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Ester catalytic hydrolysis by a tridentate N,N',N"-copper bridged cyclodextrin dimer

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ABSTRACT

A new pyridine bridged cyclodextrin dimer mono-copper complex (CuL) was synthesized and characterized. The hydrolysis of carboxylic acid esters, bis(4-nitrophenyl)carbonate (BNPC) and 4-nitrophenyl acetate (NA), and phosphate ester, a DNA model bis(4-nitrophenyl)phosphate (BNPP), promoted by CuL has been investigated. The resulting hydrolysis rate constants showed that CuL had a very high rate of catalysis for BNPC hydrolysis, yielding a 2.73×10^3 -fold rate enhancement over uncatalyzed hydrolysis at pH 7.00, compared to only a 78.2-fold rate enhancement for NA hydrolysis. The initial first-order rate constant of catalytic hydrolysis for BNPP was 1.01×10^{-7} s⁻¹ at pH 8.5, 35 °C and 0.1 mM catalyst concentration, about 1260-fold acceleration over uncatalyzed hydrolysis. The second rate constant k_{BNPP} of BNPP hydrolysis promoted by CuL was found to be 5.94×10^{-4} M⁻¹ s⁻¹.

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Phosphodiester bond is highly stable kinetically at physiological pH. The halftimes for hydrolysis of RNA and DNA at pH 7.0 and 25 °C are reported to be 110 and up to 100 billion years, respectively. Nature employs suitable enzymes like phosphodiesterases to enhance the hydrolytic rate. However, in general, enzymes are expensive, need purification, and are sensitive to the environment. So, development of artificial metallonucleases would have potential applications in biotechnology and drug development. In the past two decades, many metal complexes, including Cu²⁺, Co³⁺, Zn²⁺, and La³⁺ complexes, have been synthesized as models of metallohydrolases and/or as catalysts for the hydrolysis of DNA. RNA and peptides in which copper complexes were the most studied as artificial nucleases. Examples include small molecule copper complexes with N,N'-bidentate bipyridyl [1-5], N,N',N''-tridentate terpyridyl [6-16] and 1,4,7-triazacyclononane ligands [17-26] and their derivatives. The catalytic activities of small molecule copper complexes for ester hydrolysis are high. However, the main disadvantage is that the dimer bridged by hydroxyl group with no hydrolytic reactivity is easily formed at high pH. Recently, few investigations demonstrated copper complexes show the effectiveness of heterogeneous catalysis for phosphodiester hydrolytic reaction using polymer [27-31] or polymer-silica [32] particles as catalyst carriers. Dong-Hwang Chen et al. [33] prepared a hetero-

geneous catalyst, copper(II)-chelated chitosan magnetic nanocarrier (CMN-Cu(II)), and its calatytic rate constant for BNPP hydrolysis is higher than that of the other carrier catalysts and small molecular Cu [9]aneN₃ complexes. However, these carrier catalysts can't mimic the microenvironment of nature enzymes such as hydrophobic property and recognition to substrate very well. Compounds based on β-cyclodextrins as the models of enzyme have been paid extensive close attention due to the hydrophobic cavity and hydrophilic external surface of β -cyclodextrin, in which copper complexes have been reported. Li and Liu et al. constructed copper (II) complexes of tris(2-aminoethyl)amino-cyclodextrin (Cu-tren-CD) [34] and of CD-modified gold nanoparticles as supramolecular hydrolase models [35], both mimics showed preferable activities for BNPC hydrolysis. Breslow group designed and synthesized a cyclodextrin dimer catalyst with a N,N'-bidentate bipyridine-copper linking group, which remarkably accelerated catalytic hydrolysis of carboxylic acid diesters and phosphate diesters [36,37].

In previous papers, we reported the zinc complexes of β -cyclodextrin dimers, respectively linked by phenanthroline with tetradentate N₄ and pyridine with tridentate N₃ ligands, which demonstrated satisified activities for diester hydrolysis [38,39]. However, structure change of the bridge from tetradentate to tridentate has no obvious rate enhancement for esters hydrolysis. So, on the basis of our works, we designed and synthesized a new copper complex based on cyclodextrin dimer bridged by diaminomethyl pyridine(CuL) to investigate the hydrolytic activities affected by copper cation.

The copper complex (CuL) was prepared from the ligand (L) and copper perchlorate (Scheme 1) [40]. ESI-MS spectrometry of CuL is a

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Scheme 1. Synthetic scheme of copper complex CuL

very important method to investigate the complex formation in aqueous/MeOH solution. Fig. S1 (see Supporting Information) shows both the experimental and calculated isotopic distribution for the peak at m/z: 1216.6 and 1269.1, which were assigned to $[CuL]^{2+}$ and $[CuL+4H_2O+CH_3OH]^{2+}$ respectively. This clearly indicates the existing binding between the copper cation and L. According to the result of thermal analysis (Fig. S2, see Supporting Information), when temperature was lower than 120 °C, the copper complex lost its solvent water molecules included in the cavities of cyclodextrin with 10.8% weight(theoretical value 11.2%). And as the temperature was heated to 190 °C, the coordinated water molecular was lost and the coordinated environment of metal copper atom became unstable obviously, which caused structure collapseand of CuL directly. So the copper complex was tetra-coordinated with one coordinated water molecular. And this structure was confirmed again by the result of potentiometric pH titration (Fig. S3, see Supporting Information).

Caution: Copper perchlorate is a potentially explosive chemical, and it should be prepared in small quantities and handled with care.

The potentiometric pH titrations were performed starting at acidic pH, using a sodium hydroxide solution as the titrant. The deprotonation steps could be derived from the titration curves. The pH profiles of the titration curves, which include the distribution curves of the copper^{II} species as a function of pH (Fig. S3, see Supporting Information), were analyzed by the Hyperquad program [41]. The calculated results are summarized in Table S1, including data from the

[Cu(bamp)] (bamp = 2,6-bis(aminomethyl)pyridine) complex [42], a simple analogue of CuL. Two copper^{II} species, $[CuL(H_2O)]^{2+}$ and $[CuL(OH)]^+$, are involved in the complex formation at pH 3.0–11.5. Since the addition of NaClO₄ did not cause spectral changes, it was also confirmed that the remaining coordination site of copper^{II} was occupied by one water molecule. The formation constant (log*K*_{ML}) and *pK*_a values of CuL are similar to that of [Cu(bamp)] complex (log*K*_{ML} 12.53 ± 0.03; *pK*_{a1} 8.13 ± 0.06 for CuL and log*K*_{ML} 12.20 ± 0.04; *pK*_{a1}8.11 ± 0.09 for [Cu(bamp)]).

To determine the effect of hydrophobic interactions on the catalytic activities of CuL, 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 (Fig. 1). Monitoring the formation of 4-nitrophenolate by UV at 400 nm $(\varepsilon_{obs} = 8700 \text{ M}^{-1} \text{ cm}^{-1})$ [43], the initial hydrolysis rates of BNPC (50 $\mu M)$ and NA (400 $\mu M)$ in the presence of the catalysts were calculated (Table 1). The measured initial rate of spontaneous cleavage of BNPC (50 μ M) was very slow ($v_{control} = 2.45 \times 10^{-10}$ M s⁻¹), which was consistent with the reported value [44]. Almost no rate enhancement in the hydrolysis was observed when only the copper cation was added to the solution of BNPC or NA. However, under identical conditions, a remarkable enhancement was observed when CuL was added, which was 412-fold higher than that of BNPC self-hydrolysis. In the case of NA, however, hydrolysis rate catalyzed by CuL is just 2.87fold higher than that of the self-hydrolysis. To fully assess the hydrolysis ability of CuL for BNPC, a detailed kinetic study was undertaken.





Fig. 1. Plots of absorbance vs time during BNPC hydrolysis (a), NA hydrolysis (b) catalyzed by Cu(ClO₄)₂ and CuL in 10% MeCN solution of pH 7.00 Tris–HCl buffer at 0.1 M NaClO₄ and $(25 \pm 0.1)^{\circ}$ C. ([catalyst] = 150 μ M, [BNPC] = 50 μ M and [NA] = 400 μ M).

Saturation kinetics was observed (Fig. 2) and thus kinetic parameters deduced from the Michaelis–Menten equation for the hydrolysis are listed in Table 2. The value of k_{cat}/k_{uncat} was used to describe the catalytic ability of hydrolase mimics, and it showed values up to 2.72×10^3 for BNPC hydrolysis. For NA hydrolysis, nevertheless, the value of k_{cat}/k_{uncat} was found to be 78.2, two orders of magnitude lower than that of BNPC. Furthermore, a much better catalytic efficiency k_{cat}/K_m was obtained for BNPC hydrolysis (18.30 M⁻¹ s⁻¹) in contrast to NA hydrolysis (6.30 × 10⁻² M⁻¹ s⁻¹), which was about 2.90 × 10²-fold higher activity than that of NA.

The DNA model compound BNPP was used as substrate to investigate 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 6.50–9.00 (50 mM Good's buffer) was monitored by the appearance of 4-nitrophenolate at 400 nm [45]. Since the substrate concentration

Table 1
Initial rate (v) for ester hydrolysis promoted by different catalyst.

Catalyst ^a	BNPC		NA	
	$v (10^{-9} \mathrm{M s^{-1}})$	v/v_{contr}	$v (10^{-9} \mathrm{Ms^{-1}})$	$v/v_{\rm contr}$
Buffer Cu(II) CuL	$\begin{array}{c}(2.45\pm0.03)\!\times\!10^{-1}[44]\\(2.70\pm0.03)\!\times\!10^{-1}\\(1.02\pm0.30)\!\times\!10^{2}\end{array}$	$\begin{array}{c} 1.00 \\ 1.10 \\ 4.12 \!\times\! 10^2 \end{array}$	$\begin{array}{c} 1.35 \pm 0.10 \\ 1.37 \pm 0.20 \\ 3.88 \pm 0.40 \end{array}$	1.00 1.01 2.87

 a Reaction condition: 50 μM BNPC or 400 μM NA, 150 μM catalyst, 0.1 M NaClO₄, 50 mM pH 7.00 Tris–HCl buffer, (25 \pm 0.1) °C.

Fig. 2. Saturation kinetics of BNPC hydrolysis (a) and *p*-NA hydrolysis (b) catalyzed by CuL. Each reaction mixture contained CuL (150 μ M), Tris–HCl buffer (50 mM pH 7.00) with 0.10 M NaClO₄ at (25 \pm 0.1) °C.

was essentially constant during the measurement, the initial firstorder rate constant (k_{in} , in = initial) of the total catalyst was calculated as in Eq. (1) [46]:

$$v = k_{in}[BNPP] = (k_{BNPP}[CuL]_{total} + k_{OH^-}[OH^-])[BNPP]$$
(1)

where *v* is the 4-nitrophenolate releasing rate. At a given pH value, the $k_{\rm in}$ 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 $8.0 \times 10^{-11} \, {\rm s}^{-1}$ in water at pH 8.5 and 35 °C [47]. In the presence of 0.1 mM CuL, however, the initial first-order rate constant of the phosphate diester hydrolysis enhances up to $1.01 \times 10^{-7} \, {\rm s}^{-1}$ at the same conditions, which is about 1260-fold acceleration over the uncatalyzed hydrolysis.

Fig. 3(a) shows the effect of CuL concentrations on the k_{in} for the cleavage of BNPP at pH 8.85 and (35 ± 0.1) °C. The rate of BNPP cleavage initially increases linearly with the increase of CuL concentration but gradually deviates from the linearity. The calculated second-order rate constant of BNPP hydrolysis catalyzed by CuL, k_{BNPP} , was determined from the slope of the linear plot. Thus, the slope of k_{in} versus [CuL]_{total} from Eq. (1) resulted

able 2
Kinetic parameters for the ester hydrolysis in the presence of CuL (150 μ M) 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)	$(4.83\pm0.16)\!\times\!10^{-6}$	$(3.40\pm0.20)\!\times\!10^{-6}$
k_{cat} (s ⁻¹)	$(1.32 \pm 0.10) \times 10^{-2}$	$(2.65 \pm 0.15) \times 10^{-4}$
$K_{\rm m}$ (mM)	0.72 ± 0.03	4.68 ± 0.20
$k_{\rm cat}/K_{\rm m} ({\rm M}^{-1}{\rm s}^{-1})$	18.30	6.30×10^{-2}
k _{cat} /k _{uncat}	2.73×10^{3}	78.2



Fig. 3. (a) Dependence of the pseudo-first-order rate constant on the concentration of CuL at pH 8.85 and (35 ± 0.1) °C. I = 0.10 M NaClO₄, [BNPP] = 0.20 mM and [buffer] = 50 mM. (b) The pH dependence of the second-order rate constants of BNPP hydrolysis ([CuL] = 0.10 mM, [BNPP] = 1.00 mM, [buffers] = 50 mM, I = 0.10 M NaClO₄, T = (35 ± 0.1) °C).

in the second-order rate constant (k_{BNPP}). The dependence of the second-order rate constant (k_{BNPP}) on pH for BNPP cleavage promoted by CuL is illustrated in Fig. 3(b). k_{BNPP} increases sharply as the pH increases from 7.75 to 8.85 and then slows down at higher pH, displaying a sigmoidal curve for the cleavage reaction. The second-order rate constant k_{BNPP} value was found to be $5.94 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$.

The above results show that the hydrolysis rate of BNPC is much higher than that of NA under identical conditions. It is well-known that the 4-nitrophenolate group can bind into the hydrophobic cavity of β -cyclodextrin [48,49], and thus BNPP and BNPC with two terminal groups of 4-nitrophenol can strongly bind into both cavities of β -cyclodextrins [38,44,50]. However, such binding hardly exists for one hydrophobic group substrates NA. So, the cooperative binding action of both hydrophobic cavities with substrate plays an important role on ester cleavage. The possible intermediates of diesters hydrolysis catalyzed by CuL are proposed in Scheme 2.

Acknowledgements

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Appendix A. Supplementary material

Supplementary material to this article can be found online at doi:10.1016/j.inoche.2010.10.018.

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Scheme 2. Suggested intermediates for diesters hydrolysis catalyzed by CuL.

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- [40] Preparation of copper complex (CuL): A solution of pyridyl BisCD (0.060 g,
- 0.025 mmol) in water (2 mL) was added dropwise to a dilute aqueous solution of a slight excess of $Cu(ClO_4)_2 \cdot 6H_2O$ (0.012 g, 0.032 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 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 blue solid in 70% yield. MS (ESI, and then the due to give the pure complex as a bits solid in 70% yield in (20), H_2O/CH_3OH): m/z:calcd: 1216.8 [CuL]²⁺; 1269.3 [CuL+4H₂O+CH₃OH]²⁺ found: 1216.6; 1269.1. elemental analysis calcd (%) for C₉₁H₁₉₅N₃O₁₀₀CuCl₂: C, 35.63; H, 6.41; N, 1.37. Found: C, 35.23; H, 5.94; N 1.48.
- [41] Potentiometric pH Titration: An automatic titrator (Metrohm 702GPD Titrino) coupled to a Metrohm electrode was used and calibrated according to the Gran method. The electrode system was calibrated with buffers and checked by titration of HClO4 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 $(25+0.1)^{\circ}$ 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 Kn, the complex formation constant KML, and the deprotonation constants of the coordinated water pKa.

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- [43] The hydrolysis rates of BNPC and NA in the presence of CuL complex were measured by an initial slope method following the increase in the 400 nm absorption of the released 4-nitrophenolate. The reaction solution was maintained at (25+0.1) °C. Tris-HCl (pH 7.00) buffers were used (50 mM), and the ionic strength was adjusted to 0.10 M with NaClO₄. In a typical experiment, after substrate (NA or BNPC) and CuL complex in 10% (v/v) CH₃CN 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 kobs values were about 5%
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