

Preparation and Study of Dinuclear Zn(II) Complex for the Hydrolysis of Phosphate Esters

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It is well known that natural nucleases such as alkaline phosphatase, phospholipase C and P1 nuclease contain more than two metal ions in the active site¹ and there have been considerable research efforts in developing efficient catalysts carrying two or three metal ions for the hydrolysis of phosphate esters.² In order to achieve a possible cooperativity among metal ions, it is crucial to design effective bi- and multinucleating ligands.

During the course of our study in synthesizing hydroxy alkyl-bridged binucleating ligands, we prepared a new Zn complex with ligand L1. The reason for choosing 1,5,9-triazacyclododecane ([12]aneN₃), L2, as a monomeric unit is that the mononuclear Zn-L2 complex is known to be thermally stable and an efficient catalyst in hydrolyzing phosphate esters.³ With this respect, very recently, a series of binucleating ligands having tri- or tetraazacyclic ligands as a monomeric unit bridged by phenyl- and alkyl group, have been synthesized and chemical properties of a few of their Cu and Ni complexes have been reported.⁴ However their reactivity as a hydrolysis catalyst has never been tested. It would be worthwhile to know whether simple alkyl bridge could provide a cooperative interaction between metal ions. In this report, the reactivity of the Zn-L1 and the Zn-L2 complexes in hydrolyzing BDNPP, NPP was compared and the structural requirements of a binucleating ligand were investigated.

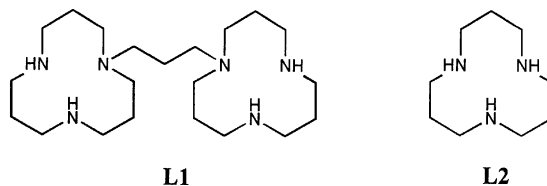
Experimental Section

Instruments. ¹H and ¹³C NMR were taken on a Varian Unity-Inova 300 MHz Spectrometer. Kinetic studies were carried out using Scinco-S2100 spectrophotometer.

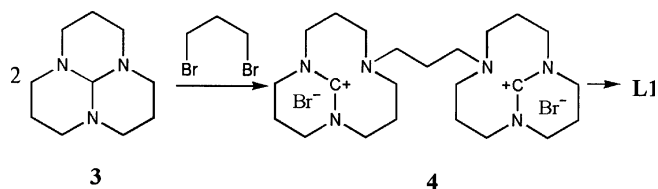
Materials. BDNPP(bis(2,4-dinitrophenyl)-phosphate) was synthesized by the known method.⁵ Most chemicals including NPP (*p*-nitrophenylphosphate, disodium salt) and solvents were purchased from Sigma-Aldrich Co. and used without further purification.

Ligand L1. 1,5,9-triazacyclododecane was synthesized by literature methods^{6a} and was selectively protected to the tricyclic orthoamide **3**, which was reacted with 1,3-dibromopropane, followed by NaOH work-up to give the ligand L1.^{6b-e} Tricyclic orthoamide **3**: To a solution of 10 g (58.4 mmol) of [12]aneN₃ dissolved in abs. ethanol 20 mL, was added 95% N,N-dimethylformamide dimethylacetal 7.4 gr (1.0 eq). The reaction mixture was refluxed for 4h, cooled to room temperature, and evaporated in vacuo at less than 40 °C to give 10.2 g of a yellowish solid **3** (95%). ¹H NMR

(CDCl₃) δ 1.40-1.45 (m, 3H), 1.9-2.2 (m, 10H), 2.32 (s, 1H), 2.7-2.9 (m, 6H). 1,3-Bis(1,5,9-triazacyclododecyl)propane L1: To a solution of 0.2 g (1.11 mmol) of tricyclic orthoamide **3** in 10 mL of dry chloroform was added 56.3 μL (0.5 eq) of 1,3-dibromopropane and the reaction mixture was refluxed for 4 days. During the course of the reaction, a white precipitate was being formed. After cooling to room temperature, the mixture was evaporated to a half of the volume and the precipitate was filtered, washed with dry CHCl₃ yielding the bicyclic amidinium salt **4** 0.33 g (51%). The salt was dissolved in 0.66 M NaOH (25 equiv. EtOH/H₂O-2 : 1) and the mixture was refluxed for 1 day. Following extraction with CHCl₃, drying with anhydrous Na₂SO₄/Na₂CO₃, and evaporation in vacuo, gives 0.3 g of transparent syrup, which solidified in refrigerator (90%). ¹H NMR (CDCl₃) δ 1.65 (m), 2.38 (bt, *J* = 7.5 Hz), 2.53 (t, *J* = 6 Hz), 2.70 (t, *J* = 5.4 Hz), 2.76 (t, *J* = 5.1 Hz) ¹³C NMR (CDCl₃) δ 26.1, 47.23, 49.21, 50.73, 53.1. **Zn(II)-L1 complex**: To a solution of 201 mg (0.53 mmol) of L1 in ethanol 10 mL was added 312.5 mg (2.0 eq) of Zn(NO₃)₂·6H₂O dissolved in ethanol 10 mL. The mixture was stirred for 2h at room temperature. The precipitate was filtered, washed with ethanol and ethyl ether to give 400 mg of a white solid. Anal. Calcd. for C₂₁H₅₂N₁₀O₃Zn₂: C, 30.92; H, 6.43; N, 17.17. Found: C, 30.78; H, 6.14; N, 17.58. **Zn(II)-L2 complex**: Zn-L2 complex was synthesized and purified by the known method.³



Kinetics. Zn complexes promoted hydrolysis of BDNPP and NPP was monitored by following the UV absorbance change at 400 nm (assigned to 2,4-dinitrophenolate, 4-nitrophenolate anions) at 34(±0.5) °C with I = 0.10 M (NaNO₃). All the reactions were carried out under pseudo-first order conditions with large excess of the Zn complexes over the phosphate esters. The rate constants were calculated by the initial rate (< 5%) method (correlation coefficient > 0.98). Buffered reaction solutions were prepared with MES (pH 6-7), HEPES (pH 7-8), TAPS or EPPS (pH 8-9), CHES (pH > 9) respectively. In a typical kinetic run, to a 2 mL of 0.5-2 mM a Zn complex solution prepared in a 20 mM buffer of a desired pH and equilibrated at 34(±0.5) °C for 10 min, was



Scheme 1

added a 5 μ L of 0.01 M stock solution of BDNPP in acetonitrile or NPP in water.

Results and Discussion

With the aid of a recent synthetic development,⁶ the binucleating ligand was prepared by reacting 2 equiv. of the 1,5,9-triazacyclododecane orthoamide **3** with 1,3-dibromopropane in chloroform followed by base work up (Scheme 1). Zn complexes of L1 and L2 were prepared by mixing ethanolic solutions of L1, L2 and $\text{Zn}(\text{NO}_3)_2$, $\text{Zn}(\text{ClO}_4)_2$, respectively. According to the elemental analysis (C, H, N) of the Zn-L2 complex, the formular structure of $\text{Zn}_2\text{L1}(\text{NO}_3)_4$ (Zn-L1) containing $3\text{H}_2\text{O}$ molecules was suggested.

Hydrolysis of BDNPP by the Zn-L1 and Zn-L2 complexes has been performed at 35 $^\circ\text{C}$ under pseudo first order condition with the metal complexes in excess. The dependence of catalyst concentration on the hydrolysis rate is shown at Figure 1. The slope is linear suggesting the first order dependence of the hydrolysis rate on the Zn complex concentration.

The relative first-order rate constants are listed in Table 1. The rate constant for BDNPP hydrolysis promoted by the Zn-L1 complex was only slightly higher than that by the Zn-L2 complex. Considering that two Zn ions exist in the Zn-L1 complex, the rate constant by the Zn-L1 complex might increase at least two fold than by the Zn-L2 complex. The rather lower reactivity of the Zn-L1 complex in hydrolyzing BDNPP is likely due to a dimerization or pKa changes of the Zn-bound water molecules.⁷ At this point, it should be men-

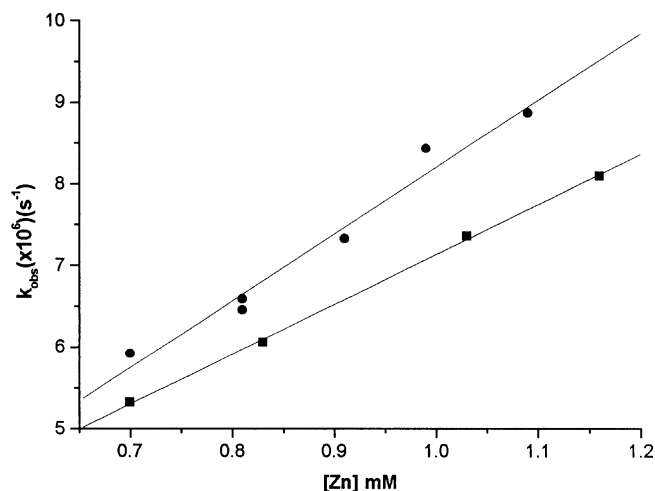


Figure 1. A plot of k_{obs} (s^{-1}) for hydrolysis of BDNPP vs the catalyst concentration (1 mM): ● = Zn-L1, ■ = Zn-L2.

Table 1. Observed pseudo-first-order rate constants (s^{-1})^a of phosphates hydrolysis mediated by Zn(II) (1.0 mM) complexes at pH 7, 34 $^\circ\text{C}$

substrate	Zn-L1	Zn-L2	Zn-L1/Zn-L2
BDNPP	8.2×10^{-6}	7.1×10^{-6}	1.2
NPP	1.4×10^{-5}	3.1×10^{-6}	4.4

^a pH 7 (20 mM HEPES), I = 0.1 M NaNO_3 , and hydrolysis by buffer have been subtracted.

tioned that at a concentration higher than 2 mM at pH 7 and at any concentrations at pH higher than 7.3, the solution became turbid, resulting in a precipitate. In the study on the crystal structure of a Cu(II) ion with a binuclear ligand, 1,2-bis(1,4,7-triaza-1-cyclononyl)ethane, it was found that the Cu(II) ion and the ligand formed 1 : 2 and 2 : 2 complexes.^{4a} It can be suggested that the same structure may form in the Zn-L1 complex. According to control experiments, the dimerization and the hydrolysis of BDNPP proceeded competitively. At concentrations less than 2mM, where the experiments were performed, absorbance changes by the Zn-L1 complex itself at 400 nm were negligible.

In contrast to the hydrolysis of a phosphate diester, BDNPP, it was observed that the hydrolysis of NPP by the Zn-L1 complex was *ca.* 4 times faster than that the Zn-L2 complex at pH 7.⁸ In bis-Co(III) complex systems, Czarnik found that a flexible dimer showed no cooperativity and that a rigid dimer showed about 10-fold rate enhancement in hydrolyzing a phosphate monoester (NPP), but not in hydrolyzing a phosphate diester (BNPP).⁹ Chin *et al.* reported that bis-Cu(II) complex showed *ca.* 300- to 500-fold rate enhancement in hydrolyzing ApA than a corresponding monomer did.¹⁰ In both cases, the double Lewis-acid type of activation mechanism was suggested to interpret the data.^{2a} It is interesting to investigate the cooperativity with a relatively flexible binucleating ligand such as L1. Although the ligands of metal complexes in the above two examples and in our study have a similar structural backbone, where two macrocyclic ligands are bridged by naphthalene, anthracene, and propyl group, respectively, the catalytic efficiency is more than two orders of magnitude different. Thus, it can be concluded that subtle changes in ligand structures affect significantly the catalytic efficiency of the metal ion-ligand systems.

In summary, there seems to be some cooperation between two metal ions in the Zn-L1 complex in hydrolyzing NPP, but not in hydrolyzing BDNPP.

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 8. A direct comparison between the maximum rate constants by each complex was not possible because the pH-rate profile by the Zn-L1 complex has not been completed due to a precipitate formation at the pH higher than 7.2. However, the pK_a of the Zn-L1 bound water molecule could be estimated to be a slightly lower than 7.3 -the reported pK_a of the Zn-L2 complex bound water molecule. (ref. 3a) Under this assumption, the hydrolysis rate by the Zn-L1 complex at pH 7, reaches its maximum, while the same by the Zn-L2 complex does not. Therefore the ratio could be smaller if the rate constants were measured at the pHs corresponding to pK_as of the each complex.
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