and RNA. Determine reaction rates and possible mechanisms. Examine the effects of lanthanide ionic radius and charge of the complexes.

Progress Report

This is a research program originally started nine years ago (since 1995). Many 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, 萬錦, 潘美容, 李亮緯, 許呈安, 林孟嘉, 管佈雲, 鄭昇沛, 謝明宏, 許地利, 張志傑, 陳家翎, 宋婉貞, 劉維健, 蔡政憲, 羅千婷, 吳柏宏, 劉銘傑, 張雅真, 林玉淳, 蕭志祥, 蔡政憲, 林穎男, 邱明慧, 謝明宏, 李智凱) have been trained. Recent research progress is briefly discussed below:

A. Kinetics of BNPP Phosphodiester Hydrolysis by Some Macroyclic and Linear Polyaminopolycarboxylate Lanthanide Complexes

In this paper, we report the study of the use of the europium complex, EuDO2A⁺ (DO2A is 1,7-dicarboxymethyl-1,4,7,10-tetraazacyclododecane) and other lanthanide complexes, as catalysts for the hydrolysis of phosphodiester bond of the model compound BNPP. EuDO2A⁺ is the quickest catalysts in terms of BNPP hydrolysis among LaDO2A⁺, EuDO2A⁺ and YbDO2A⁺, indicating charge density is not the only factor controlling the reaction. Among EuDO2A⁺, EuK21DA⁺, EuEDDA⁺ and EuHEDTA⁺, EuEDDA⁺ with the greatest number of water-coordinated sites hydrolyzes BNPP more efficiently at pH below 8. (At pH > 8, EuEDDA⁺ solution becomes misty and precipitates form). At pH 11.0, the hydrolysis rate of BNPP in the presence of EuDO2A⁺ is 100 times faster than that of EuHEDTA⁺, presumably due to the additional positive charge of EuDO2A⁺. These results indicate the high stability and outstanding hydrolysis rate toward BNPP of EuDO2A⁺. At 25°C, pH 9.35, an second order dependence on [EuDO2A⁺] for the BNPP hydrolysis reaction followed by a incomplete kinetic saturation at higher concentration suggests the involvement of two metal complexes and the formation of catalyst-substrate complex in the mechanism of BNPP hydrolysis. Fitting the data into monomer-dimer equilibrium (2EuDO2A⁺ = (EuDO2A⁺)₂) and pre-equilibrium kinetic model, the first order rate constants kcat = 3.1×10⁻⁴ s⁻¹, 2.3×10⁻² s⁻¹ for the hydrolysis of EuDO2A⁺ - BNPP and (EuDO2A⁺)₂ - BNPP were obtained respectively. If another model, BNPP hydrolysis through 1:1 and 1:2 complexation with EuDO2A⁺, is used to fit the data, the first order rate constant kcat = 6.3×10⁻³ s⁻¹, 1.4×10⁻² s⁻¹ for hydrolysis of EuDO2A⁺ - BNPP and EuDO2A⁺ - BNPP - EuDO2A⁺ were obtained respectively. All of these kcat values calculated are higher than those reported for BNPP hydrolysis by other metal complexes. The hydrolysis rates of EuDO2A⁺ and CeDO2A⁺ toward DNA and RNA is comparable to the rates reported. The cleavage rates are decaying with time. Potentiometry indicates there are slow equilibrium reactions in EuDO2A⁺(aq). Freshly-prepared complex solution is important for repeating the experimental results (cf. Appendix 1, a paper submitted for publication).
B. Synthesis of Ln(NO2A)+ Complexes and the Study of Their Hydrolytic Activity toward Phosphodiester Bond

The preliminary study of some lanthanide-NO2A (1,4 dicarboxymethyl-1,4,7-triazacyclononane) complexes, including EuNO2A⁺ and YbNO2A⁺, for the BNPP phosphodiester hydrolysis has been accomplished. The observed rates are smaller as compared with those of EuDO2A⁺. We are in the process to understand the reasons behind it (one of the three poster presentations at the 2003 Annual Meeting of the Chinese Chemical Society (Taipei), Chungli, Taiwan, November 29-30, Appendix 2).

C. The Protonation Constants and Selected Lanthanide Complex Formation Constants of NO2A

Figure 1 shows the ligand NO2A titration curve and its complex formation titration curve with Cu(II) ion. Table 1 lists the calculated NO2A protonation constants and those of NOTA for comparison.

**Table 1** The protonation constants of NOTA, NO2A and [9]aneN₃.

<table>
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<th>NOTA</th>
<th>NO2A</th>
<th>[9]aneN₃</th>
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<tbody>
<tr>
<td>Log K₁</td>
<td>13.0ᵃ</td>
<td>11.4₁ᵇ</td>
<td>11.3ᶜ</td>
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<tr>
<td>Log K₂</td>
<td>5.6</td>
<td>5.7₄</td>
<td>5.5₉</td>
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<tr>
<td>Log K₃</td>
<td>2.₅</td>
<td>3.₁₆</td>
<td>2.₈₈</td>
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<td>Log K₄</td>
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<td>1.₇₁</td>
<td>1.₉₆</td>
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<td>Log K₅</td>
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<tr>
<td>Log K₆</td>
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ᵃ Ref. 1,ᵇ Ref. 2,ᶜ Ref. 3,ᵈ Ref. 4,ᵉ Ref. 5,ᶠ Ref. 6
The protonation scheme of NO2A is proposed below:

The selected, calculated lanthanide complex formation constants of NO2A are listed in Table 2.

**Table 2**  The selected lanthanide Complex formation constant of NO2A and NOTA.

<table>
<thead>
<tr>
<th>Log K_{ML}</th>
<th>NOTA</th>
<th>NO2A</th>
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<tbody>
<tr>
<td>La^{3+}</td>
<td>13.5a</td>
<td>9.13±0.12</td>
</tr>
<tr>
<td>Nd^{3+}</td>
<td>13.2</td>
<td>10.60±0.16</td>
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<tr>
<td>Eu^{3+}</td>
<td>13.8</td>
<td>11.08±0.06</td>
</tr>
<tr>
<td>Gd^{3+}</td>
<td>14.3</td>
<td>10.77±0.12</td>
</tr>
<tr>
<td>Dy^{3+}</td>
<td>15.1</td>
<td>11.02±0.05</td>
</tr>
<tr>
<td>Er^{3+}</td>
<td>15.2</td>
<td>11.12±0.08</td>
</tr>
<tr>
<td>Yb^{3+}</td>
<td>15.3</td>
<td>11.52±0.07</td>
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</table>
D. Synthesis Macrocyclic Ligands to be Used for Lanthanide Artificial Nucleases and MRI Contrast Agents

We have designed a number of additional macrocyclic ligands to form lanthanide complexes for DNA/RNA phosphodiester hydrolysis. The synthetic details are depicted below:

\[ \begin{align*}
&\text{(1) Me}_2\text{NCH(OMe)}_2, \text{benzene, reflux, 2-4 h (100\%) } \\
&\text{(2) EtOH-H}_2\text{O, room temp., 2 h (70-85\%) } \\
&\text{(3) BrCH}_2\text{COOR}', \text{iPr}_2\text{NEt, MeCN (90\%) } \\
&\text{(4) H}^+ \\
\end{align*} \]

When \( n = 2 \), the ligand is NO2A and it has been prepared and characterized. When \( n = 3 \), the ligand possesses a 12-membered ring and the synthesis is more difficult. The 12-membered macrocycle is very stable when reacted with the acetal and it won’t hydrolyze to give the mono-N-protected macrocyclic. We are trying to solve this problem in order to obtain the final compound with two carboxylic acid functional group attached. The dimeric ligand incorporating two NO2A is also in preparation.

E. Publications (refereed, 1998-present)  
Total publications = 97


11. **C.A. Chang***, Bo-Hong Wu, Pu-Yun Kuan, Chia-Ling Chen. “Macrocyclic Lanthanide Complexes as Artificial Nucleases and Ribonucleases1. 1. Unusual High Hydrolysis Rates of Phosphodiester Bonds by Eu(DO2A) at High pH.” Submitted, 2004 (Appendix 1).


1. 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，2000年11月。
2. 張菀倫,“萬丈高樓平地起,交大生物科技之父張正教授診,交大友聲雜誌，401期，9-14頁，2003年12月01日出刊。
3. 張正,“台灣生技未來契機”、生技創業管理教戰首冊，國家生技醫療產業策進會，預定2004年6月出刊。


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

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<tr>
<th>學生姓名</th>
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<td>1. M.S. 謝發坤 F.K. Shieh</td>
<td>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 &amp; Polyamino Polycarboxylates</td>
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<td>2. M.S. 郭永斌 Y.B. Kuo</td>
<td>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</td>
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<td>3. M.S. 萬磊 L. Wan</td>
<td>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</td>
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<td>4. M.S. 潘美蓉 M.R. Pan</td>
<td>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</td>
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<td>5. M.S. 李亮緯 L.W. Lee</td>
<td>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</td>
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<td>6. M.S. 許呈安 T.A. Hsu</td>
<td>甲狀腺腫瘤標記之研究 The Search of Tumor Markers for Thyroid Carcinoma</td>
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<td>7. M.S. 鄭昇沛 S.P. Cheng</td>
<td>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</td>
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<td>8. M.S. 謝明宏 M.H. Shieh</td>
<td>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</td>
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In addition to the above-mentioned publications, abstracts and meetings, the PI has been invited by various institutions to present more than 80 seminars for the last 9 years.
Recently considerable effort has been extended to develop small molecules (mostly metal complexes) as artificial nucleases and ribonucleases to hydrolyze DNA and RNA, respectively. These include a number of mononuclear and dinuclear transition metal complexes (e.g. divalent zinc and copper ions) and trivalent lanthanide (Ln) complexes. It is found that the design of ligands and the choice of metal ion could have profound effect in artificial nuclease and ribonuclease activity and an effective agent should have the following properties: (1) specific and effective hydrolytic cleavage ability under at least physiologic conditions, (2) both thermodynamically and kinetically stable, (3) high catalytic turnover rates and preferably without the need of cofactors, and (4) relatively easy preparation. Trivalent macrocyclic lanthanide complexes with high coordination number and coordination unsaturation are therefore excellent candidates for these considerations because of their high thermodynamic stability and low kinetic lability.

A number of lanthanide complexes with or without linking to oligonucleotide (used for specific DNA/RNA binding) have been reported to hydrolyze RNA or DNA, e.g. Ln-oligonucleotide-IDA by Komiyama, Ln-“texaphyrins” by Sessler, hexadentate Schiff bases by Morrow and Hall, and a number of cyclen based lanthanide complexes such as Eu(THED), by Morrow. Uncoordinated lanthanide ion solutions have also been reported to hydrolyze DNA/RNA or model compounds by Komiyama, Chin, and Schneider. From the mechanistic point of view, Chin claimed that during RNA hydrolysis, the bimetallic hydroxo cluster, [La₂(OH)₅]⁺, was the most effective promoter. A report by L. Que et al demonstrated that a bimetallic Ce(III) complex was able to hydrolyze double-strand DNA molecules to linear forms. This is the first reported DNA double strand scission to linear forms.

Previously, we have reported the solution coordination properties of Ln(DO2A)⁺ where DO2A is 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid. Because DO2A has six donor atoms and when it forms a lanthanide complex with 8-9 coordination number, two or three coordinated water molecules would occupy the remaining uncoordinated metal sites. In order to develop new and efficient macrocyclic lanthanide complexes as artificial nucleases, we have used Ln(DO2A)⁺ (Ln = Eu(III) and La(III)) to carry out the hydrolysis of the phosphate diester bond of a model compound, BNPP (sodium bis(4-nitrophenyl)-phosphate). For comparison purpose, the Eu(III) complexes with a 7-coordination ligand, K21DA (1,7-diaza-4,10,13-trioxacyclopentadecane-N,N'-diacetic acid), and a 4-coordination ligand, EDDA (ethylenediamine-N,N'-diacetic acid) were also studied (Scheme 1). Laser excited fluorescence studies confirmed that Eu(DO2A)⁺ and Eu(K21DA)⁺ have 3 and 2 coordinated water molecules, respectively.

It was found that BNPP hydrolysis was promoted by Eu(DO2A)$^+$, La(DO2A)$^+$, and Eu(K21DA)$^+$, as expected. Figure 1 shows that the observed rate constants increased with increasing pH in a titration-like fashion. However, unlike the general precipitation problems for free lanthanide ion solutions at high pH, an unusual high rates were observed for Eu(DO2A)$^+$.

Figure 1. pH-rate profile of BNPP cleavage by lanthanide complexes at 25°C, $\mu = 0.10$ M (CH$_3$)$_4$NCl, $[\text{LnL}]= 1.0$ mM, and $[\text{BNPP}] = 0.10$ mM.

This discovery is the first of its kind. Previously we have determined that the first pK$_h$ value of Eu(DO2A)(H$_2$O)$_3$$^+$ is about 8.1. Thus, it is very likely that the rate “jump” for Eu(DO2A)$^+$ reaction with BNPP at pH 8.1 was due to the formation of Eu(DO2A)(H$_2$O)$_2$(OH) which is a better nucleophile than Eu(DO2A)(H$_2$O)$_3$$^+$. This is consistent with the finding by Breslow et al. on phosphate ester bond hydrolysis by macrocyclic Zn(II) complexes$^{3a}$. The other rate “jumps” at higher pH could be due to the formation of hydroxo-bridged clusters, i.e. $[\text{Ln}_2$(OH)$_2$]$^{3+}$, $[\text{Ln}_n$(OH)$_3$]$^{3(n-3)+}$, etc. Note that the crystal structure of a lanthanide aminoacetate complex with a cubane-like $[\text{Ln}_n$(OH)$_4$]$^{8+}$ core has only recently been reported$^6$. The rates for the reaction of BNPP with Eu(K21DA)$^+$ was relatively slower which may be due to the fact that Eu(K21DA)$^+$ has only two inner-sphere coordinated water molecules$^7$.

The observed BNPP hydrolysis rate constants with EuDO2A$^+$ were about 1.4-10 times greater than those with LaDO2A$^+$ in the pH range 6.8-10.6, and 74-1300 times greater than those of EuK21DA$^+$ in the pH range 6.5-9.9. The lanthanide contraction dictates that Eu(III) ion has a smaller ionic radius than that of La(III), consequently greater ionic potential and better Lewis acidity. The pK$_h$ values$^8$ for the respective aqua La(III) and Eu(III) ions are 9.33 and 8.58. Thus, Eu(DO2A)(H$_2$O)$_3$$^+$ is expected to have a lower pK$_h$ value than La(DO2A)(H$_2$O)$_3$$^+$, and the resulting hydroxo species is a better nucleophile. However, based on the k$_{obs}$-pH profile (Figure 1), the pK$_h$ value of La(DO2A)(H$_2$O)$_3$$^+$ is estimated to be ~ 7.9 and it is lower than that of Eu(DO2A)(H$_2$O)$_3$$^+$. This indicates that upon complexation, the Lewis acidity of lanthanide ions may be modified.

The BNPP hydrolysis rates (data not shown) by Eu(EDDA)$^+$ were also interesting in that they were faster at lower pH (e.g. < pH ~8) due presumably to its larger number of coordinated water molecules (i.e. 5). However, beyond pH 8, the formation of precipitate prevents further investigation.

Schneider et al. reported the BNPP hydrolysis by free trivalent lanthanide salt solutions at pH 7, 25$\degree$C.$^4n$. The observed rate constant data at different lanthanide concentrations were fitted to the Michaelis-Menten model, and it was found that the trend for k$_{cat}$ values was Yb$^{3+}$ > Eu$^{3+}$ > La$^{3+}$, i.e. 7.8×10$^{-4}$ s$^{-1}$, 2.5×10$^{-4}$ s$^{-1}$, and 0.13×10$^{-4}$ s$^{-1}$, respectively; and the K$_M$ (Michaelis-Menten constant) values were similar, i.e. 2.3×10$^{-3}$ M, 2.8×10$^{-3}$ M, and 2.6×10$^{-3}$ M, for Yb$^{3+}$, Eu$^{3+}$, and La$^{3+}$, respectively. In our hands, the preliminary studies using YbDO2A$^+$ for the hydrolysis of BNPP (data to be published) showed that the rates were the slowest among the three LnDO2A$^+$ (Ln = La(III), Eu(III), and Yb(III)).

At pH 9.35, the rate-concentration data for
Eu(DO2A)\(^{+}\) were obtained and shown in Figure 2. Fitting these data to the monomer-dimer reaction model in Scheme 2 gives \(k_1 = 9.1 \times 10^{-3} \text{ M}^{-1} \text{s}^{-1}\), \(k_2 = 4.0 \text{ M}^{-1} \text{s}^{-1}\), and \(K_f = 8.2 \text{ M}^{-1}\) \((R^2 = 0.997)\). The dimer rate constant \(k_2\) is about 400 times greater than that of monomeric species \(k_1\). However, the small \(K_f\) value indicates that the dimer concentration is relatively small for a [Eu(DO2A)]\(^{+}\) range 1.00-4.75 mM.

\[R = k_1 [\text{Eu(DO2A)}] [\text{BNPP}] + k_2 [\text{Eu(DO2A)}]^2 [\text{BNPP}]\]

In conclusion, the observations presented by this initial publication such as (1) unusual high pH BNPP hydrolysis rates promoted by Eu(DO2A)\(^{+}\), (2) different trend of Ln(DO2A)\(^{+}\)+ BNPP hydrolysis reaction rates (as compared to free lanthanide salt solutions), (3) variation of lanthanide hydrolysis pK\(_b\) values upon complexation, (4) possible dimeric or higher order lanthanide metal ion clusters involvement in BNPP hydrolysis at high pH, and (5) the equilibrium as well as kinetics of hydroxo-bridged lanthanide clusters formation, all warrant more detailed studies. We are in the process to obtain more data to answer the above-mentioned questions and further details will be reported in the near future.

**Acknowledgment**

The authors would like to thank National Science Council of Taiwan for financial support of this research (No. 93-2113-M-009-004).

**Experimental**

The ligands, DO2A and K21DA were synthesized in our laboratory by published procedures. Both ligands have been purified and characterized by NMR (H and C), elemental analysis, mass spectroscopy, and acid base titrations. Other reagent grade chemicals were purchased from various vendors and were used as received. Potentiometric pH titrations were performed using an automatic titrator (Brinkmann 670 Metrohm Titroprocessor) to calculate the pKa values and to determine the ligand concentrations according to published procedures. Pure ligand and metal nitrate stock solutions were prepared and standardized by standard techniques. The rates of BNPP hydrolysis by lanthanide complexes were measured using a Hewlett Packard 8453 UV-Vis diode-array spectrophotometer at specified experimental conditions by observing the spectral intensity growth at 400 nm due to the formation of nitrophenolate ion.

**References**

1. This is the first of a series of papers that we plan to publish under this title.


ABSTRACT FOR WEB PUBLICATION

Unusual high BNPP phosphate ester bond hydrolysis reaction rate was promoted by Eu(DO2A)$^{3+}$ over La(DO2A)$^{3+}$ and Yb(DO2A)$^{3+}$. The rate constant-[Eu(DO2A)$^{3+}$] data were fitted to a monomer-dimer reaction model and the dimer rate constant is 400 times greater than that of the monomer.
Objectives

Our laboratory has been interested in studying artificial DNA nuclease by employing some macrocyclic and linear lanthanide complexes, e.g. EuNO2A+, Eu(HEDTA), and Eu(EDDA)⁺. These complexes function as catalysts for the hydrolysis of phosphodiester bond of the DNA model compound BNPP. In this paper, we report the study of NO2A(1,4 dicarboxymethyl-1,4,7-triazacyclononane) complexes, including EuNO2A⁺, YbNO2A⁺, LnNO2A⁺. We study the different ligand’s number how to affect the hydrolysis rate of phosphodiester bond by comparing two compounds EuNO2A⁺, EuDO2A⁺. And we study the coordinate stabilities of NO2A⁺ between lanthanide metal meanwhile search for possible mechanisms to describe the process of hydrolysis phos-phodiester bond by NO2A complexes.
LnDO2A$^+$ and LnNO2A$^+$

<table>
<thead>
<tr>
<th>Ligand coordination number</th>
<th>Number of Coordinated Water Molecules</th>
<th>Total Charge in Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln(DO2A)$^+$</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Ln(NO2A)$^+$</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Hydrolysis of BNPP

Pseudo-first order reaction

$\text{Rate} = k [\text{LnNO2A}]^m [\text{BNPP}]^n$

$[\text{LnNO2A}] >> [\text{BNPP}], n=1$

$\text{Rate} = k_{\text{obs}} [\text{BNPP}]$

$\text{wavelength: 400 nm}$
pH profile of BNPP cleaved by EuDO2A and YbNO2A at 25°C, $\mu = 0.1((\text{CH}_3)_4\text{NCl}), [\text{LnL}] = 1.0 \text{ mM}, [\text{BNPP}] = 0.1 \text{ mM}$

YbNO2A at pH=8.9 and EuNO2A at pH=9.5 have good $k_{obs}$, so we pick pH 9.6(EuNO2A) and pH 8.9(YbNO2A) for the latter discussions.

Dependence of $k_{obs}$ on the concentration of EuNO2A at 25°C, pH 9.6, 20 mM CHES, $\mu = 0.5$, and 0.1 mM BNPP

The relationship of $k_{obs}$ and [EuNO2A] isn’t linear, so we try to suppose different models which can fit the curve.
Model 1

\[
\begin{align*}
\text{EuNO2A}^+ + \text{BNPP} & \xrightleftharpoons[k_1]{k_2} P \\
2 \text{EuNO2A}^+ + \text{BNPP} & \xrightarrow{k_2} P \\
R &= k_1[\text{EuNO2A}^+] [\text{BNPP}] + k_2[\text{EuNO2A}^+]^2 [\text{BNPP}] \\
R_i &= k_1[\text{EuNO2A}^+] T [\text{BNPP}] T + k_2[\text{EuNO2A}^+] T^2 \\
\kappa_{obs} &= k_1[\text{EuNO2A}^+] T + k_2[\text{EuNO2A}^+] T^2 \\
\end{align*}
\]

we get \( k_1 = 4.76 \times 10^{-12} \text{ M}^{-1} \text{s}^{-1} \) and \( k_2 = 0.527 \text{ M}^{-2} \text{s}^{-1} \)

Model 2 (consider the dimer exists)

\[
\begin{align*}
2 \text{EuNO2A}^+ & \xrightarrow[K_f]{K_f} (\text{EuNO2A}^+)_2 \\
\text{EuNO2A}^+ + \text{BNPP} & \xrightleftharpoons[k_1]{k_2} P \\
(\text{EuNO2A}^+)_2 + \text{BNPP} & \xrightarrow{k_2} P \\
R &= k_1[\text{EuNO2A}^+] [\text{BNPP}] + k_2[(\text{EuNO2A}^+)_2][\text{BNPP}] \\
K_f &= \frac{[(\text{EuNO2A}^+)_2]}{[\text{EuNO2A}^+]^2} \\
R &= k_1[\text{EuNO2A}^+] [\text{BNPP}] + k_2K_f[\text{EuNO2A}^+]^2 [\text{BNPP}] \\
[\text{Eu}^{3+}] &= [\text{EuNO2A}^+] + 2[(\text{EuNO2A}^+)_2] \\
K_f &= -\frac{1 + (1 + 8K_f[\text{Eu}^{3+}])^{1/2}}{4K_f} \\
[\text{EuNO2A}^+] &= -0.5 + (0.25 + 2K_f[\text{Eu}^{3+}])^{1/2} \frac{1}{2K_f}
\end{align*}
\]
\[ k_{\text{obs}} = k_1 \left[ \frac{0.5 + (0.25 + 2Kf \cdot [\text{Eu}^3^+]_T)^{1/2}}{2K_i} \right] + k_2 K_f \left[ \frac{0.5 + (0.25 + 2Kf \cdot [\text{Eu}^3^+]_T)^{1/2}}{2K_i} \right]^2 \]

Assume \( y = k_{\text{obs}}, [\text{Eu}^3^+]_T = X, a = k_1, b = K_f, c = k_2 \) and compute by Sigma Plot, we get the figure:

- \( k_1 = 5.16 \times 10^{-12} \text{ M}^{-1}\text{s}^{-1} \)
- \( k_2 = 1.03 \text{ M}^{-1}\text{s}^{-1} \)
- \( K_f = 0.52 \text{ M}^{-1} \)

Dependence of \( k_{\text{obs}} \) on \([\text{EuNO2A}]\) at 25°C, pH 8.9, 20 mM CHES, \( \mu = 0.5 \), and 0.1 mM BNPP

\[ \frac{K}{(\text{YbNO2A} + \text{BNPP})} \rightarrow \text{P} \quad k \]

\[ k_{\text{obs}} = \frac{kk [\text{YbNO2A}]}{1 + K[\text{YbNO2A}]} \]

- \( K = 66.8 \text{ M}^{-1} \)
- \( k = 6.58 \times 10^{-5} \text{ s}^{-1} \)

The curve above isn't like the dependence of \( k_{\text{obs}} \) on \([\text{EuNO2A}]\), if we make a radical expression on \([\text{YbNO2A}]\), then we get a new plot.
There is a linear relationship between kobs and [YbNO2A]^{1/2}

Half order relationship between kobs and [YbNO2A] may be caused by YbNO2A dimer formation.

Compare of EuDO2A^+ and EuNO2A^+
(At 25°C, \( \mu = 0.1((\text{CH}_3)_4\text{N})\text{Cl} \), [LnL] = 1.0 mM, [BNPP] = 0.1 mM)

The macrocyclic ring of DO2A is bigger than NO2A that can chelates metal more tightly. Water ligands of NO2A complex enhance multimer formation, and reduce the cleavage ability.
The data of LnDO2A and LnNO2A hydrolysis BNPP

<table>
<thead>
<tr>
<th></th>
<th>EuNO2A</th>
<th>EuDO2A</th>
</tr>
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<tbody>
<tr>
<td>Mod 1</td>
<td>( k_1 = 4.76 \times 10^{-12} \text{ M}^{-1} \text{s}^{-1} )</td>
<td>( k_1 = 0.017 \text{ M}^{-1} \text{s}^{-1} )</td>
</tr>
<tr>
<td></td>
<td>( k_2 = 0.527 \text{ M}^{-2} \text{s}^{-1} )</td>
<td>( k_2 = 26.6 \text{ M}^{-2} \text{s}^{-1} )</td>
</tr>
<tr>
<td>Mod 2</td>
<td>( k_1 = 5.16 \times 10^{-12} \text{ M}^{-1} \text{s}^{-1} )</td>
<td>( k_1 = 9.1 \times 10^{-3} \text{ M}^{-1} \text{s}^{-1} )</td>
</tr>
<tr>
<td></td>
<td>( k_2 = 1.03 \text{ M}^{-1} \text{s}^{-1} )</td>
<td>( k_2 = 3.9 \text{ M}^{-1} \text{s}^{-1} )</td>
</tr>
<tr>
<td></td>
<td>( K_f = 0.52 \text{ M}^{-1} )</td>
<td>( K_f = 8.2 \text{ M}^{-1} )</td>
</tr>
</tbody>
</table>

\( k \) of dimer is always higher than moner in all models of two complexes, but the ratio of dimer is very low. DO2A has better cleavage ability than NO2A.

Conclusion

- There are more ligand binding sites for H₂O in NO2A than DO2A, but these water molecules may not be at the best position to help the cleavage of phosphodiester bond. And the NO2A molecules very likely form multipolynuclear species that lessen the cleavage ability.
- EuNO2A in solution can form a small amount dimer that has outstanding ability to cleavage the phosphodiester bond.
- YbNO2A in solution can form dimer which has no cleavage ability, and that causes the half order relationship between the concentration of YbNO2A and the rate of cleavage BNPP.