

# Hydrolysis of phosphodiester with hydroxo- or carboxylate-bridged dinuclear Ni(II) and Cu(II) complexes

Kazuya Yamaguchi,<sup>a\*</sup> Fumio Akagi,<sup>a</sup> Shuhei Fujinami,<sup>b</sup> Masatatsu Suzuki,<sup>b</sup> Mitsuhiro Shionoya,<sup>c</sup> and Shinnichiro Suzuki<sup>a</sup>

<sup>a</sup> Department of Chemistry, Graduate School of Science, Osaka University, 1-16 Machikaneyama, Toyonaka, Osaka 560-0043, Japan. E-mail: kazu@ch.wani.osaka-u.ac.jp

<sup>b</sup> Department of Chemistry, Faculty of Science, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

<sup>c</sup> Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Received (in Cambridge, UK) 8th November 2000, Accepted 15th January 2001

First published as an Advance Article on the web 8th February 2001

A hydroxo- or carboxylate-bridged dinuclear Ni(II) complexes with *N,N,N',N'*-tetrakis(6-methyl-2-pyridylmethyl)-1,3-diaminopropan-2-ol has been synthesized as models for Ni(II)-substituted phosphotriesterase, which are more active catalysts for hydrolysis of phosphodiester than the corresponding dinuclear Cu(II) and Zn(II) complexes.

It has recently been shown that a number of phosphate esterases are activated by two metal ions.<sup>1</sup> For example, phosphotriesterases which hydrolyze organophosphate triesters, known to be potent insecticides and neurotoxins, require several metal ions [Zn(II), Cd(II), Ni(II), Co(II) and Mn(II)] for their activities.<sup>2</sup> *Pseudomonas diminuta* phosphotriesterase has two Zn(II) atoms per subunit. The binuclear metal site was confirmed by the 2.1 Å resolution X-ray crystal structure of the enzyme.<sup>3</sup> The structure of the active site is very similar to urease, a Ni(II) enzyme catalyzing the hydrolysis of urea, characterized by a bridging carbamylated Lys, two His ligands for each metal ion, and a bridging solvent ligand.<sup>4</sup> Interestingly, Ni(II)-substituted phosphotriesterase has a higher specific activity than the native Zn(II) enzyme, while the Cu(II)-substituted enzyme has a similar activity to the native one.<sup>2</sup> Although there have been numerous reports of esterase model systems using well defined metal complexes [Zn(II), Cu(II), Co(III) and Ln(III)],<sup>5</sup> only a few Ni(II) model complexes have been studied in relation to the structure and reactivity in the hydrolysis of phosphodiesters.<sup>6</sup> In order to understand the mechanistic roles of the metal ions in phosphate ester hydrolysis, we have examined hydrolysis catalyzed by hydroxo- or carboxylate-bridged dinuclear Ni(II) and Cu(II) complexes. We have found that the hydrolysis activities of the dinuclear Ni(II) complexes are significantly greater than those of the Cu(II) and Zn(II) complexes.

[Ni<sub>2</sub>(Me<sub>4</sub>-tpdp)(MeCO<sub>2</sub>)(H<sub>2</sub>O)<sub>2</sub>](ClO<sub>4</sub>)<sub>2</sub> **1** was prepared by the reaction of [Ni<sub>2</sub>(Me<sub>4</sub>-tpdp)(OH)](ClO<sub>4</sub>)<sub>2</sub> **2** with equimolar MeCO<sub>2</sub>H in acetone at room temp. and was recrystallized from MeCN–H<sub>2</sub>O as light green crystals.<sup>7</sup> The X-ray crystal structure<sup>8</sup> of **1** reveals that the two hexacoordinate Ni(II) ions bridged by alkoxide and acetate anions are 3.62 Å apart. The geometries of both the Ni(II) sites are octahedral, with oxygen atoms of water molecules at the sixth coordination site. [Cu<sub>2</sub>(Me<sub>4</sub>-tpdp)(CH<sub>3</sub>CO<sub>2</sub>)](ClO<sub>4</sub>)<sub>2</sub> **3** was prepared by the reaction of [Cu<sub>2</sub>(Me<sub>4</sub>-tpdp)(OH)(H<sub>2</sub>O)](ClO<sub>4</sub>) **4** with equimolar MeCO<sub>2</sub>H in acetone at room temperature and was recrystallized from methanol–diethyl ether as dark green crystals. The X-ray crystal structure<sup>8</sup> of **3** reveals that the two pentacoordinate Cu(II) ions bridged by alkoxide and acetate anions are 3.54 Å apart. The geometries of both the Cu(II) centers are distorted square pyramidal.

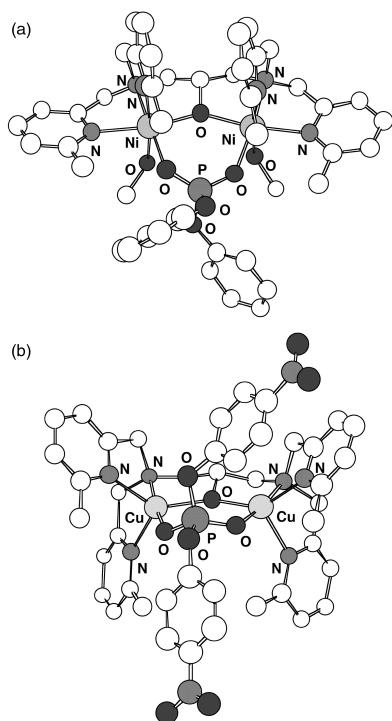
As a model reaction of phosphotriesterase, we examined the hydrolysis of bis(4-nitrophenyl)phosphate (BNP) with the dinuclear metal complexes **1–4**. The hydrolysis rates (*v*) catalyzed by the complexes (2.0 mM) were measured by the initial slope method following an increase in the 395 nm

absorption band of released 4-nitrophenolate in 20% MeCN aqueous solution at 35.0 ± 0.1 °C. Buffer solutions (20 mM HEPES, pH 6.5–8.0; TAPS, pH 8.5–9.0; CAPS, pH 9.5–10.7)<sup>9</sup> were used, and the ionic strength adjusted to 0.10 with NaNO<sub>3</sub>. The observed first-order rate constant, *k*<sub>obs</sub>/s<sup>−1</sup> for the hydrolysis reaction was calculated from the dependency of *v* on the initial concentration (2.0–8.0 mM) of BNP. The second-order rate constants, *k*<sub>BNP</sub>/M<sup>−1</sup> s<sup>−1</sup> for BNP hydrolysis are given by fitting of the kinetic eqn (1).

$$v = k_{\text{obs}}[\text{BNP}] = k_{\text{BNP}}[\text{complex}][\text{BNP}] \quad (1)$$

The *k*<sub>BNP</sub> values for the Ni(II) and Cu(II) complexes (**1**, **2**, **3**, and **4**) were determined to be (3.4 ± 0.2) × 10<sup>−2</sup>, (3.5 ± 0.1) × 10<sup>−2</sup>, (1.8 ± 0.1) × 10<sup>−4</sup> and (1.8 ± 0.1) × 10<sup>−4</sup> M<sup>−1</sup> s<sup>−1</sup> at pH 10.1 and 35.0 °C, respectively. The results demonstrate that the nucleophilic reactions catalyzed by the Ni(II) complexes are *ca.* 200 times faster than those by the Cu(II) complexes. Koike and Kimura reported that mononuclear Zn(II) complexes [Zn([12]aneN<sub>3</sub>)(OH)] and Zn([12]aneN<sub>4</sub>)(OH)]<sup>10</sup> catalyze the hydrolysis of BNP, with *k*<sub>BNP</sub> of 2 × 10<sup>−5</sup> and 13 × 10<sup>−5</sup> M<sup>−1</sup> s<sup>−1</sup>, respectively, at 35.0 °C.<sup>5d</sup> The second-order rate constant for BNP hydrolysis with the Zn<sub>2</sub>(Me<sub>4</sub>-tpdp) complex is 6.7 × 10<sup>−4</sup> M<sup>−1</sup>s<sup>−1</sup> under almost the same conditions.<sup>11</sup> It is interesting that the Ni(II) complexes are more effective than the Cu(II) and Zn(II) complexes for phosphate ester hydrolysis. It should be noted that the hydrolysis activity of a mononuclear Ni(II) complex (Ni(Me<sub>2</sub>-bpa)(H<sub>2</sub>O)(ClO<sub>4</sub>)<sub>2</sub>, **5**)<sup>12</sup> is lower than those of dinuclear **1** and **2**; *k*<sub>BNP</sub> for the mononuclear Ni(II) complex (6.4 × 10<sup>−3</sup> M<sup>−1</sup>s<sup>−1</sup> at pH 10.1) is 5–6 times smaller than those for the dinuclear Ni(II) complexes **1** and **2**. The result suggests that the dinuclear Ni(II) complexes are the cooperative hybrid catalysts.<sup>5d</sup>

The DPP-bridged dinickel complexes,<sup>13</sup> [Ni<sub>2</sub>(Me<sub>4</sub>-tpdp)(DPP)](ClO<sub>4</sub>)<sub>2</sub> **5**, and the BNP-bridged dicopper complex, [Cu<sub>2</sub>(Me<sub>4</sub>-tpdp)(BNP)](ClO<sub>4</sub>)<sub>2</sub> **6**, were obtained by the reaction of **2** with equimolar DPP, and **4** with equimolar BNP in acetone solution, respectively. For X-ray crystal structure analysis, **5** was recrystallized from dry methanol while **6** was recrystallized from dry acetonitrile–diethyl ether. As shown in Fig. 1, the X-ray crystal structures of **5** and **6** reveal that the metal ions are bridged by alkoxide and phosphate diester.<sup>8</sup> The geometries of both the Cu sites in **6** are distorted square pyramidal, while the geometries of both the Ni sites in **5** are octahedral, with oxygen atoms of methanol at the sixth coordination site. The crystal structures of **1**, **3**, **5**, **6** and [Zn<sub>2</sub>(Me<sub>4</sub>-tpdp)(BNP)]<sup>2+</sup> indicate that the Ni(II) centers are hexacoordinate, whereas the Cu(II) and Zn(II)<sup>11</sup> centers are pentacoordinate; the hexacoordinate Ni(II) sites would be more favorable for the formation of an active intermediate complex bound BNP than the pentacoordinate Cu(II) and Zn(II) sites. The substrate could coordinate to Ni(II) ions under conditions that the nucleophile remains bound, and might react with the nucleophile, whereas the substrate

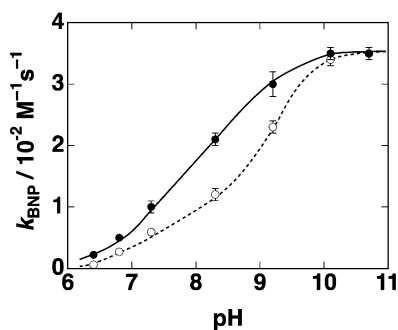


**Fig. 1** Perspective views of  $[\text{Ni}_2(\text{Me}_4\text{-tpdp})(\text{DPP})(\text{MeOH})_2]^{2+}$  (a) and  $[\text{Cu}_2(\text{Me}_4\text{-tpdp})(\text{BNP})]^{2+}$  (b). Hydrogen atoms are omitted for clarity.

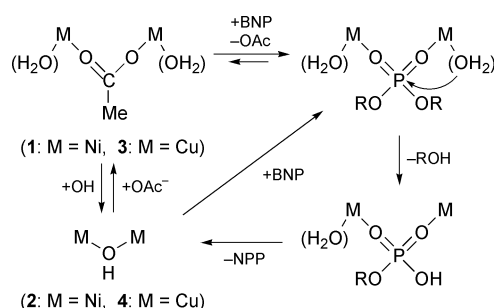
coordinated to Cu(II) or Zn(II) ions could react only with exogenous nucleophile.

$k_{\text{BNP}}$  Values for **1** and **2** are plotted against pH in Fig. 2. The inflection point at pH 8.0 for **2** almost coincides with the  $\text{pK}_a$  value of 7.9 for the coordinated water of **2**. Therefore, the OH<sup>−</sup> ligand in **2** must be a good nucleophile for phosphodiester. On the other hand, the inflection point for **1** is shifted to a higher pH. These findings might be caused by ligand exchange of acetate with the substrate. The pH dependence of the UV–VIS spectrum of **1** was measured at  $25.0 \pm 0.1$  °C. The UV–VIS spectrum of **1** was similar to that of **2** at high pH, with isosbestic points being observed (data not shown). The equilibrium constant for acetate binding was calculated as  $25 \pm 2$  from the absorbance change at 488 nm in the pH range 7.5–11.0 and suggests that the acetate ligand in **1** readily dissociates and exchanges with hydroxide at high pH. This is consistent with the fact that the reactivity of **1** is similar to that of **2** at high pH while acetate ion in **1** would be a competitive inhibitor of the hydrolysis of BNP at low pH. Complex **2** behaves as a catalyst as revealed by the yield of product (>200% relative to complex) when the reaction of 8.0 mM BNP with 2.0 mM complex was carried out for 12 h at pH 10.1 and 35.0 °C. However, BNP hydrolysis with **2** inhibited (by 30%) upon the addition of an equimolar amount of 4-nitrophenyl phosphate (NPP), the hydrolysis product of BNP, at pH 10.1 and 35.0 °C NPP is also a competitive inhibitor with the substrate.

In conclusion, the mechanism of BNP hydrolysis with the dinuclear Ni(II) and Cu(II) complexes can be summarized by



**Fig. 2** Dependence of  $k_{\text{BNP}}$  on pH for the hydrolysis with complex **1** (○) and complex **2** (●) in 20% MeCN aqueous solution at 35.0 °C.



**Scheme 1** Suggested mechanism of BNP hydrolysis with hydroxo- or carboxylate-bridged dinuclear complexes ( $M = \text{Ni}$  or  $\text{Cu}$ ;  $R = \text{C}_6\text{H}_4\text{NO}_2$ ).

Scheme 1. The hexacoordinate dinuclear Ni(II) complexes are more active catalysts for phosphate diester hydrolysis than the corresponding pentacoordinate dinuclear Cu(II) and Zn(II) complexes. The coordination number of the metal ions in an intermediate complex would be important for phosphodiester hydrolysis.

This work was supported by Grant-in-Aids for Scientific Research on Priority Area No.12640538 from the Ministry of Education, Science, Sports and Culture of Japan, to whom we express our thanks.

## Notes and references

- D. E. Wilcox, *Chem. Rev.*, 1992, **96**, 2435.
- G. A. Omburo, J. M. Kuo, L. S. Mullins and F. M. Raushel, *J. Biol. Chem.*, 1992, **267**, 13278.
- J. L. Vanhooke, M. M. Benning, F. M. Raushel and H. M. Holden, *Biochemistry*, 1996, **35**, 6020.
- E. Jabri, M. B. Carr, R. P. Hausinger and P. A. Karplus, *Science*, 1995, **268**, 998.
- For example: (a) E. L. Hegg and J. N. Burstyn, *Coord. Chem. Rev.*, 1998, **173**, 133; (b) J. R. Morrow and W. C. Troglor, *Inorg. Chem.*, 1988, **27**, 3387; (c) N. H. Williams, B. Takasaki, M. Wall and J. Chin, *Acc. Chem. Res.*, 1999, **32**, 485; (d) T. Koike and E. Kimura, *J. Am. Chem. Soc.*, 1991, **113**, 8935; (e) D. H. Vance and A. W. Czarnik, *J. Am. Chem. Soc.*, 1993, **115**, 12 165.
- M. A. De Rosch and W. C. Troglor, *Inorg. Chem.*, 1990, **29**, 2409; J. R. Morrow, L. A. Buttrey and K. Berback, *Inorg. Chem.*, 1992, **31**, 16.
- $\text{Me}_4\text{-tpdp}$ :  $N,N,N',N'$ -tetakis[(6-methyl-2-pyridyl)methyl]-1,3-diaminopropan-2-ol (Y. Hayashi, T. Kayatani, H. Sugimoto, M. Suzuki, K. Inomata, A. Uehara, Y. Mizutani, T. Kitagawa and Y. Maeda, *J. Am. Chem. Soc.*, 1995, **117**, 11 220).
- Crystal data*: for **1**:  $\text{C}_{33}\text{H}_{46}\text{O}_{14}\text{N}_6\text{Cl}_2\text{Ni}_2$ ,  $M = 936.06$ , monoclinic,  $P2_1/n$  (no. 14),  $a = 17.336(1)$ ,  $b = 13.045(1)$ ,  $c = 18.060(1)$  Å,  $\beta = 100.938(5)^\circ$ ,  $V = 4010.0(5)$  Å<sup>3</sup>,  $Z = 4$ ,  $\mu(\text{Mo-K}\alpha) = 11.45$  cm<sup>−1</sup>, 6698 reflections measured, 5724 unique reflections collected [ $I > 3\sigma(I)$ ],  $T = 153$  K,  $R_{\text{int}} = 0.0264$ ,  $R = 0.060$ ,  $R_w = 0.096$ . For **3**:  $\text{C}_{37}\text{H}_{50}\text{O}_{12}\text{N}_6\text{Cl}_2\text{Cu}_2$ ,  $M = 968.83$ , monoclinic,  $P2_1/c$  (no.14),  $a = 17.577(3)$ ,  $b = 17.387(2)$ ,  $c = 14.257(2)$  Å,  $\beta = 100.63(1)^\circ$ ,  $V = 4282(1)$  Å<sup>3</sup>,  $Z = 4$ ,  $\mu(\text{Mo-K}\alpha) = 11.84$  cm<sup>−1</sup>, 7300 reflections measured, 6088 unique reflections collected [ $I > 3\sigma(I)$ ],  $T = 153$  K,  $R_{\text{int}} = 0.0305$ ,  $R = 0.053$ ,  $R_w = 0.079$ . For **5**:  $\text{C}_{46}\text{H}_{59}\text{Cl}_2\text{N}_6\text{O}_{16}\text{Ni}_2\text{P}$ ,  $M = 1171.28$ , monoclinic,  $P2_1/n$ ,  $a = 13.5091(4)$ ,  $b = 20.927(1)$ ,  $c = 18.0299(9)$  Å,  $\beta = 96.4758(5)^\circ$ ,  $V = 5064.6(4)$  Å<sup>3</sup>,  $Z = 4$ ,  $\mu(\text{Mo-K}\alpha) = 9.956$  cm<sup>−1</sup>, 11459 reflections measured, 8547 unique reflections collected [ $I > 3\sigma(I)$ ],  $T = 138.2$  K,  $R_{\text{int}} = 0.02299$ ,  $R = 0.052$ , and  $R_w = 0.078$ . For **6**:  $\text{C}_{48}\text{H}_{59.5}\text{Cl}_2\text{Cu}_2\text{N}_{8.5}\text{O}_{19.5}\text{P}$ ,  $M = 1296.52$ , orthorhombic,  $Pnma$ ,  $a = 22.941(4)$ ,  $b = 36.360(4)$ ,  $c = 14.160(2)$  Å,  $V = 11812(2)$  Å<sup>3</sup>,  $Z = 8$ ,  $\mu(\text{Mo-K}\alpha) = 9.15$  cm<sup>−1</sup>, 7732 reflections measured, 5142 unique reflections collected [ $I > 2.60\sigma(I)$ ],  $T = 288.2$  K,  $R = 0.081$ ,  $R_w = 0.114$ . CCDC 153180–153183. See <http://www.rsc.org/suppdata/cc/b0/b008994j/> for crystallographic data in .cif or other electronic format.
- HEPES:  $N$ -2-hydroxyethylpiperazine- $N'$ -2-ethanesulfonic acid; TAPS:  $N$ -tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid; CAPS: cyclohexylaminopropanesulfonic acid.
- [12]aneN<sub>3</sub>: 1,5,9-triazacyclododecane; [12]aneN<sub>4</sub>: 1,4,7,10-tetraazacyclododecane.
- M. Suzuki *et al.*, unpublished data.
- $\text{Me}_2\text{-bpa}$ : bis[(6-methyl-2-pyridyl)methyl]amine.
- DPP: diphenylphosphate.