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Efficient promotion of phosphate diester cleavage by a face-to-face cyclodextrin dimer without metal†

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An organic face-to-face cyclodextrin dimer promotes the cleavage of bis(4-nitrophenyl) phosphate efficiently in neutral pH without the addition of metal. Both of the phosphate diester bonds can be cleaved.

Phosphate diesters in nucleic acids are stable under physiological conditions[1] and a series of phosphodiesterases[2] have been developed by nature to maintain the metabolism of nucleic acids. To investigate the mechanism and structure–activity relationship (SAR) of native enzymes, chemists have developed abundant bio-inspired mimics.[3] Many of them are based on the mimicking of first[4] and second[5] coordination spheres. Recently, various cyclodextrin (CD) metal complexes with notable phosphatediester cleavage activity have been reported because CD can support a hydrophobic environment and a substrate-binding site to mimic the second coordination sphere.

In these CD derivates, the CD dimers possess higher substrate affinities than the CD monomer.[6] Most of them are linked on the primary face ("back to back" like, see Scheme S1, ESI†) and they exhibit considerable promotion to bis(4-nitrophenyl)phosphate (**BNPP**)[7] cleavage in the presence of one[8] or more[9] transition metal ions. Nevertheless, in some cases the face-to-face[10] dimers linked on the secondary face demonstrated higher activities than back-to-back CD dimers as mentioned by Breslow *et al.*[8a,c]

Based on this inspiration, we synthesize a face-to-face CD dimer ligand (**L**) (**L** = 2,6-bis(3-mono-amino-β-cyclodextrin-methyl)-pyridine). Unexpectedly, without the addition of metal ions, this ligand itself can exhibit the efficient promotion of **BNPP** cleavage. Its cleavage activity is not only more than that of its Cu(II) complex but also comparable with those of transition metal complexes. Furthermore, it can also cleave both of the phosphate diester bonds of **BNPP**, which is rarely found in organic mimics. All of these are different from the back-to-back CD dimers with the same bridge[8d,e] which promote the cleavage of **BNPP** only in the presence of metal. So we present this unexpected result in the details below.

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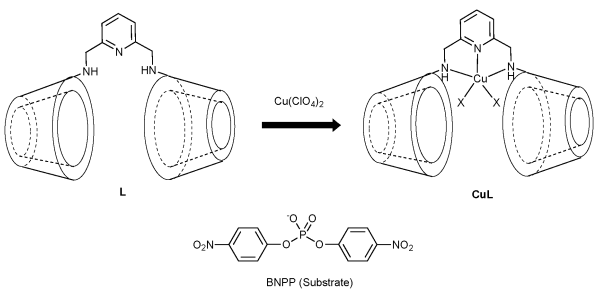
The synthesis and characterization of **L** and **CuL** (as shown in Scheme 1) have been described in the ESI.† The species distribution plot of **L** is shown in Fig. 1 which is based on the data from potentiometric titrations. The NH groups on the bridge have lower pK (Table 1) values than normal primary amines. So this bridge is deprotonated at high pH. The first deprotonation constant of free **L** (pK = 11.34) is slightly lower than that of the OH group on native CD (pK = 12).[11] The species distribution, the formation constant (K_f) and pK_a of **CuL** are shown in the ESI (Fig. S1 and Table S1†). The 1 : 1 complex of **CuL** is found in electrospray ionization mass spectrometry (ESI-MS) (Fig. S2, ESI†) and the K_f (log K_f = 12.40) is similar to the CD dimer Cu(II) complex with the same bridge, so the Cu^{2+} could coordinate with the bridge group on **L** as reported.[8e]

To investigate the cleavage product, ^{31}P-NMR spectroscopy was used and the existence of the phosphorylated **L** monoester (**PL**) was found in the ^{31}P-NMR spectra[12] (Fig. S3, ESI†) and confirmed by ESI-MS (Fig. S4, ESI†). After we determined that the final ratio of [4-nitrophenoxide ($\mathbf{NP^-}$)]/[**PL**] was 1.98 in the mixture, it was confirmed that both of the diester bonds on **BNPP** were cleaved.

To find the active species, we investigated the pH–k_{obs} relationship and the results are shown in Fig. 1. Then we fitted k_{obs} and pH to Eqn 1 (ESI†) and got the k_{BNPP} of all the species (Table 1). They are comparable with high activity first transition metal complexes[5a,b] whose k_{BNPP} lie in the range 10^{-2}–10^{-1} M^{-1} s^{-1}. In particular, the $\mathbf{L^-}$ exhibits a remarkably high activity which could be due to the deprotonated OH groups. As far as we know it is rare that an organic compound, without the addition of a metal, can promote the



Scheme 1 **L** and **CuL** with the phosphate ester substrate (**BNPP**) used in this work. X would be water, ClO_4^- or OH groups on CD.

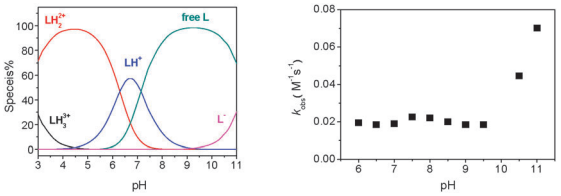


Fig. 1 Species distribution of **L** (left) and second order rate constants (k_{obs}) of **BNPP** cleavage at pH 6–11 in buffer 50 mM, I = 0.1 M ($NaClO_4$) and T = 308 ± 0.1 K (right).

Table 1 The pK and k_{BNPP} of the **L** species

Species	pK	k_{BNPP} (M^{-1} s^{-1})
LH_2^{2+}	6.28	(1.7 ± 0.2) × 10^{-2}
LH^{+}	7.16	(2.4 ± 0.3) × 10^{-2}
free L	11.34	(1.8 ± 0.1) × 10^{-2}
L^{-a}	—[b]	(1.2 ± 0.04) × 10^{-1}

[a] The OH-deprotonated **L**. [b] The determination of this pK needs a very high pH which is hard to reach in aqueous potentiometric titrations.

cleavage both of the diester bonds of **BNPP** as efficiently as metal complexes.

However, it is unexpected that $\mathbf{LH}^{2+}$, $\mathbf{LH}^{+}$ and free **L** have similar activities. To gain a further understanding, the Michaelis–Menten behavior of **L** is also investigated at different pH at which the dominant species is not same (Fig. 2). As is shown in Table 2, the k_{cat}/K_M of **L** is higher than that of organic compounds[13] and CD dimer metal complexes.[9] However, notably the k_{cat} (s^{-1}) at pH 7.0 ($\mathbf{LH}^{+}$ is dominant) and 9.0 (free **L** is dominant) are almost identical, but the substrate binding constant (K_b, $K_b = 1/K_M$) at pH = 7.0 is higher than that at pH = 9.0. It is clear that whether the bridge group is protonated or not, it could not affect the cleavage rate of the ester bonds significantly, though a positively charged bridge by protonation could enhance the substrate attraction. A probable explanation is proposed: **BNPP** is activated mainly by the numerous serried OH groups on CD groups,[14,15] because the number of OH groups is more than that of the NH groups and they are stronger H-bond donors than NH. They occupy the H-bond acceptor sites of the phosphate groups on **BNPP** so the activation of NH is limited.

Based on a comprehensive consideration of the time dependence of ^{31}P-NMR, ESI-MS and UV-vis spectra, a probable reaction process is proposed as shown in Fig. 3: (1) the **BNPP** is trapped by **L** (Fig. S5, ESI†), then activated; (2) the OH groups on **L** and their conjugated base (deprotonated OH groups) act as nucleophiles to attack the phosphate diester to form a new diester and one $\mathbf{NP}^{-}$ is released. This is the rate-determining step. (3) This new phosphate diester transforms into the **PL** monoester and releases another $\mathbf{NP}^{-}$. Further cleavage of **PL** does not happen.

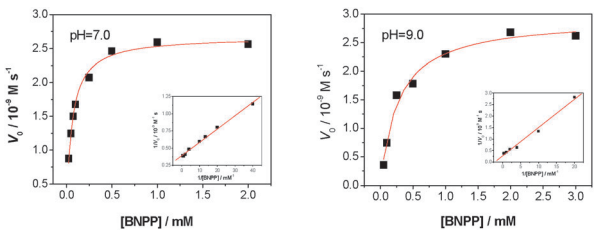


Fig. 2 The effect of **BNPP** concentration on V_0 in the presence of 0.1 mM **L** in buffer 50 mM, I = 0.1 M ($NaClO_4$) and T = 308 ± 0.1 K (left: pH = 7.0, right: pH = 9.0). The solid lines show the fit of data to the Michaelis–Menten equation. Inset: the Lineweaver–Burk double-reciprocal plot.

Table 2 The kinetic parameters from the Michaelis–Menten equation

	pH = 7.0	pH = 9.0
k_{cat} (s^{-1})	(2.7 ± 0.04) × 10^{-5}	(2.9 ± 0.1) × 10^{-5}
K_M (M)	(5.8 ± 0.4) × 10^{-5}	(2.4 ± 0.3) × 10^{-4}
K_b (M^{-1})[a]	(1.7 ± 0.1) × 10^{4}	(3.7 ± 0.5) × 10^{3}
k_{cat}/K_M (M^{-1} s^{-1})	(4.7 ± 0.3) × 10^{-1}	(1.1 ± 0.1) × 10^{-1}
k_{cat}/k_{uncat}[b]	6.8 × 10^{5}	7.2 × 10^{5}

[a] $K_b = 1/K_M$. [b] k_{uncat} = 4.0 × 10^{-11} s^{-1}, ref. 9*b*.

To exclude the possibility of metal-induced activity, we added Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+} and Pb^{2+}, which are usually used to construct hydrolase mimics, to the solution of **L** separately with molar ratio = 1 : 1 in neutral pH. We found that all the additions of metal ions reduce the rate constant (Fig. 4) and indicate that the bridge is not metallized by these metals in our experimental conditions.

The SAR of **L** is also investigated after the mechanism is primarily demonstrated and we focus on: the roles of the bridge group and the CD groups. To investigate the role of the bridge, Cu^{2+} is used as a probe because it could coordinate with the N atoms on the bridge. We find that the k_{obs} of **CuL** are lower than those of **L** (Fig. S6, ESI†) and the addition of Cu^{2+} to **L** reduces the initial rate constant gradually (Fig. 5). To try to explain this, we propose that the Cu^{2+} coordinates with the bridge which could induce the unsuitable location of substrate on **L** just like the enzymes inhibited by metals. We also find that the CD monomer, 3-mono-amino-β-cyclodextrin (3-**ACD**), has no significant activity at neutral pH and more basic conditions until the CD groups are linked suitably (Fig. S7, ESI†). According to these results, we propose that the bridge could adjust the position of two CD groups and gain an optimized conformation in the process of the cleavage reaction.

The role of the CD cavities of **L** is also studied and we used *meso*-tetra(4-sulfonatophenyl)porphine (**TPPS**) as the inhibitor

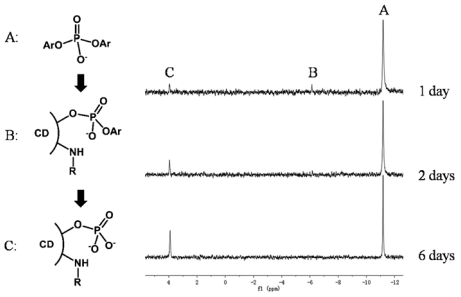


Fig. 3 The suggested mechanism (left) and the time-dependant ^{31}P-NMR spectra (right) of the cleavage of **BNPP** promoted by **L** in buffer (50 mM CHES, with 10% v/v D_2O), I = 0.1 M ($NaClO_4$), pH = 9.0 and T = 308 ± 0.1 K. CHES, N-cyclohexyl-2-aminoethanesulfonic acid.

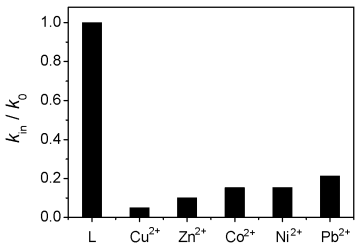


Fig. 4 The initial rate constants (k_{in}) of **BNPP** cleavage in the presence of **L** (0.1 mM) with or without metal ions ([M] = 0.1 mM) in buffer (50 mM MOPSO), I = 0.1 M ($NaClO_4$), pH = 7.0 and T = 308 ± 0.1 K. k_0 is the **BNPP** cleavage rate constant without the addition of metal. MOPSO, 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid.

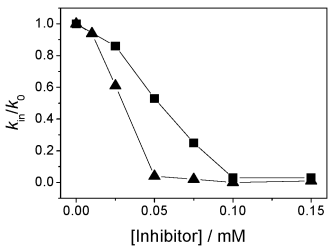


Fig. 5 The Cu^{2+} (■) and **TPPS** (▲) dependence of initial rate constants (k_{in}) of **BNPP** cleavage in the presence of 0.1 mM **L** in buffer (50 mM CHES), I = 0.1 M ($NaClO_4$), pH = 9.0 and T = 308 ± 0.1 K. k_0 is the **BNPP** cleavage rate constant without inhibitor.

since it can form a complex with CD through the secondary face.[16] We found that the addition of **TPPS** to **L** reduced the initial rate constant gradually (Fig. 5) when the **L** can also trap **TPPS** (Fig. S8, ESI†). So we propose that the cavities of **L** could catch the substrate in the cleavage process just like native enzymes.

In conclusion, we present the first organic CD dimer which can cleave both of the diester bonds of **BNPP** at neutral pH without the addition of metal. Its rate constants are comparable with those of high activity metal complexes. Further investigations to understand the SAR of **L** are still in progress and it could be useful to improve the design of phosphodiesterase mimics.

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