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Ester Hydrolysis by a Cyclodextrin Dimer Catalyst with a Tridentate N,N′,N″-Zinc Linking Group

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Abstract: A new β-cyclodextrin dimer, 2,6-dimethylpyridine-bridged-bis(6-monoammonio-β-cyclodextrin) (pyridyl BisCD, L), is synthesized. Its zinc complex (ZnL) is prepared, characterized, and applied as a catalyst for diester hydrolysis. The formation constant ($\log K_{ML} = 7.31 \pm 0.04$) of the complex and deprotonation constant ($pK_{a1} = 8.14 \pm 0.03$, $pK_{a2} = 9.24 \pm 0.01$) of the coordinated water molecule were determined by a potentiometric pH titration at (25 ± 0.1)°C, indicating a tridentate N,N′,N″-zinc coordination. Hydrolysis kinetics of carboxylic acid esters were determined with bis(4-nitrophenyl)carbonate (BNPC) and 4-nitrophenyl acetate (NA) as the substrates. The resulting hydrolysis rate constants show that ZnL has a very high rate of catalysis for BNPC hydrolysis, yielding an 8.98×10^3-fold rate enhancement over uncatalyzed hydrolysis at pH 7.00, compared to only a 71.76-fold rate enhancement for NA hydrolysis. Hydrolysis kinetics of phosphate esters catalyzed by ZnL are also investigated using bis(4-nitrophenyl)phosphate (BNPP) and disodium 4-nitrophenyl phosphate (NPP) as the substrates. The initial first-order rate constant of catalytic hydrolysis for BNPP was $1.29 \times 10^{-7}\,s^{-1}$ at pH 8.5, 35 °C and 0.1 mM catalyst concentration, about 1600-fold acceleration over uncatalyzed hydrolysis. The pH dependence of the BNPP cleavage in aqueous buffer was shown as a sigmoidal curve with an inflection point around pH 8.25, which is nearly identical to the pK_a value of the catalyst from the potentiometric titration. The k_{BNPP} of BNPP hydrolysis promoted by ZnL is found to be $1.68 \times 10^{-3}\,M^{-1}s^{-1}$, higher than that of NPP, and comparatively higher than those promoted by its other tridentate N,N′,N″-zinc analogues.

Keywords: cyclodextrins · hydrolysis · kinetics · tridentate ligands · zinc complex

Introduction

During the past two decades, many β-cyclodextrin dimers (bisCDs) have been constructed with various linkers.[1] Compared with native cyclodextrin and monomodified CDs, bridged bisCDs show significantly higher binding abilities and molecular selectivities through the cooperative binding of two adjacent CD units.[2–4] This fascinating property enables bisCDs to be successfully utilized in molecular recognition,[5–7] and as drug carriers,[8] such as bis(β-CD)s/paclitaxel complex displaying a higher antitumor activity and satisfactory water solubility than the parent paclitaxel. The bis(β-CD)s with a carbon–carbon double bond in the linker have a potential use in photodynamic therapy.[9]

Furthermore, the pioneering work of Breslow et al. provided valuable insights into the design of metallo-bisCDs and their applications in enzyme mimics.[10,11] He and his coworkers synthesized metallo-bisCDs bridged by a bipyridine unit with a N,N′-bidentate ligand as metallohydrolase models, which held the functional group of the substrate di-

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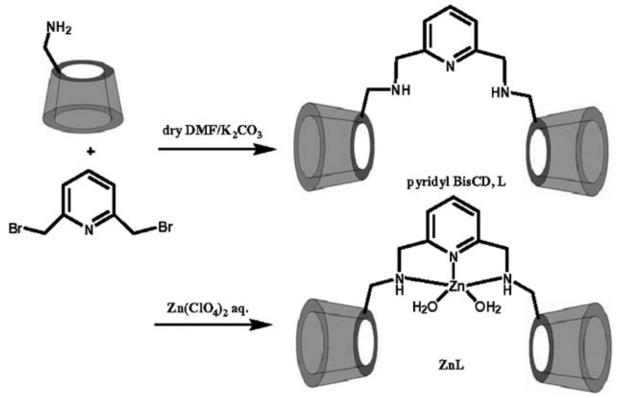
rectly above the metal ion bound to the linker through the cooperative binding of the substrate with two β-CD cavities, and remarkably accelerated catalytic hydrolysis of carboxylic acid diesters and phosphate diesters. Another β-cyclodextrin dimer linked by telluroxides exhibits good catalytic hydrolysis activity of carboxylic acid diesters, and bridged by ditellurium shows enzymatic specificity and mimics the glutathione peroxidase, exhibiting a high efficiency of catalyzing the reduction of cumene peroxide and hydroperoxide in the presence of thiol substrates.[12] Other kinds of enzyme mimics involving metallo-bisCDs linked by porphyrins show selective oxidation of substrates with good catalytic turnover numbers.[13]

We have recently reported several cyclodextrin inclusion complexes constructed by metal complexes binding into β-CDs or their derivatives as supramolecular metalloenzyme models.[14–17] Their catalytic activities are greatly improved since the β-CD or its derivatives are able to mimic the environment of the second coordination sphere in the active site of an enzyme. On the basis of the above work, we have just reported that the zinc complex of a β-cyclodextrin dimer, linked by phenanthroline with a tetradentate N,N′,N″,N‴ ligand, was demonstrated as a promising catalyst for diester hydrolysis.[18] In a systematic study, we designed and synthesized a cyclodextrin dimer bridged by 2,6-bis(ammoniomethyl) pyridine (pyridyl BisCD, L) and its zinc complex (ZnL) with a tridentate N,N′,N″-zinc coordination, which was reasoned to have a more appropriate catalytic site for hydration or hydrolysis.[19] The investigation of esterase activity of ZnL was performed to promote hydrolysis of the carboxylic acid esters, bis(4-nitrophenyl)carbonate (BNPC) and 4-nitrophenyl acetate (NA), and phosphate esters, bis(4-nitrophenyl)phosphate (BNPP) and disodium 4-nitrophenyl phosphate (NPP).

Results and Discussion

Synthesis and Characterization of ZnL

As illustrated in Scheme 1, the dimer (L) was synthesized in 35 % yield by the reaction of 6-monodeoxy-6-monoamino-β-cyclodextrin with 2,6-bis(bromomethyl)pyridine. The dimer (L) was characterized by NMR spectroscopy and Mass Spectrometry (MS) (Figures S1–S2 in the Supporting Information).



Scheme 1. Synthetic Scheme of the catalyst ZnL.

Abstract in Chinese:

本文合成并表征一个新的 2，6-二胺甲基吡啶桥连双环糊精（**L**）及其锌配合物(**ZnL**)，电位滴定结果表明锌配合物的稳定常数 $\log K_{ML}$=7.31±0.03，两个配位水的电离常数分别为 pK_{a1}=8.14±0.03, pK_{a2}=9.24 ± 0.01。在 pH 7.00, 锌配合物催化水解对硝基苯酚碳酸二酯 BNPC，衡量水解酶模型的催化能力的 k_{cat}/k_{uncat} 值高达 8.98×10^{3}，而在同样条件下，催化水解对硝基苯酚碳酸单酯 NA， k_{cat}/k_{uncat} 值仅为 71.76。锌配合物催化水解对硝基苯酚磷酸二酯 BNPP，在 pH 8.5, 35 ºC 下，初始速率常数（1.29×10^{-7} s^{-1}）约为自水解的 1600 倍左右。二级速率常数 k_{BNPP} 随着 pH 的增加而呈 “S”曲线，并分别在 pH 8.25 出现拐点，非常接近 pH 电位滴定得出的$[ZnL(H_2O)_2]^{2+}$ 的 pK_{a1} 值。锌配合物催化水解 BNPP 的的二级速率常数为 1.68×10^{-3} M^{-1} s^{-1}，高于单酯 NPP，也远高于大多数有相近 pK_a 的锌配合物的值。

The further reaction of L and zinc(II) perchlorate gave the zinc(II) complex in a moderate yield (60 %). ESI-MS spectrometry of ZnL is a very important method to investigate the complex formation in aqueous/MeOH solution. Figure 1 shows both the experimental and calculated isotopic distribution for the peak at *m*/*z*: 1217.8, which was assigned to $[ZnL]^{2+}$. This clearly indicates the existing binding between the zinc cation and L. To probe the complexation process, H NMR was used to investigate and distinguish the difference before and after L complexation. Some changes in chemical shifts of the pyridine and amine protons in ZnL were observed relative to those in L: the H_a, H_b, and H_c protons shifted by approximately 0.32, 0.20, and 1.0 ppm, respectively (Figure S2 in the Supporting Information), which also indicated that a complex had formed between the L and zinc(II) cation. Moreover, protons of two coordinated water molecules H_d were observed.

Formation and Deprotonation Constants of ZnL

The protonation constants (K_n) of the ligand L, its complex formation constants (K_{ML}), and the deprotonation constant (pK_a) of the coordinated water molecules as well as species distribution in solution were determined by the pH potentiometric titration at I = 0.10 M $NaClO_4$ and (25 ± 0.1) °C. The pH profiles of the titration curves, which include the distribution curves of the Zn^{II} species as a function of pH (Figure 2), were analyzed by the Hyperquad program. The calculated results are summarized in Table 1, including data from the [Zn(bamp)] (bamp = 2,6-bis(aminomethyl)pyridine) complex,[20] a simple analogue of ZnL.

Three Zn^{II} species, $[ZnL(H_2O)_2]^{2+}$, $[ZnL(H_2O)(OH)]^{+}$, and $[ZnL(OH)_2]$, corresponding to Equations (1–3), respectively, are involved in the complex formation at pH 3–11 (Figure 2). Since the addition of $NaClO_4$ did not cause spectral changes, it was also confirmed that the remaining coordination sites of Zn^{II} were occupied by two water molecules.[16]

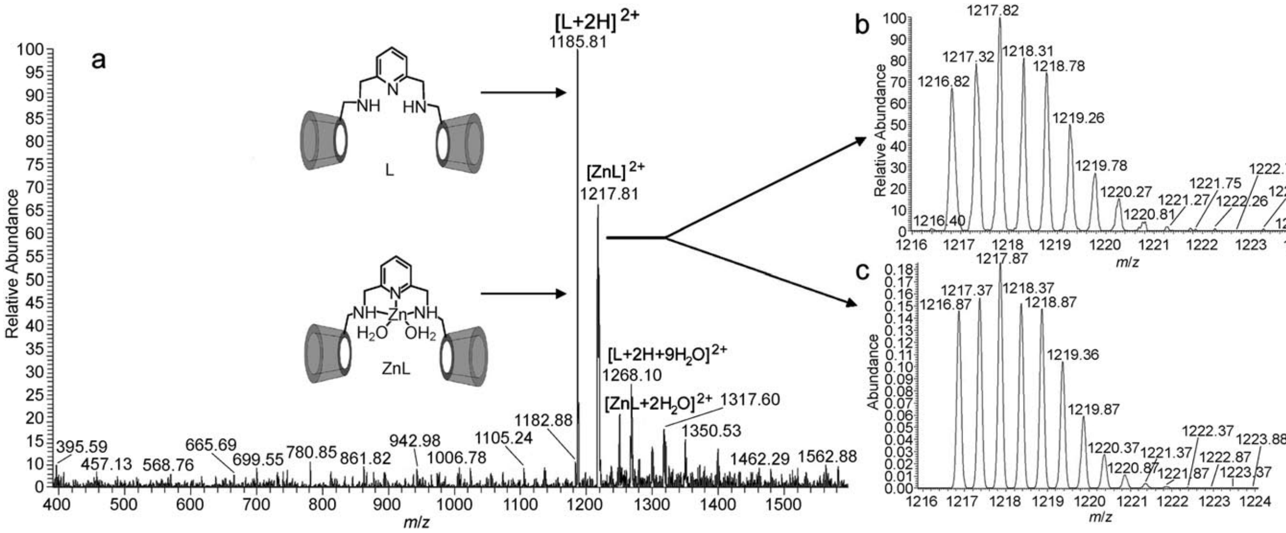


Figure 1. ESI-MS spectra of ZnL. a) Full range spectra. b) Bivalence detected ion isotopes spectra. c) Bivalence simulated ion isotopes spectra .

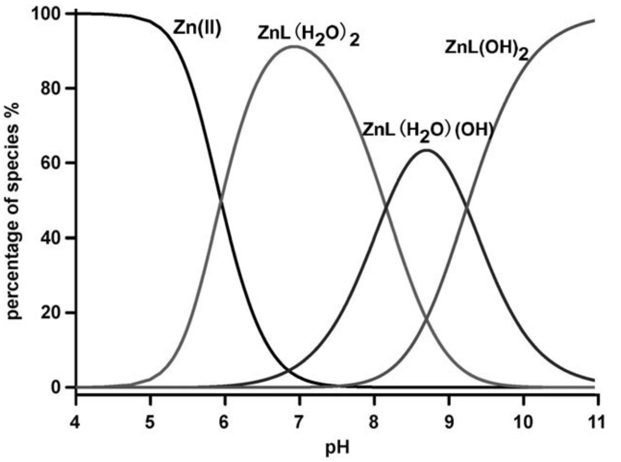


Figure 2. Distribution plots of species with ZnL (1.0 mM) as a function of pH at 0.1 M $NaClO_4$ and (25 ± 0.1) °C.

$$Zn^{2+}+L = [ZnL(H_2O)_2]^{2+} \qquad K_{ZnL} \tag{1}$$

$$[ZnL(H_2O)_2]^{2+} = [ZnL(H_2O)(OH)]^{+}+H^{+} \qquad K_{a1} \tag{2}$$

$$[ZnL(H_2O)(OH)]^{+} = [ZnL(OH)_2]+H^{+} \qquad K_{a2} \tag{3}$$

It might be interesting to find out that the formation constant ($\log K_{ML}$) of ZnL is much higher than that of the [Zn(bamp)] complex (7.31 ± 0.03 for ZnL and 5.56 ± 0.02 for [Zn(bamp)]). This change is probably caused by the existence of cyclodextrins which strongly enhances the stability of the zinc complex. Additionally, the pK_a values of ZnL (p$K_{a1}=8.14\pm0.03$, p$K_{a2}=9.24\pm0.01$) are similar to those of [Zn(bamp)] (p$K_{a1}=7.94\pm0.1$, p$K_{a2}=9.63\pm0.09$).

Hydrolysis of Carboxylic Acid Esters

To determine the effect of hydrophobic interactions on the catalytic activities of ZnL, BNPC and NA were selected as testing substrates because of their structural features. Studies on the hydrolysis kinetics of BNPC and NA were performed in a 10% MeCN solution of Tris-HCl (50 mM, pH 7.00) at (25 ± 0.1)°C (Figure 3). Monitoring the formation of 4-nitrophenolate by UV at 400 nm ($\varepsilon_{obs}=8700\,M^{-1}cm^{-1}$),[21] 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 ($v_{control}=2.45\times10^{-10}\,M\,s^{-1}$), which was consistent with the reported value.[12a] Almost no rate enhancement in the hydrolysis was observed when only the zinc cation was added to the solution of BNPC or NA. However, under identical conditions, a remarkable enhancement was observed when ZnL was added, which was 530-fold higher than that of BNPC self-hydrolysis. In the case of NA, however, the hydrolysis rate for the reaction catalyzed by ZnL is just 2.09-fold higher than that of the self-hydrolysis. To fully assess the hydrolysis ability of ZnL for BNPC, a detailed kinetic study was undertaken. Saturation kinetics were observed (Figure 4) and thus, kinetic parameters deduced

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

Chemical equilibrium		Equilibrium constant	
		bamp[a]	pyridylBisCD
$H_3L^{3+}=H_2L^{2+}+H^+$	pK_1	7.50 ± 0.04	7.52 ± 0.06
$H_2L^{2+}=HL^{+}+H^+$	pK_2	8.32 ± 0.02	8.54 ± 0.05
$Zn^{2+}+L=[ZnL(H_2O)_2]^{2+}$	$\log K_{ML}$	5.56 ± 0.02	7.31 ± 0.03
$[ZnL(H_2O)_2]^{2+}=[ZnL(H_2O)(OH)]^{+}+H^+$	pK_{a1}	7.94 ± 0.1	8.14 ± 0.03
$[ZnL(H_2O)(OH)]^{+}=[ZnL(OH)_2]+H^+$	pK_{a2}	9.63 ± 0.09	9.24 ± 0.01

[a] Ref. [20], bamp = 2,6-bis(aminomethyl)pyridine.

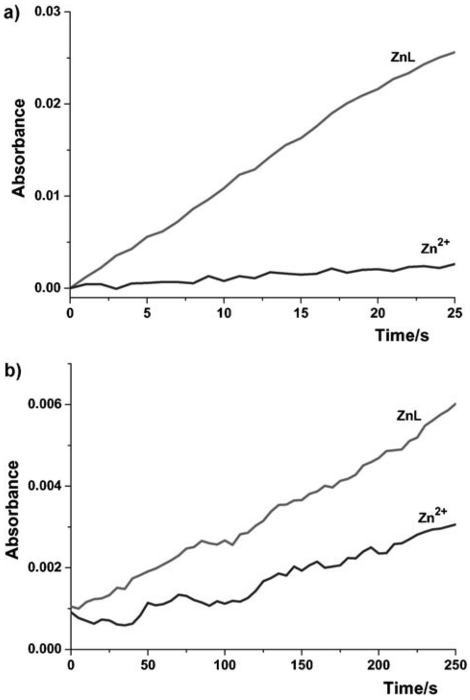


Figure 3. Plots of absorbance versus time during BNPC hydrolysis (a) and NA hydrolysis (b) catalyzed by $Zn(ClO_4)_2$ and ZnL in 10 % MeCN solution of pH 7.00 Tris-HCl buffer at 0.1 M $NaClO_4$ and (25 ± 0.1) °C. ([catalyst] = 50 μM, [BNPC] = 50 μM and [NA] = 400 μM).

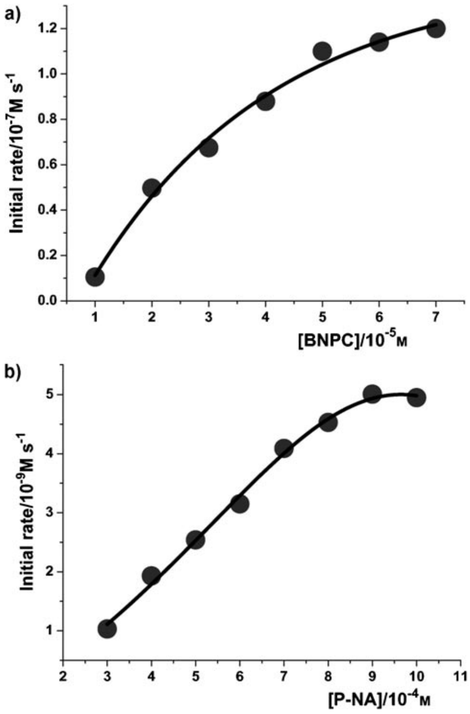


Figure 4. Saturation kinetics of BNPC hydrolysis (a) and NA hydrolysis (b) catalyzed by ZnL. Each reaction mixture contained ZnL (50 μM), Tris-HCl buffer (50 mM, pH 7.00) with 0.10 M $NaClO_4$ at (25 ± 0.1) °C.

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

Catalyst[a]	BNPC v [10^{-9} M s^{-1}]	v/v_{contr}	NA v [10^{-9} M s^{-1}]	v/v_{contr}
Buffer	$(2.45 \pm 0.03) \times 10^{-1}$[b]	1.00	1.35 ± 0.10	1.00
Zn^{II}	$(2.62 \pm 0.03) \times 10^{-1}$	1.07	1.45 ± 0.13	1.07
ZnL	$(1.30 \pm 0.30) \times 10^{2}$	5.30×10^{2}	2.82 ± 0.30	2.09

[a] Reaction condition: 50 μM BNPC or 400 μM NA, 50 μM catalyst, 0.1 M $NaClO_4$, 50 mM pH 7.00 Tris-HCl buffer, (25 ± 0.1) °C. [b] Ref. [12a], v = 0.0147 μM min^{-1}.

Table 3. Kinetic parameters for the ester hydrolysis in the presence of ZnL (50 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}]	$(4.34 \pm 0.20) \times 10^{-2}$	$(2.44 \pm 0.30) \times 10^{-4}$
K_m [mM]	$(9.60 \pm 0.30) \times 10^{-2}$	1.72 ± 0.02
k_{cat}/K_m [M^{-1} s^{-1}]	4.52×10^{2}	1.42×10^{-1}
k_{cat}/k_{uncat}	8.98×10^{3}	71.76

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 it showed values up to 8.98×10^3 for BNPC hydrolysis. For NA hydrolysis, nevertheless, the value of k_{cat}/k_{uncat} was found to be 71.76, which is two orders of magnitude lower than that of BNPC. Furthermore, a much better catalytic efficiency k_{cat}/K_m was obtained for BNPC hydrolysis (4.52×10^2 M^{-1} s^{-1}) in contrast to NA hydrolysis (1.42×10^{-1} M^{-1} s^{-1}), which was about a 3.18×10^3-fold higher activity than that of NA.

Hydrolysis of Phosphate Esters

BNPP is often used as a DNA model compound in the investigation of phosphodiesterase activity. The test was carried out in buffers to mimic biological conditions. The initial phosphorylation rate in aqueous solution at (35 ± 0.1) °C and pH 6.50–9.50 (50 mM Good's buffer) was monitored by the appearance of 4-nitrophenolate at 400 nm.[22] Since the substrate concentration was essentially constant during the measurement, the initial first-order rate constant (k_{in}, in = initial) of the total catalyst was calculated as in Equation (4):[16]

$$v = k_{in}[\mathrm{BNPP}] = (k_{\mathrm{BNPP}}[\mathrm{ZnL}]_{total} + k_{\mathrm{OH^-}}[\mathrm{OH^-}])[\mathrm{BNPP}] \qquad (4)$$

where v is the 4-nitrophenolate releasing rate. At a given pH value, the k_{in} values were measured at different concentrations of the catalyst. Koike et al. even reported the initial first-order rate constant of BNPP spontaneous hydrolysis was 8.0×10^{-11} s^{-1} in water at pH 8.5 and 35 °C.[23] In the presence of 0.1 mM ZnL, however, the initial first-order rate constant of the phosphate diester hydrolysis is enhanced up to 1.29×10^{-7} s^{-1} under the same conditions, which is about a 1600-fold acceleration over the uncatalyzed hydrolysis.

Figure 5a shows the effect of ZnL concentrations on the k_{in} for the cleavage of BNPP at pH 8.85 and (35 ± 0.1) °C.

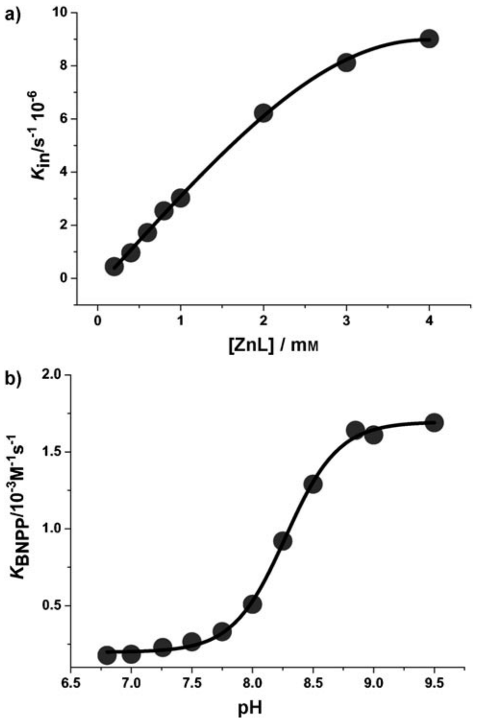


Figure 5. a) Dependence of the initial first-order rate constants on the concentration of ZnL at pH 8.85 and (35±0.1) °C (I = 0.10 M NaClO$_4$, [BNPP] = 0.20 mM, [buffer] = 50 mM). b) The pH dependence of the second-order rate constants of BNPP hydrolysis ([ZnL] = 0.10 mM, [BNPP] = 1.00 mM, [buffers] = 50 mM, I = 0.10 M NaClO$_4$, T = (35±0.1) °C.

The rate of BNPP cleavage initially increases linearly with the increase of ZnL concentration but gradually deviates from the linearity. The calculated second-order rate constant of BNPP hydrolysis catalyzed by ZnL, k_{BNPP}, was determined from the slope of the linear plot. Thus, the slope of k_{in} versus $[ZnL]_{total}$ from Equation (4) resulted in the second-order rate constant (k_{BNPP}). For comparison, phosphate monoester NPP hydrolytic cleavage was performed under the same conditions. The dependence of the second-order rate (k_{BNPP} and k_{NPP}) on pH for BNPP and NPP cleavage promoted by ZnL are illustrated in Figure 5b and Figure S3 in the Supporting Information, respectively. k_{BNPP} increases sharply as the pH increases from 7.50 to 8.85 and then slows down at higher pH, displaying a sigmoidal curve for the cleavage reaction, whereas k_{NPP} shows a slow increase as pH increases from 7.50 to 8.85. 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 8.25 for BNPP and 8.15 for NPP. This is almost the same as the pK_{a1} value of the coordinated water deprotonation in $[ZnL(H_2O)_2]^{2+}$ obtained from the potentiometric pH titration (Table 1). The catalytic activity of ZnL to phosphate diester BNPP is higher than that to the monoester NPP ($k_{BNPP} = 1.68 \times 10^{-3}$ M^{-1}s^{-1}, $k_{NPP} = 5.75 \times 10^{-4}$ M^{-1}s^{-1}); the main reason is that the binding ability of the ditopic hydrophobic substrate BNPP with the two cavities of cyclodextrin is more stronger than that of the monotopic hydrophobic substrate NPP.

Moreover, for BNPP hydrolysis, the k_{BNPP} value is comparatively higher than triamine and macrocyclic polyamine ZnN_3 analogues[24–27] with a similar pK_a in the catalytic hydrolysis of BNPP (see Table 4), and is slightly higher than that of our previous reported Zn-phenCD$_2$ complex with N_4 ligand.[18]

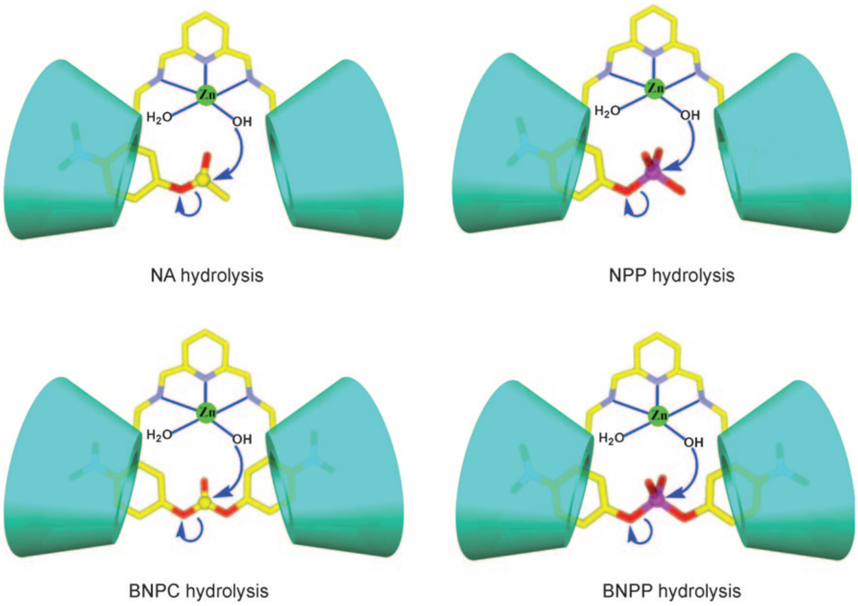
The rate of hydrolysis of the phosphate esters increased with pH increase. According to the species distribution determined by the pH potentiometric titration and analysis of the k_{BNPP}/pH and k_{NPP}/pH profiles, the active species should be $[ZnL(OH)(H_2O)]^+$ and $[ZnL(OH)_2]$, and the Zn−OH in ZnL acts as the nucleophile. The second-order rate constants, k_{BNPP} and k_{NPP}, exhibiting a sigmoidal curve versus pH, however, is different from that of our previous work, in which the second-order rate constant k_{NA} increased exponentially with an increase in pH, and NA hydrolysis was catalyzed by a supramolecular inclusion complex $[Zn(L^1)(H_2O)_2(\beta\text{-CD})](ClO_4)_2\cdot 9.5\,H_2O$ (L^1 = 4-(4′-tert-butyl)benzyldiethylenetriamine).[16] Thus, the effective nucleophile should be only one of the Zn−OH groups and the other hydroxyl group in $[ZnL(OH)_2]$ is not functional during the catalysis. This may be ascribed to the orientation of the second hydroxyl deviated from the phosphorus atom of the esters, resulting in the inappropriate distance between the second hydroxyl group and the phosphorus atom of the esters.

Table 4. The second-order rate constants k_{BNPP} of BNPP hydrolysis promoted by Zn^{II} complexes at (35±0.1) °C.[a]

Species	type	k_{BNPP} [M^{-1}s^{-1}]	pK_a	Ref.
$[Zn(dien)(OH)]^+$	N,N′,N″-Zn	2.3×10^{-5}	8.93	[24]
$[Zn(epd)(OH)]^+$	N,N′,N″-Zn	6.4×10^{-5}	8.90	[24]
$[Zn(dpt)(OH)]^+$	N,N′,N″-Zn	2.3×10^{-5}	8.58	[24]
$[Zn(tach)(OH)]^+$	N,N′,N″-Zn	9.7×10^{-5}	8.13	[24]
$[Zn(taci)(OH)]^+$	N,N′,N″-Zn	1.2×10^{-4}	8.56	[24]
$[Zn(tmca)(OH)]^+$	N,N′,N″-Zn	1.1×10^{-4}	8.51	[24]
$[Zn(OH)(N,N',N''\text{-}[12]ane)]^+$	N,N′,N″-Zn	8.5×10^{-5}	7.3	[25]
$[Zn(OH)(N,N',N''\text{-}[12]ane)]^+$	N,N′,N″-Zn	8.5×10^{-5}	7.2	[26]
$[Zn(bmxd)(OH)]^+$	N,N′,N″-Zn	1.0×10^{-5}	6.94	[27]
$[Zn(L)(OH)]^+$	N,N′,N″-Zn	1.68×10^{-3}	8.14	this work
$[Zn(phenCD_2)(OH)]^+$	N,N′,N″,N‴-Zn	9.9×10^{-4}	8.18	[18]

[a] dien = diethylenetriamine, epd = N-(2-aminoethyl)-1,3-propanediamine, dpt = dipropylenetriamine, tach = 1,3,5-triaminocyclohexane, taci = 1,3,5-triamino-1,3,5-trideoxy-cis-inositol), tmca = 2,4,6-trimethoxycyclohexane-1,3,5-triamine, N,N′,N″-[12]ane = 1,5,9-triazacyclododecan, bmxd = 3,6,9,17,20,23-hexaazatricyclotriaconta-1,11,13,15,25,27-hexaene.

The above results show that under identical conditions, the hydrolysis rate of BNPC is much higher than that of



NA hydrolysis

NPP hydrolysis

BNPC hydrolysis

BNPP hydrolysis

Scheme 2. Suggested intermediates for ester hydrolysis catalyzed by ZnL.

NA, and the BNPP hydrolysis rate is higher than that of NPP. It is well-known that the 4-nitrophenolate group can bind into the hydrophobic cavity of β-cyclodextrin,[28,29] and thus BNPP and BNPC with two ditopic groups of 4-nitrophenol can strongly bind into both cavities of β-cyclodextrins.[11c,12a,18] However, such binding hardly exists for the one hydrophobic group substrates NA and NPP. Therefore, the enhanced second-order rate constant should be contributed to the cooperative binding action of both hydrophobic cavities and zinc hydroxyl active species of the complex, and a possible intermediate of the esters hydrolysis catalyzed by ZnL is proposed in Scheme 2.

Conclusions

A new cyclodextrin dimer with a tridentate ligand (N,N′,N″) and its tridentate N,N′,N″-zinc complex have been synthesized and characterized. The zinc complex exhibits a good catalytic ability for bis(4-nitrophenyl) substrates in the hydrolysis of carboxylic acid ester and phosphate esters, especially for the carboxylic acid diester. The hydrophobic interactions between catalyst and substrate play important roles in the hydrolysis.

Experimental Section

Materials

NA, BNPC, BNPP, and 2,6-bis(bromomethyl)pyridine were purchased from Aldrich. NPP (disodium 4-nitrophenyl phosphate) was purchased from TCI. 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. Water used in all physical measurement experiments was Milli-Q grade. The materials of 6-monodeoxy-6-monoamino-β-cyclodextrin was prepared from 6-monodeoxy-6-monoazido-β-cyclodextrin according to a previous procedure reported by Jicsinszky et al.,[30] with a minor modification, whereas 6-mono(p-toluenesulfonyl)-β-cyclodextrin was prepared in dry pyridine solution rather than in aqueous solution.[31] All compounds were confirmed by their elemental analyses and ESI-MS and ^{1}H NMR spectra.

General Methods

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

Syntheses

2,6-dimethylpyridyl-bridged-bis(6-monoammonio-β-cyclodextrin) (L): A solution of 2,6-bis(bromomethyl)pyridine (0.261 g, 0.985 mmol) in dry DMF (5 mL) was added to a solution of 6-monodeoxy-6-monoamino-β-cyclodextrin (2.461 g, 2.168 mmol) and anhydrous potassium carbonate (1.193 g, 8.63 mmol) in dry DMF with stirring. The mixture was then heated to 80 °C for 8 h under an argon atmosphere. The reaction was cooled to room temperature, and then mixture was evaporated to dryness under reduced pressure. The resulting residue was dissolved in a small amount of hot water, and the aqueous solution was 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 with distilled deionized water as the eluent to give the pure compound in 35.2 % yield. ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 7.62$–7.68 (m, 1 H; pyridine-H-4), 7.23 (d, $J = 7.8$ Hz, 2 H; pyridine-H-3,5), 5.84–5.72 (m, 28 H; OH-2,3), 4.82 (m, 14 H; H-1), 4.56–4.46 (m, 12 H; OH-6), 3.63–3.31 (m, 88 H; H-3,5,6, pyridine-CH_2), 2.05 ppm (brs, 2 H; NH); MS (ESI, H_2O/CH_3OH): *m/z*: calcd:1186.5 $[L+2H]^{2+}$, 1204.5 $[L+2H+2H_2O]^{2+}$; found: 1186.2, 1204.9; elemental analysis calcd (%) for $C_{91}H_{147}N_3O_{68}{\cdot}12H_2O$: C 42.24, H 6.66, N 1.62; found: C 42.54, H 7.071, N 1.819.

Zinc complex (ZnL): A solution of pyridyl BisCD (0.101 g, 0.040 mmol) in water (2 mL) was added dropwise to a dilute aqueous solution of a slight excess of $Zn(ClO_4)_2{\cdot}6H_2O$ (0.020 g, 0.053 mmol) with stirring at room temperature. The resulting solution was stirred for 3 h, and then the solution was evaporated under reduced pressure. Ethanol was added to form a precipitate which was collected by filtration and washed successively with a small amount of ethanol, and then dried in vacuo to give the pure complex as a white solid in 60 % yield. ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 8.00$–7.95 (m, 1 H; pyridine-H-4), 7.46–7.42 (m, 2 H; pyridine-H-3,5), 5.82–5.77 (m, 28 H; OH-2,3), 4.84 (m, 14 H; H-1), 4.52–4.34 (m, 12 H; OH-6), 3.65–3.32 (m, 88 H; H-3,5,6, pyridine-CH_2), 1.05 ppm (brs, 6 H; NH; H_2O); MS (ESI, H_2O/CH_3OH): *m/z*: calcd: 1217.4 $[ZnL]^{2+}$; 1253.4 $[ZnL+4H_2O]^{2+}$ found: 1217.8; 1253.8; elemental analysis calcd (%) for $C_{91}H_{151}N_3O_{78}ZnCl_2{\cdot}22H_2O$: C 35.63, H 6.41, N 1.37; found: C 35.55, H 6.072, N 1.499.

Potentiometric pH Titration

An automatic titrator (Metrohm 702GPD Titrino) coupled to a Metrohm electrode was used and calibrated according to the Gran method.[32] The electrode system was calibrated with buffers and checked by titration of

$HClO_4$ 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 an 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, for which the experimental error was below 1 %. The titration data were fitted from the raw data with 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.

Kinetics of BNPC and NA Hydrolysis

The hydrolysis rates of BNPC and NA in the presence of ZnL complex were measured by an initial slope method following the increase in the 400 nm absorption of the released 4-nitrophenolate.[18] The reaction solution was maintained at (25 ± 0.1) °C. Tris-HCl (pH 7.00, 8.85) buffers were used (50 mM), and the ionic strength was adjusted to 0.10 M with $NaClO_4$. In a typical experiment, after substrate (NA or BNPC) and ZnL complex in 10 % (v/v) CH_3CN solution at an appropriate pH were mixed, the UV absorption decay was recorded immediately and was followed generally until 2 % decay of 4-nitrophenyl acetate. Errors on k_{obs} values were about 5 %.

Kinetics of BNPP and NPP Hydrolysis

The rate of hydrolysis of BNPP and NPP to give mono(4-nitrophenyl)-phosphate and 4-nitrophenolate was measured by an initial slope method following the increase in the 400 nm absorption of the released 4-nitrophenolate in aqueous solution at (35 ± 0.1) °C.[22] At this wavelength, the absorbance of the ester substrate was negligible. MES (pH 6.00–6.60), MOPS (pH 6.60–7.40), HEPES (pH 7.40–8.20), TAPS (pH 8.20–8.90), and CHES (pH 8.90–9.50) buffers were used (50 mM), 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, NPP, buffers, and ZnL in aqueous solution were freshly prepared. The reactions were initiated by injecting a small amount of BNPP and NPP into the buffer solutions of ZnL and followed by fully mixing at (35 ± 0.1) °C. The visible absorption increase was recorded immediately and was followed generally until 2 % formation of 4-nitrophenolate, in which ε values for 4-nitrophenolate were 7106 (pH 6.80), 9137 (pH 7.00), 11 715 (pH 7.26), 13 720 (pH 7.50), 15 270 (pH 7.75), 16 306 (pH 8.00), 16 953 (pH 8.25), 17 340 (pH 8.50), 17 626 (pH 8.85), 17 694 (pH 9.00), and 17 810 (pH 9.50) at 400 nm. The initial first-order rate constants, k_{in} (s^{-1}), for the cleavage of BNPP and NPP, 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}, k_{NPP}) for the catalyzed reactions were determined as the slope of the linear plots of k_{in} versus ZnL concentration. To correct the spontaneous cleavage of BNPP and NPP, each reaction was measured against a reference cell that was identical to the sample cell in composition except for the absence of ZnL. Errors on k_{BNPP} and k_{NPP} values were about 5 %.

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