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Carboxy and diphosphate ester hydrolysis promoted by di- or tri-nuclear zinc(II) complexes based on β-cyclodextrin

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ABSTRACT

A new ligand (L), 6-mono-(2-(2-hydroxy-3-(hydroxymethyl)-5-methyl benzylamino)-ethylamino)-β-cyclodextrin, based on β-cyclodextrin derivatives with dinucleating units was synthesized and used to prepare a trimetallic bis-ligands zinc complex ($Zn_3(L^{2-})_2$). The esterase activity of the complex was investigated by the hydrolysis of two carboxylic acid esters, bis(4-nitrophenyl)carbonate (BNPC) and 4-nitrophenyl acetate (NA), and a DNA model bis(4-nitrophenyl)phosphate (BNPP) as a phosphate ester. The catalytic rate for BNPC was very high, which was found to be a 5.63×10^3-fold rate enhancement over uncatalyzed hydrolysis and 1.62×10^2-fold rate enhancement over uncatalyzed hydrolysis for NA hydrolysis at pH = 7.0. For the catalytic hydrolysis of BNPP, the initial first-order rate constant of 0.1 mM catalyst was 5.85×10^{-8} s^{-1} at pH = 8.50 and 35 °C, which is a 731-fold acceleration over uncatalyzed hydrolysis. The second rate constant (k_{BNPP}) was found to be 1.22×10^{-3} M^{-1} s^{-1} at pH = 10.0. According to the potentiometric titration study, the zinc complex exists in a dinuclear single ligand coordinated mode and a trinuclear bis-ligands system at pH ≥ 7.0. The ester hydrolysis activity was attributed to the cooperative interaction of the two metal centers and the hydrophobic cavity of β-cyclodextrin with substrates.

1. Introduction

Many metalloenzymes such as phospholipase C, nuclease P1 and exonuclease site of DNA polymerase use two or three metal ions in the active site to catalyze the hydrolytic cleavage of phosphate diester bonds in nucleotides (e.g., RNA and DNA). The metal ions act cooperatively as Lewis acid sites in the activation of a nucleophile and the substrate, and in the stabilization of the pentacoordinate phosphorus transition state and the leaving group. In the last two decades, many model systems for small molecule dinuclear or trinuclear hydrolytic metalloenzymes have been designed and studied for the hydrolysis of carboxylate esters and phosphate esters. Examples include macrocyclic polyamine dinuclear [1–7] and trinuclear metal complexes [8,9], rigid pyridine [10] or pyrazol [11–14] bridged polyamine ligands dinuclear metal complexes. Reinhoudt and co-workers reported synthetic dinuclear and trinuclear metallophosphodiesterases based on calix[4]arenes [15–18], which exhibited a very high catalytic activity in the transesterification of the RNA model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate. However, the complexes based on calix[4]arenas are not active in the hydrolysis of the phosphate triester diethyl *p*-nitrophenyl phosphate (DEPNP), the diester EPNP and the monoester *p*-nitrophenyl phosphate (PNP). Metalloenzyme models of mononuclear complexes based on cyclodextrin (CD) dimers exhibit high hydrolytic activities due to the cooperative binding of two β-CD cavities with the substrates. For example, Breslow and Zhang synthesized metallo-bisCDs bridged by a bipyridine unit with a N,N′-bidentate ligand as metallohydrolase models, which remarkably accelerated catalytic hydrolysis of carboxylic acid diesters and phosphate diesters [19,20]. β-Cyclodextrin dimer linked by telluroxides prepared by Liu et al. [21] exhibited good catalytic hydrolysis activity of carboxylic acid diesters.

We previously reported the zinc complexes of β-CD dimers, either linked by phenanthroline with tetradentate N_4 or pyridine with tridentate N_3 ligands, which demonstrated satisfactory activities for diester hydrolysis [22,23]. However, changing the bridge ligands from tetradentate to tridentate had no obvious rate enhancement in esters hydrolysis. Research on the hydrolytic activity influenced by poly nuclear metal complexes based on cyclodextrins will aid the design of highly active artificial hydrolase. Therefore, we designed and synthesized a dinucleating ligand based on a cyclodextrin, and prepared its trinuclear bis-ligand

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zinc complex. We also investigated the hydrolytic activities of this zinc complex affected by the cooperative action between metal centers and cooperative binding of two β-CD cavities with substrates. The investigation of esterase activity of the zinc complex was performed to promote hydrolysis of the carboxylic acid esters, bis(4-nitrophenyl)carbonate (BNPC) and 4-nitrophenyl acetate (NA), and phosphate ester, bis(4-nitrophenyl)phosphate (BNPP).

2. Experimental

2.1. Materials

2-Hydroxy-5-methylisophthalaldehyde, NA, BNPC, and BNPP were purchased from Sigma Aldrich. Ethylene diamine was purchased from Acros. β-CD (reagent grade) was recrystallized twice from H_2O and dried in vacuo for 12 h at 100 °C. DMF was dried over CaH_2 for 2 days and then distilled under reduced pressure prior to use. Common organic reagents were reagent grade and redistilled before use. 6-Mono(*p*-toluenesulfonyl)-β-cyclodextrin was synthesized by a literature procedure [24]. Water used in all physical measurement experiments was Milli-Q grade. All compounds were confirmed by elemental analyses, ESI-MS and ^{1}H NMR spectroscopy.

2.2. Instrumentation

^{1}H NMR spectra were recorded on a Varian INOVA-300NB or Mercury plus 300 spectrometers. Elemental analyses were performed on a Perkin-Elemer 240 elemental analyzer. ESI-MS spectra were performed on a Thremo LCQ-DECA-XP spectrometer. UV–vis spectra were obtained on a Varian Cary 300 UV/Vis spectrophotometer equipped with a temperature controller (±0.1 °C).

2.3. Synthesis

2.3.1. Preparation of 6-mono-(N-aminoethyl) amino-β-cyclodextrin

A solution of 6-mono(*p*-toluenesulfonyl)-β-cyclodextrin (4.000 g, 3.10 mmol) in dry DMF (12 mL) was added to 10 mL ethylene diamine with stirring. The mixture was heated to 65 °C for 3 h under argon atmosphere. After cooling to room temperature, excess ethylene diamine was removed by evaporation under reduced pressure, and then 400 mL acetone was poured into the resulting residue to give a white precipitate. The crude product was recrystallized twice from H_2O to give the pure compound (3.898 g) in 98.4% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.70–5.69 (m, 14H; OH-2,3), 4.82–4.81 (m, 7H; H-1), 4.45–4.44 (m, 6H, OH-6), 3.31–3.62 (m, 46H; H-2,3,4,5,6; CH_2), MS (ESI, H_2O/CH_3OH): m/z: calcd: 1177.3 $[M+H]^+$; found: 1177.6; elemental analysis calcd (%) for $C_{44}H_{76}N_2O_{34}\cdot 7H_2O$: C, 40.55; H, 6.96; N, 2.15. Found: C, 40.69; H, 6.93; N, 2.22.

2.3.2. Preparation of 6-mono-(2-(2-hydroxy-3-(hydroxymethyl)-5-methylbenzylamino)-ethylamino)-β-cyclodextrin (L)

A solution of 2-hydroxy-5-methylisophthalaldehyde (0.210 g, 1.30 mmol) in 5 mL dry DMF was added to the solution of 6-mono-(N-aminoethyl) amino-β-cyclodextrin (1.521 g, 1.29 mmol) in 20 mL dry DMF and methanol with stirring. The mixture was heated to 50 °C for 48 h under argon. After cooling to room temperature, the mixture was chilled in an ice-water bath and added with $NaBH_4$ (0.284 g, 7.51 mmol), then allowed to slowly warm to room temperature and stirred overnight. A small amount of hot water was then added to the mixture and filtered. The filtrate was evaporated to dryness under reduced pressure. The resulting residue was dissolved in a small amount of hot water and poured into acetone (200 mL) to give a light-yellow precipitate. The crude product was dried and purified by column chromatography over Sephadex G-25 and eluted with distilled deionized water to give 0.450 g (26.3%) of the pure compound as white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.78–6.74 (m, 2H; phenyl-H), 6.58–6.61 (brs, 1H; phenyl-OH), 5.69–5.68 (m, 14H; OH-2,3), 4.82 (m, 9H; H-1, hydroxymethylene-CH_2), 4.56–4.46 (m, 6H; OH-6), 3.63 (m, 30H; H-3,5,6, pyridine–CH_2, methylene–CH_2), 3.36 (m, 18H; H-2,4, methylene–CH_2), 2.16(s, 3H; methyl–CH_3), 1.07 (brs, 2H; NH, CH_2OH). MS (ESI, H_2O/CH_3OH): m/z: calcd: 1327.3 $[L+H]^+$, 1349.3 $[L+Na]^+$; found: 1327.6, 1349.3; elemental analysis calcd (%) for $C_{53}H_{86}N_2O_{36}\cdot 10H_2O$: C, 41.98; H, 7.11; N, 1.85. Found: C, 41.57; H, 6.60; N, 2.08.

2.3.3. Preparation of trinuclear zinc complex ($Zn_3(L^{2-})_2$)

A solution of L (0.047 g, 0.035 mmol) in water (2 mL) was added dropwise to a dilute aqueous solution of $Zn(ClO_4)_2\cdot 6H_2O$ (0.020 g, 0.054 mmol) with stirring at room temperature. The mixture was adjusted to pH = 9–10 and stirred for 2 h, and then concentrated. Acetone was added to precipitate the product, which was collected by centrifugation and washed with 100 mL acetone, followed by drying in vacuo to give the pure complex as a white solid (0.030 g, 47.6%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.86 (m, 2H; phenyl–H), 6.65 (m, 2H; phenyl–H), 5.82–5.64 (m, 28H; OH-2,3), 4.82 (m, 18H; H-1, hydroxymethylene–CH_2), 4.56–4.46 (m, 6H; OH-6), 3.62 (m, 60H; H-3,5,6, pyridine–CH_2, methylene–CH_2), 3.32 (m, 36H; H-2,4, methylene–CH_2), 2.12 (s, 3H; methyl–CH_3), 2.07 (s, 3H; methyl–CH_3); elemental analysis calcd (%) for $C_{106}H_{232}Cl_2N_4O_{112}Zn_3$: C, 35.15; H, 6.46; N, 1.55. Found: C, 34.74; H, 6.33; N 1.63. ICP, Zn, calcd: 4.98%; found: 5.33%.

CAUTION: Zinc perchlorate is potentially explosive. It should be prepared in small quantities and handled with care.

2.4. Potentiometric titration

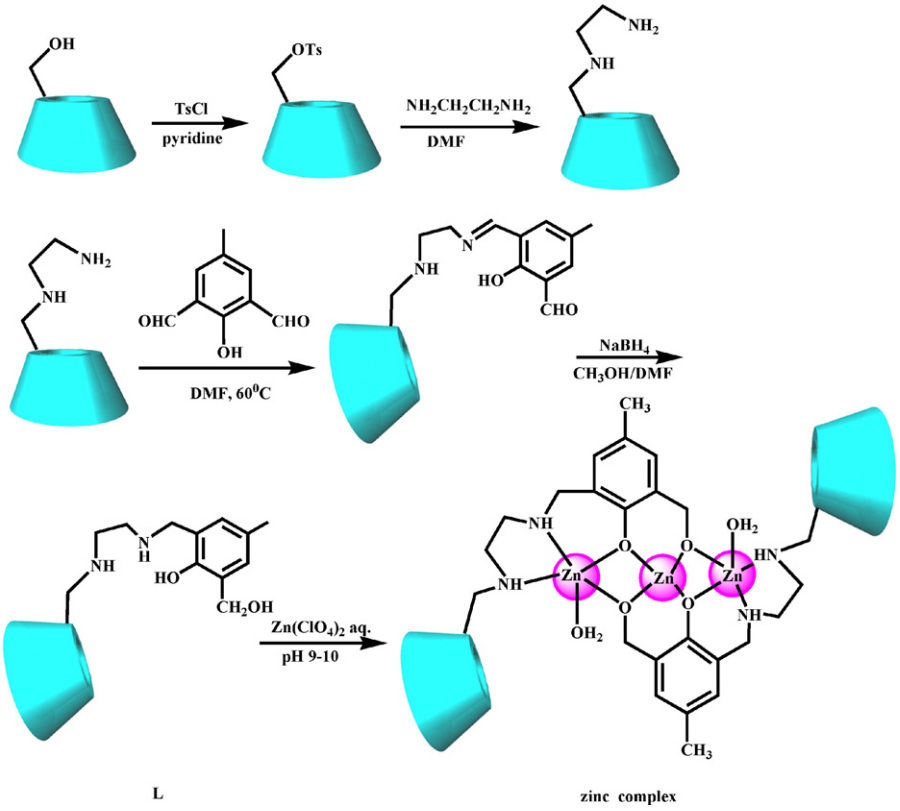
An automatic titrator (Metrohm 702GPD Titrino) coupled to a Metrohm electrode was used and calibrated according to Gran's method [25], then checked by titrating $HClO_4$ with NaOH solution (0.10 M). The thermostated cell contained 25 mL of 1.00 mM species in aqueous solutions with the ionic strength maintained at 0.10 M by sodium perchlorate. All titrations were carried out in the aqueous solutions under argon atmosphere at 25 ± 0.1 °C, and initiated by adding fixed volumes of 0.10 M standard NaOH in small increments to the titrated solution. Duplicate measurements were performed and the experimental error was below 1%. The titration data were fitted from the raw data using the Hyperquad 2000 program to calculate the ligand protonation constants K_n, the complex formation constant K_{ML}, and the deprotonation constants of the coordinated water pK_a.

2.5. Kinetics of BNPC and NA hydrolysis

The hydrolysis rates of BNPC and NA in the presence of zinc complex were measured by an initial slope method that follows the increase in absorption at 400 nm of the released 4-nitrophenolate [22]. 50 mM Tris–HCl (pH = 7.00) buffers were used; the ionic strength was adjusted to 0.10 M with $NaClO_4$ and the reaction solution was maintained at 25 ± 0.1 °C. In a typical experiment, after substrate (NA or BNPC), zinc(II) cation and ligand in 10% (v/v) CH_3CN solution at pH = 7.0 were mixed, the UV absorption decay was recorded immediately and monitored until 2% decay of 4-nitrophenyl acetate. Errors on k_{obs} values were about 5%.

2.6. Kinetics of BNPP hydrolysis

The rate of hydrolysis of BNPP to give mono(4-nitrophenyl)phosphate and 4-nitrophenolate was measured



Scheme 1. Synthetic scheme of ligand and its trinuclear zinc complex.

by an initial slope method that follows the increase in the 400 nm absorption of the released 4-nitrophenolate in aqueous solution at 35 ± 0.1 °C [26]. At this wavelength, the absorbance of the ester substrate was negligible. Buffer solutions (50 mM) of HEPES (pH = 7.40–8.20), TAPS (pH = 8.20–8.90), and CHES (pH = 8.90–10.50) were used, and the ionic strength was adjusted to 0.10 M with $NaClO_4$. The pH of the solution was measured after each run, and all kinetic runs with pH variation larger than 0.1 were excluded. BNPP, buffers, zinc(II) cation and ligand in aqueous solution were freshly prepared. The reactions were initiated by injecting a small amount of BNPP into the zinc(II) cation and ligand buffer solutions, and then mixed at 35 ± 0.1 °C. The increase in visible absorption was recorded immediately and monitored until 2% formation of 4-nitrophenolate, in which ε values for 4-nitrophenolate were 13,720 (pH = 7.50), 15,270 (pH = 7.75), 16,306 (pH = 8.00), 17,340 (pH = 8.50), 17,626 (pH = 8.85), 17,694 (pH = 9.00), 17,810 (pH = 9.50), 17,846 (pH = 10.0) and 17,858 (pH = 10.50) at 400 nm. The initial first-order rate constants, k_{in} (s^{-1}), for the cleavage of BNPP were obtained directly from a plot of the 4-nitrophenolate concentration versus time by the method of initial rates, which was linear with $R > 0.996$. The second-order rate constants (k_{BNPP}) for the catalyzed reactions were determined as the slope of the linear plots of k_{in} versus ($Zn_3(L^{2-})_2$) concentration. To correct for the spontaneous cleavage of BNPP, each reaction was measured against a reference cell that was identical to the sample cell in composition except for the absence of catalyst. Errors on k_{BNPP} values were about 5%.

3. Results and discussion

3.1. Characterization of $Zn_3(L^{2-})_2$

As illustrated in Scheme 1, the ligand (L) was synthesized in 26.3% yield by the reaction of 2-hydroxy-5-methylisophthalaldehyde and 6-mono-(N-aminoethyl)amino-β-cyclodextrin, and was further characterized by NMR spectroscopy and mass spectrometry (MS) (Fig. S1–S2 in the Supporting Information (SI)). By reacting L with zinc(II) perchlorate at pH = 9.0–10.0, a trinuclear zinc(II) complex was obtained in a moderate yield (47.6%), which was characterized by ICP and NMR spectroscopy. Compared with the ligand, the ^{1}H NMR spectrum of the complex showed two kinds of protons on the phenyl and two kinds of methyl protons with the same peak areas; however, the phenolic proton peak had disappeared. Moreover, the obvious peak splitting of the protons of OH–2,3 and OH–6 were observed (Fig. S3 in the SI), which indicated that the trimetalic bis-ligands complex was formed.

3.2. Zn(II) coordination in aqueous solution

Artificial metallohydrolase activity is preferentially studied in buffered aqueous solutions to most closely mimic biological conditions. Hence, knowledge of the species distribution in solution is crucial for understanding the trends in hydrolytic reactivity. Potentiometric titrations were performed to determine the pK_a values of the ligand as well as the stability constants of its zinc complexes and the pK_a values of zinc-bound water molecules in these complexes (Table 1).

The deprotonation steps of the material could be derived from their titration curves by titrating the corresponding acidic solutions with a sodium hydroxide solution. In the case of the ligand, three deprotonation steps were found in the pH range of 2.0–11.5, including the deprotonation of phenolic proton. Formation of both dinuclear single ligand and trinuclear bis-ligands complexes were detected in aqueous solution (Fig. 1). In the pH range of 6.0–8.0, deprotonation of the ligand's phenolic hydroxyl leads to $[Zn_2L^-]^{3+}$, and then deprotonation of the hydroxymethyl induced by the metallic zinc ion would lead to $[Zn_2L^{2-}]^{2+}$ species. At higher pH (8.0–11.5), three more Zn^{II} species are fromed:

Table 1
Equilibrium constants of the ligand and its zinc complexes.

Chemical equilibrium	Equilibrium constant	
$H_2L^{2+} = HL^+ + H^+$	pK_1	1.30 ± 0.03
$HL^+ = L + H^+$	pK_2	5.52 ± 0.02
$L = L^- + H^+$	pK_3	8.55 ± 0.02
$2Zn^{2+} + L^- = [Zn_2L^-]^{3+}$	$\log K_{Zn2L}$	6.96 ± 0.04
$[Zn_2L^-]^{3+} = [Zn_2L^{2-}]^{2+} + H^+$	pK_{a1}	6.54 ± 0.02
$3Zn^{2+} + 2L^{2-} + 2H_2O = [Zn_3(L^{2-})_2(H_2O)_2]^{2+}$	$\log K_{Zn3L2}$	16.32 ± 0.06
$[Zn_3(L^{2-})_2(H_2O)_2]^{2+} = [Zn_3(L^{2-})_2(H_2O)(OH)]^+ + H^+$	pK_{a1}	9.09 ± 0.01
$[Zn_3(L^{2-})_2(H_2O)(OH)]^+ = [Zn_3(L^{2-})_2(OH)_2] + H^+$	pK_{a2}	10.79 ± 0.01

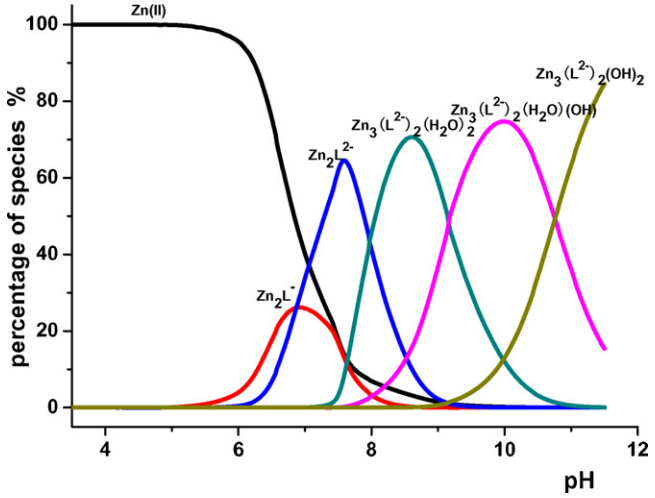


Fig. 1. Distribution plots of species with zinc complex as a function of pH at 0.1 M $NaClO_4$ and 25 ± 0.1 °C.

Table 2
Initial rate (v) for ester hydrolysis promoted by different catalyst.

Catalyst[a]	BNPC		NA	
	v ($\times 10^{-9}$ M s^{-1})	v/v_{contr}	v ($\times 10^{-9}$ M s^{-1})	v/v_{contr}
Buffer	$(2.45 \pm 0.03) \times 10^{-1}$ [21]	1.00	1.35 ± 0.10	1.00
Zn(II)	$(2.65 \pm 0.03) \times 10^{-1}$	1.10	1.45 ± 0.13	1.07
Zn_2L	$(2.12 \pm 0.30) \times 10^{2}$	8.65×10^{2}	6.52 ± 0.30	4.82

[a] Reaction condition: 50 μM BNPC or 400 μM NA, 150 μM catalyst, 0.1 M $NaClO_4$, 50 mM pH = 7.00 Tris–HCl buffer, 25 ± 0.1 °C.

$[Zn_3(L^{2-})_2(H_2O)_2]^{2+}$, $[Zn_3(L^{2-})_2(H_2O)(OH)]^+$ and $[Zn_3(L^{2-})_2(OH)_2]$. The higher stability constant of trizinc complex compared to dinuclear single ligand complex indicates that trinuclear system is more stable at higher pH, which can be ascribed to distribution in two terminals of the two cyclodextrins from two ligands.

3.3. Hydrolysis of carboxylic acid esters

BNPC and NA hydrolysis promoted by the zinc complex were monitored by the appearance of the *p*-nitrophenate anion at 400 nm (ε_{obs} = 8700 M^{-1} cm^{-1}) [27] in a 10% MeCN solution in Tris–HCl (50 mM, pH = 7.00) at 25 ± 0.1 °C (Fig. 2). The initial hydrolysis rates of BNPC (50 μM) and NA (400 μM) in the presence of the catalysts were calculated (Table 2). The measured initial rate of spontaneous cleavage of BNPC (50 μM) was very slow ($\upsilon_{control}$ = 2.45 × 10^{-10} M s^{-1}), which was consistent with the reported value [21]. Almost no hydrolysis rate enhancement was observed when only zinc cation was added to either BNPC or NA solution. However, under identical conditions, a remarkable 865-fold increase in hydrolysis rate over BNPC self-hydrolysis was observed when the zinc complex catalyst was added to BNPC. For NA, the catalyzed hydrolysis rate was 4.82-fold higher than that

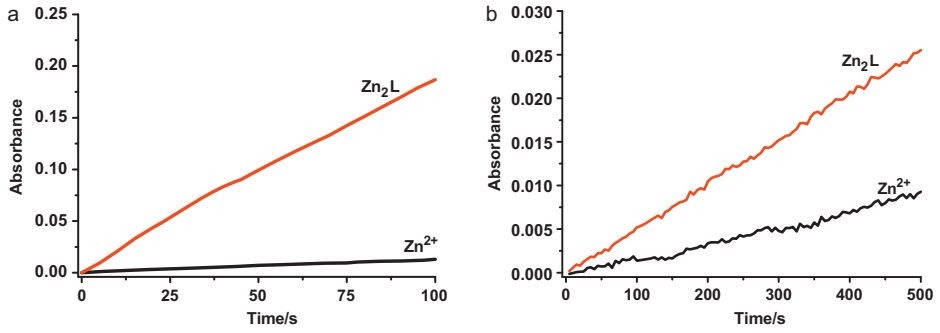


Fig. 2. Plots of absorbance vs time during BNPC hydrolysis (a), NA hydrolysis (b) catalyzed by $Zn(ClO_4)_2$, and Zn_2L in 10% MeCN solution of pH = 7.00 Tris–HCl buffer at 0.1 M $NaClO_4$ and 25 ± 0.1 °C. ([L] = 150 μM, [Zn^{2+}] = 300 μM, [BNPC] = 50 μM and [NA] = 400 μM).

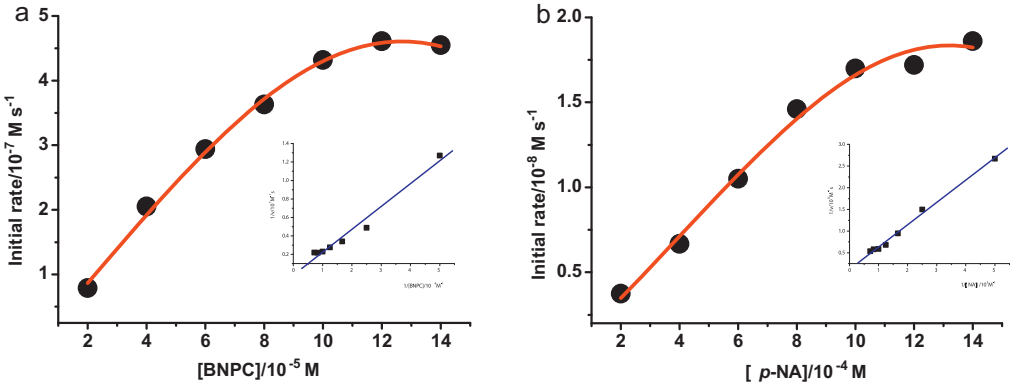


Fig. 3. Saturation kinetics of BNPC hydrolysis (a) and *p*-NA hydrolysis (b) catalyzed by Zn_2L. Each reaction mixture contained Zn_2L (150 μM), Tris–HCl buffer (50 mM pH = 7.00) with 0.10 M $NaClO_4$ at 25 ± 0.1 °C.

Table 3
Kinetic parameters for the ester hydrolysis in the presence of Zn_2L (150 μM) in 10% MeCN solution of Tris–HCl (50 mM pH = 7.00) buffer at 25 ± 0.1 °C.

Substrate	BNPC	NA
k_{uncat} (s^{-1})	$(4.83 \pm 0.16) \times 10^{-6}$	$(3.40 \pm 0.20) \times 10^{-6}$
k_{cat} (s^{-1})	$(2.72 \pm 0.10) \times 10^{-2}$	$(5.52 \pm 0.15) \times 10^{-4}$
K_m (mM)	1.01 ± 0.03	42.58 ± 0.02
k_{cat}/K_m ($M^{-1}\ s^{-1}$)	26.7	1.29×10^{-2}
k_{cat}/k_{uncat}	5.63×10^3	1.62×10^2

of self-hydrolysis. To fully assess the hydrolysis ability of the catalyst for BNPC, a detailed kinetic study was carried out. Saturation kinetics was observed (Fig. 3) and thus kinetic parameters deduced from the Michaelis–Menten equation for the hydrolysis are listed in Table 3. The value of k_{cat}/k_{uncat} was used to describe the catalytic ability of hydrolase mimics, and the values for BNPC hydrolysis and NA hydrolysis were 5.63×10^3 and 1.62×10^2, respectively.

3.4. Hydrolysis of phosphate esters

The DNA model compound BNPP was used as substrate to investigate phosphodiesterase activity. The initial phosphorylation rate in 50 mM Good's buffer at 35 °C and pH = 7.50–10.50 was monitored by the appearance of 4-nitrophenolate at 400 nm. Since the substrate concentration was essentially constant during the measurement, the initial first-order rate constant (k_{in}) of the total catalyst was calculated using Eq. (1) [28]:

$$v = k_{in}[\text{BNPP}] = (k_{\text{BNPP}}[Zn_3(L^{2-})_2]_{total} + k_{OH^-}[OH^-])[\text{BNPP}] \quad (1)$$

where v is the 4-nitrophenolate releasing rate. At a given pH value, the k_{in} values were measured at different catalyst concentrations. Koike et al. reported the initial first-order rate constant of BNPP spontaneous hydrolysis was $8.0 \times 10^{-11}\ s^{-1}$ in water at pH = 8.5 and 35 °C [29]. In the presence of 0.20 mM ligand and 0.30 mM zinc(II)

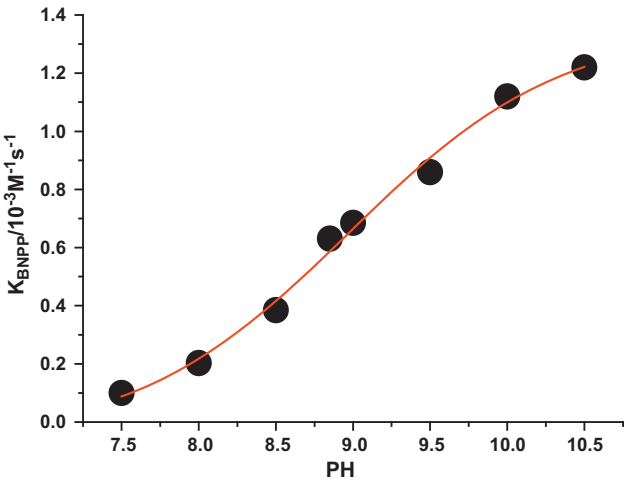
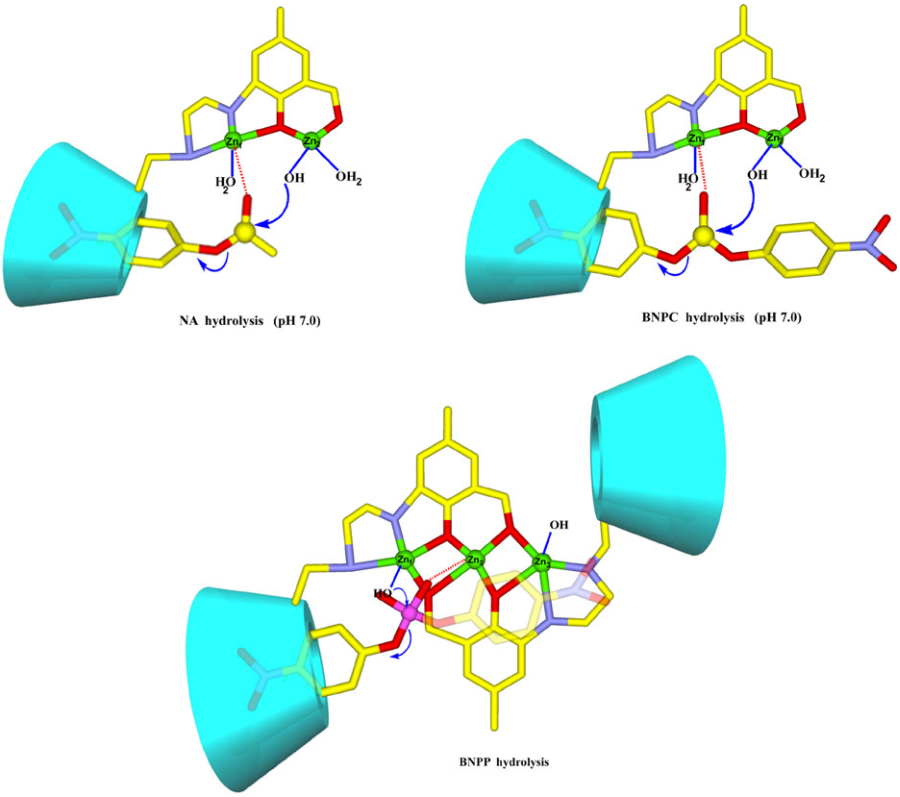


Fig. 4. The pH dependence of the second-order rate constants of BNPP hydrolysis ([L] = 0.20 mM, $[Zn^{2+}]$ = 0.30 mM, [BNPP] = 1.00 mM, [buffers] = 50 mM, I = 0.10 M $NaClO_4$, T = 35 ± 0.1 °C).

cation, the initial first-order rate constant of the phosphate diester hydrolysis was $5.85 \times 10^{-8}\ s^{-1}$ under the same conditions, which is about 731-fold acceleration over the uncatalyzed hydrolysis.

Fig. 4 displays the dependence of the second-order rate (k_{BNPP}) on pH for BNPP hydrolysis promoted by $Zn_3(L^{2-})_2$ as a sigmoid curve for the cleavage reaction. The results indicate a kinetic process controlled by an acid/base equilibrium. The data were fitted by a Boltzman model, which resulted in an inflection point at 9.00 for BNPP. This is almost the same as the pK_{a1} value of the coordinated water deprotonation in $[Zn_3(L^{2-})_2(H_2O)_2]^{2+}$ obtained from the potentiometric pH titration (Table 1). The second-order rate constants of $Zn_3(L^{2-})_2$ for phosphate diester BNPP (k_{BNPP}) is $1.22 \times 10^{-3}\ M^{-1}\ s^{-1}$ at pH = 10.0.



Scheme 2. Suggested intermediates of ester hydrolysis catalyzed by zinc complexes.

The hydrolysis rate of the phosphate diester increased with increasing pH. According to the species distribution as determined by the pH potentiometric titration and analysis of the k_{BNPP}/pH profiles, the active species should be $[Zn_3(L^{2-})_2\ (H_2O)(OH)]^+$ and $[Zn_3(L^{2-})_2(OH)_2]$, while the Zn–OH in $Zn_3(L^{2-})_2$ acted as the nucleophile. The second-order rate constants (k_{BNPP}) were plotted as a sigmoid curve over pH; however, this is different from of our previous work in which the second-order rate constant (k_{NA}) increased exponentially with increasing pH and NA hydrolysis was catalyzed by a supramolecular inclusion complex $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]\ (ClO_4)_2{\cdot}9.5H_2O$ (L^1 = 4-(4′-tert-butyl)benzyldiethylenetriamine) [28]. Thus, only one of the Zn–OH groups was the effective nucleophile and the other hydroxyl group in $[Zn_3(L^{2-})_2(OH)_2]$ was not active during the catalysis.

These results show that the zinc complex has good hydrolytic activities for both monoester NA and diesters BNPC and BNPP, which may be attributed to its polynuclear structures at different pH. At pH = 7.00, the zinc complex was found to exist in the dinuclear single ligand molecular structure. In the catalytic hydrolysis of carboxylic acid esters, the substrates bind to the hydrophobic cavity of β-cyclodextrin, and Zn_1 interacts with the oxygen atom of ester and stabilizes the substrate, which held the functional group of the substrate directly above Zn_2. The Zn_2–OH active species can then nucleophilically attack the carbonyl. When pH > 8.5, the zinc complexes exist in a trimetal bis-ligands structure. In BNPP hydrolysis, one hydrophobic cavity binds with the substrate, and Zn_2 interacts with the oxygen atom of phosphonyl ester and stabilizes the substrate simultaneously. Consequently, the Zn_1–OH active species nucleophilic attacks phosphonyl. The two cyclodextrins at the terminals of the complex cannot bind with the substrate cooperatively due to their large distance and unsuitable direction. Thus the possible intermediates of the esters hydrolysis catalyzed by zinc complexes are proposed in Scheme 2.

4. Conclusion

A trinuclear bis-cyclodextrins zinc complex based on a new β-cyclodextrin derivatized ligand was synthesized and characterized. The zinc complex displayed good hydrolytic activities for both monoester and diesters, which could be attributed to the special polynuclear structures of the zinc complex at different pH. The cooperative interaction of two metal centers and hydrophobic cavity of β-cyclodextrin with substrates play an important role on ester cleavage.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcata.2010.11.037.

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