Effect of hydrophobic interaction cooperating with double Lewis acid activation in a zinc(II) phosphodiesterase mimic†

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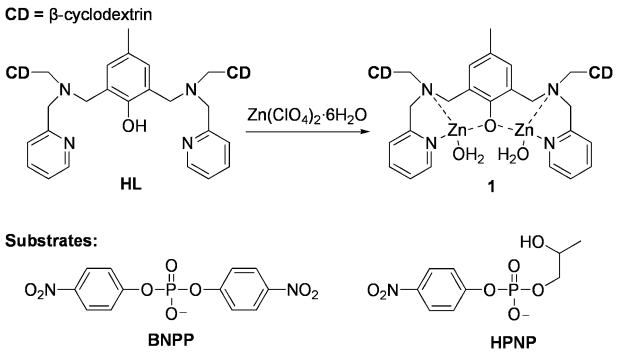
The novel dinuclear Zn(II) complex (1) containing a β-CD dimer could accelerate BNPP (a DNA substitute) hydrolysis more efficiently than catalyze HPNP (a RNA substitute) transesterification with different mechanisms involved; the β-CDs played remarkably different roles.

Under normal circumstances, the phosphodiester bonds in DNA and RNA are very stable,[1] but in the presence of phosphodiesterase, they could be hydrolyzed very easily. Significant efforts have been dedicated to build bio-inspired and smart phosphodiesterase mimics.[2] Inorganic chemists have synthesized simple mono- or di-nuclear complexes imitating active sites of phosphodiesterase to understand the cooperative role of metal ions[3] and weak interaction effect between a metal site and organic groups.[4] Meanwhile, for the first time, the supramolecular method was applied to the studies of mimetic hydrolase by organic chemists.[5] One of the most typical examples is a type of artificial enzyme constructed by modified β-cyclodextrins (β-CDs).[6,7] In these studies, the β-CD molecule could efficiently catch the substrate by its cavity to stabilize the transition state. Based on the above-mentioned inorganic and organic approaches, herein we present a novel hydrolase mimic, which is a dinuclear Zn(II) complex (**1**), derived from a modified β-CD dimer, 2,6-bis{[6-mono(2-pyridylmethylamino)-β-cyclodextrin]-methyl}-4-methylphenol (**HL**) (ESI†).

Incubation of **HL** with 2 equivs of Zn(II) in water at room temperature yielded the dinuclear complex, **1** (Scheme 1), which was structurally characterized (ESI†). Compared with other reported hydrolase mimics, **1** is the first complex that possesses both double Lewis activation sites and double hydrophobic cavities, which implies that the multiple interactions could function cooperatively to result in remarkable reactivity.

First, we investigated the thermodynamic properties of **1** in aqueous solution using potentiometric titration to determine the active species. In total, three deprotonation events were observed (Fig. 1 and Table S1, ESI†). The obtained pK_{a1} of 5.45 ± 0.02 was assigned to the proton release from the acidic phenolic hydroxyl group. The pK_{a2} of 7.49 ± 0.04 assigned to a terminal Zn(II) bound water was lower than most reported ones which commonly give pK_a values above 8.0. The increased acidity might be due to the hydrogen bond between Zn(II)-water and the nearby C6-hydroxyl group of β-CD, which could assist the Zn(II) center to facilitate the deprotonation.[8] The pK_{a3} of 9.91 ± 0.05 assigned to a terminal Zn(II) bond was somewhat higher due to the reduced positive charge at the metal core.[9] This analysis revealed that **1** generated two sets of Zn(II) bound hydroxyl groups in solution which might be the active species responsible for the hydrolysis of phosphoric ester bonds.[10]

Then, **BNPP** was used as a substrate to test the catalytic activity of **1** mimicking metallohydrolases. The Machaelis complex formed by **1** with **BNPP** (1 : 1) was detected using ESI-MS (Fig. S1 and Table S2, ESI†), suggesting enhanced affinity between the catalyst and the substrate. ^{31}P NMR spectroscopy monitoring of the hydrolysis reaction showed that the final product of the catalyzed reactions was 4-nitrophenyl phosphate (**NPP**), and no further hydrolysis of **NPP** was observed (Fig. S2, ESI†). The dependence of the second-order rate constant (k_{obs}), as determined by varying catalyst concentration, on pH was investigated (Fig. 2a). Compared to the distribution plots in Fig. 1, it was assumed that the kinetic process was controlled by two acid–base equilibria. The data was fit to eqn 1 (ESI†) in which the pK_a values were fixed as determined from the titration experiment, giving (1.1 ± 0.1) × 10^{-3} M^{-1} s^{-1} and (4.9 ± 0.6) × 10^{-3} M^{-1} s^{-1} for k^1_{BNPP} and k^2_{BNPP}, respectively. The k^2_{BNPP} corresponding to $[Zn_2L(OH)_2]^{+}$ was higher than k^1_{BNPP} corresponding to $[Zn_2L(OH)]^{2+}$, which could be explained by the fact that a higher pK_a for metal coordinated water usually accompanies better nucleophilic properties.[11] Note that the more nucleophilic species, $[Zn_2L(OH)_2]^{+}$, showed even higher reactivity than the most active Zn(II)-bound hydroxyl species ever reported.[12] Compared with either reported simple dinuclear Zn(II) analogues or mononuclear Zn(II)-β-CD complex, the reactivity enhancement was remarkable in our study. Thus, we concluded that



Scheme 1 Synthesis of catalyst **1** and the phosphate diester substrates used in this study.

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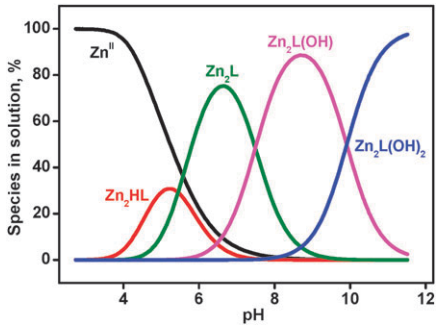

Fig. 1 Distribution plots of species with 1.0 mM **1** as a function of pH at 0.1 M $NaClO_4$ and 298 ± 0.1 K.

combined Lewis activation and hydrophobic interaction could result in synergistic effects on **BNPP** hydrolysis.

Another substrate, **HPNP**, serving as an RNA substitute, was also used to test the reactivity of **1**. ^{31}P NMR spectroscopy showed that the catalytic reaction followed an accepted intramolecular pathway producing cyclic propylene phosphate (**CPP**) as the final product (Fig. S3, ESI†). A complex formed by **1** with the product **CPP** (1 : 1) was also detected using ESI-MS (Fig. S4 and Table S2, ESI†). The data in the pH-rate profile (Fig. 2b) were fitted to eqn 2 (ESI†) in which the pK_a value was fixed as determined from the titration experiment, giving a k_{HPNP} of 0.36 ± 0.01 M^{-1} s^{-1}. A cursory comparison with reported simple dinuclear analogues indicated that the reactivity of **1** toward **HPNP** was moderate but lower than the most efficient one.[4a] This fact implied that β-CDs in **1** might not produce efficient positive effects toward **HPNP** transesterification.

The dependence of initial rate of **BNPP** and **HPNP** on substrate concentration was also measured at the quasi-optimal

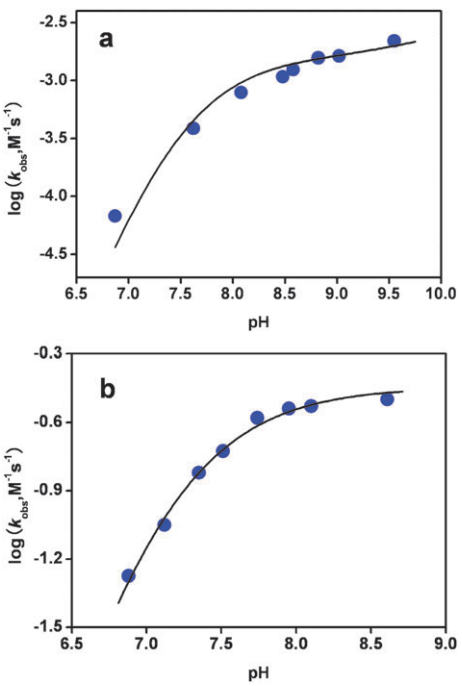

Fig. 2 The pH dependence of the second-order rate constants for (a) **BNPP** hydrolysis and (b) **HPNP** transesterification catalyzed by **1** at 308 ± 0.1 K, I = 0.10 M $NaClO_4$, and [buffer] = 50 mM.

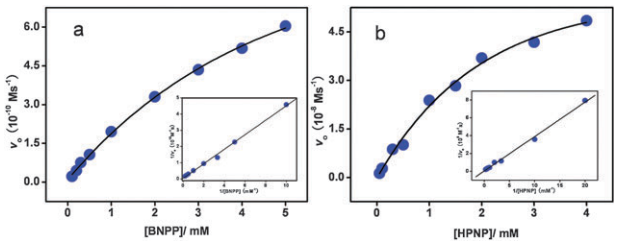

Fig. 3 Saturation kinetics catalyzed by 0.1 mM **1** in buffer (50 mM TAPS and HEPES), I = 0.1 M ($NaClO_4$) at 308 ± 0.1 K: (a) **BNPP** hydrolysis at pH 8.82; (b) **HPNP** transesterification at pH 7.95.

pH (Fig. 3). Saturation kinetics were observed for both at higher concentrations. The data were fitted to the Michaelis–Menten equation with K_m = 10 mM and k_{cat} = 2.2 × 10^{-5} s^{-1} for **BNPP**, and K_m = 7.4 mM and k_{cat} = 1.9 × 10^{-3} s^{-1} for **HPNP**, which were determined from Lineweaver–Burk double-reciprocal plots. The k_{cat}/k_{uncat} for **BNPP** (2.0 × 10^6) was one order of magnitude higher than that for **HPNP** (1.6 × 10^5). Thus, **1** not only showed high catalytic activity toward phosphodiester hydrolysis, but also had a preference for **BNPP**. The binding constant (K_b = 100 M^{-1}) of **BNPP** was much higher than those of the simple dinuclear Zn(II) analogues,[13] which might be due to the additional hydrophobic interactions caused by β-CDs. However, since **BNPP** is a much weaker ligand than **HPNP**,[4a,13] the binding constant of **BNPP** was a little lower than that of **HPNP** (K_b = 135 M^{-1}).

To further understand the roles of β-CDs, a ditopic compound di(*p*-*tert*-butylbenzyl) amine (**DBBA**)[7b] was employed. **DBBA** could form an inclusion complex with β-CD[6e,f] as characterized by ESI-MS and ROESY spectra (Fig. S5 and S6, ESI†). Thus, the cavities were occupied. Therefore, **DBBA** could be applied as a β-CD inhibitor. In each run, **DBBA** was added to the reaction system at increased concentrations in the presence of the substrates. As a result, **BNPP** hydrolysis was strongly inhibited by **DBBA** (Fig. 4), and the profile fitted to eqn 3 (ESI†) gave a K_i value of 0.017 mM. The strong inhibition for **BNPP** was also observed at a lower pH with a K_i value of 0.014 mM (Fig. S7), which excluded the pK_a effects of the amine inhibitor. This indicated that the hydrophobic interaction offered by β-CDs played a crucial role during catalyzed **BNPP** hydrolysis. Very unexpectedly, as for **HPNP** transesterification, **DBBA** did not show any evident inhibition effect (Fig. 4), indicating β-CDs were not involved in this catalytic cycle. Thus, the metal sites provided the primary driving force to attract and activate **HPNP**. In contrast, the bulky β-CDs might introduce the steric hindrance which decreased the binding affinity between **HPNP** and zinc(II) ions. This might prevent the C2-hydroxyl chain from forming the assumed pseudocyclic conformation, which is required for the intramolecular nucleophilic addition to the phosphorus.[14]

Based on the above results, we proposed possible catalytic intermediates which are illustrated in Fig. 5. In the case of **BNPP** hydrolysis, hydrophobic and electrostatic interactions functioned cooperatively to anchor and activate substrates. In tandem, the two hydroxyl groups coordinated to Zn(II) ions could both intervene in the nucleophile attack. The hydrophobic interaction was proposed to assist the positive metal core to stabilize the catalytic transition states. In the case of

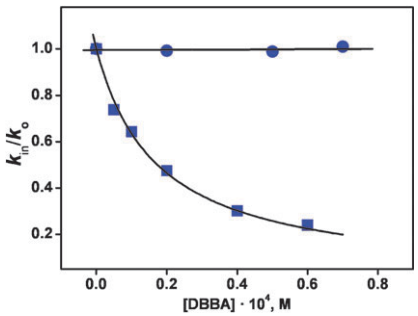

Fig. 4 **DBBA** concentration dependence of normalized pseudo-first-order rate constants of **BNPP** (■) and **HPNP** (●) cleavage catalyzed by **1** (0.1 mM) at pH 8.82 (50 mM TAPS buffer) and pH 7.95 (50 mM HEPES buffer), respectively, where k_0 is the pseudo-first-order rate constant without inhibition.

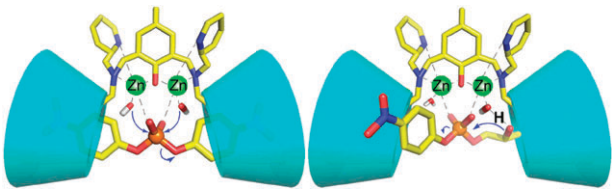

Fig. 5 The proposed intermediates of **BNPP** hydrolysis (left) and **HPNP** transesterification (right).

HPNP transesterification, the binding and activation of substrates mainly depended on the concerted double Zn(II) ions. The cavities of β-CDs failed to catch the substrate during the reaction of **HPNP**. The different affinities of **BNPP** and **HPNP** with β-CDs might be due to the special arrangement of two cavities which suited the ditopic hydrophobic substrate **BNPP** much better than **HPNP**.

In summary, the mimic in our study is the first one characterized by a dinuclear metal core containing hydrophobic β-CD domains for substrates. The kinetic investigation of **BNPP** hydrolysis revealed that the catalytic rates were dependent on both the dinuclear core and the hydrophobic domains, which is the major reason for the higher catalytic acceleration. In the study of **HPNP** transesterification, the catalytic rates were independent of the hydrophobic domains. Our results provided novel insights into the development of bio-inspired and smart artificial metallohydrolases.

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Notes and references

1 N. H. Williams, B. Takasaki, M. Wall and J. Chin, *Acc. Chem. Res.*, 1999, **32**, 485.
2 (*a*) J. Weston, *Chem. Rev.*, 2005, **105**, 2151; (*b*) J. Chin, *Curr. Opin. Chem. Biol.*, 1997, **1**, 514; (*c*) D. E. Wilcox, *Chem. Rev.*, 1996, **96**, 2435; (*d*) N. Sträter, W. N. Lipscomb, T. Klabunde and B. Krebs, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 2024; (*e*) A. L. Gavrilova and B. Bosnich, *Chem. Rev.*, 2004, **104**, 349.
3 (*a*) B. Bauer-Siebenlist, F. Meyer, E. Farkas, D. Vidovic and S. Dechert, *Chem.–Eur. J.*, 2005, **11**, 4349; (*b*) C. He and S. J. Lippard, *J. Am. Chem. Soc.*, 2000, **122**, 184; (*c*) O. Iranzo, A. Y. Kovalevsky, J. R. Morrow and J. P. Richard, *J. Am. Chem. Soc.*, 2003, **125**, 1988.
4 (*a*) G. Feng, D. Natale, R. Prabaharan, J. C. Mareque-Rivas and N. H. Williams, *Angew. Chem., Int. Ed.*, 2006, **45**, 7056; (*b*) A. M. Piątek, M. Gray and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 9878; (*c*) R. Krämer, *Coord. Chem. Rev.*, 1999, **182**, 243; (*d*) H. Aït-Haddou, J. Sumaoka, S. L. Wiskur, J. F. Folmer-Andersen and E. V. Anslyn, *Angew. Chem., Int. Ed.*, 2002, **41**, 4013; (*e*) R. Bonomi, G. Saielli, U. Tonellato, P. Scrimin and F. Mancin, *J. Am. Chem. Soc.*, 2009, **131**, 11278.
5 R. Breslow and L. E. Overman, *J. Am. Chem. Soc.*, 1970, **92**, 1075.
6 (*a*) R. Breslow and S. D. Dong, *Chem. Rev.*, 1998, **98**, 1997; (*b*) R. R. French, P. Holzer, M. G. Leuenberger and W. D. Woggon, *Angew. Chem., Int. Ed.*, 2000, **39**, 1267; (*c*) R. Villalonga, R. Cao and A. Fragoso, *Chem. Rev.*, 2007, **107**, 3088; (*d*) D. Q. Yuan, Y. Kitagawa, K. Aoyama, T. Douke, M. Fukudome and K. Fujita, *Angew. Chem., Int. Ed.*, 2007, **119**, 5112; (*e*) H. Fu, Y.-H. Zhou, W.-L. Chen, Z.-G. Deqing, M.-L. Tong, L.-N. Ji and Z.-W. Mao, *J. Am. Chem. Soc.*, 2006, **128**, 4924; (*f*) Y.-H. Zhou, H. Fu, W.-X. Zhao, W.-L. Chen, C.-Y. Su, H. Sun, L.-N. Ji and Z.-W. Mao, *Inorg. Chem.*, 2007, **46**, 734.
7 (*a*) Y. Liu and Y. Chen, *Acc. Chem. Res.*, 2006, **39**, 681; (*b*) Y.-H. Zhou, M. Zhao, Z.-W. Mao and L.-N. Ji, *Chem.–Eur. J.*, 2008, **14**, 7193; (*c*) Z.-Y. Dong, X. Huang, S.-Z. Mao, K. Liang, J.-Q. Liu, G.-M. Luo and J.-C. Shen, *Chem.–Eur. J.*, 2006, **12**, 3575.
8 J. C. Mareque-Rivas, R. Prabaharan and R. Torres Martín de Rosales, *Chem. Commun.*, 2004, 76.
9 C. Bazzicalupi, A. Bencini, E. Berni, A. Bianchi, P. Fornasari, C. Giorgi and B. Valtancoli, *Inorg. Chem.*, 2004, **43**, 6255.
10 W. N. Lipscomb and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375.
11 I. Bertini, C. Luchinat, M. Rosi, A. Sgamellotti and F. Tarantellilc, *Inorg. Chem.*, 1990, **29**, 1460.
12 M. Acra, A. Bencini, E. Berni, C. Caltagirone, F. A. Devillanova, F. Isaia, A. Garau, C. Giorgi, V. Lippolis, A. Perra, L. Tei and B. Valtancoli, *Inorg. Chem.*, 2003, **42**, 6929.
13 (*a*) J. Chen, X. Wang, Y. Zhu, J. Lin, X. Yang, Y. Li, Y. Lu and Z. Guo, *Inorg. Chem.*, 2005, **44**, 3422; (*b*) N. V. Kaminskaia, C. He and S. J. Lippard, *Inorg. Chem.*, 2000, **39**, 3365.
14 R. Cacciapaglia, A. Casnati, L. Mandolini, D. N. Reinhoudt, R. Salvio, A. Sartori and R. Ungaro, *J. Am. Chem. Soc.*, 2006, **128**, 12322.