## Hydrolytic Activity of A Fe<sup>III</sup>Zn<sup>II</sup> Complex toward Di(*p*-nitrophenyl) Phosphate: A Functional Model of Heterobimetallic Phosphodiestelase

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A Fe<sup>III</sup>Zn<sup>III</sup> complex, [FeZn(L)(AcO)<sub>3</sub>]BPh<sub>4</sub>·H<sub>2</sub>O, of 2-{N-[2-(dimethylamino)ethyl]iminomethyl}-6-[N, N-di(2-pyridyl-methyl)aminomethyl]-4-methylphenolate (L<sup>-</sup>) hydrolyses tri(p-nitrophenyl) phosphate (TNP) into di(p-nitrophenyl) phosphate (DNP<sup>-</sup>) and DNP<sup>-</sup> into mono(p-nitrophenyl) phosphate (MNP<sup>2-</sup>) in aqueous DMF.

Bimetallic cores exist at the active sites of many metalloenzymes and play an essential role in biological systems.<sup>1</sup> Dinuclear Zn cores are found at the active sites of phosphoesterases.<sup>1-4</sup> It is known that phosphotriesterase has only two Zn ions at the active site<sup>5</sup> whereas phospholipase C<sup>6</sup> and P1 nuclease<sup>7</sup> have an additional Zn ion to hydrolyze phosphodiester. It is supposed that these phosphodiesterases require a trinuclear Zn core to bind a substrate at the dinuclear Zn unit and to provide the nucleophile (OH $^-$  or water) on the third Zn center (Figure 1, A). Moreover, heterodinuclear FeZn core was recognized at the active sites of purple acid phosphatase<sup>8</sup> and human calcineurin<sup>9</sup> that facilitate the hydrolysis of phosphodiesters into phosphomonoesters. It is considered that these enzymes employ a FeZn core instead of trinuclear Zn core to accommodate a substrate in chelating mode on the Fe center and provide the nucleophile on the Zn center<sup>10</sup> (Figure 1, B). Here we report a FeZn complex that has a hydrolytic function relevant to phosphodiesterase.



**Figure 1.** Supposed interation of phosphodiester with (A) triuclear Zn core and (B) dinuclear FeZn core in biological phosphodiester.

The FeZn complex  $[FeZn(L)(AcO)_3]BPh_4 \cdot H_2O$  of the endoff compartmental ligand HL (Fig. 2) was prepared as reddish brown crystals by the reaction of HL with Fe(AcO)\_3 and Zn(AcO)\_2 \cdot 2H\_2O in methanol in the presence of sodium tetraphenylborate.<sup>11</sup>

The crystal structure of the FeZn complex was determined by single-crystal X-ray analysis.<sup>12</sup> An ORTEP view of the complex is shown in Fig. 2.<sup>13</sup> The two metal ions are bridged by the phenolic oxygen atom of L<sup>-</sup> and two acetate groups in a '*syn-syn*' mode in the Fe–Zn separation of 3.385(1) Å. The Fe is bound to the bidentate arm and has a six-coordinate geometry together with

two oxygen atoms (O2 and O4) from the bridging acetate groups and the oxygen atom (O6) from a unidentate acetate group. The Zn is bound to the tridentate arm and has a six-coordinate geometry together with two oxygen atoms (O3 and O5) from the bridging acetate groups. The average of the Fe-to-donor bond distances is 2.038 Å and the average of the Zn-to-donor distances is 2.123 Å. The site specificity of metal ions in the FeZn complex is in accord with our recent finding that a smaller metal ion is bound to the bidentate arm and a larger metal ion to the tridentate arm.<sup>14</sup>



Figure 2. Crystal structure of [FeZn(L)(AcO)<sub>3</sub>]BPh<sub>4</sub>·H<sub>2</sub>O.

The complex shows two absorption bands at 340 nm ( $\mathcal{E}$ : 5500 M<sup>-1</sup>cm<sup>-1</sup>) and 480 nm ( $\mathcal{E}$ : 1000 M<sup>-1</sup>cm<sup>-1</sup>) in DMF. The spectrum is virtually invariant at a moderate complex concentration ( $1.0 \times 10^{-3}$ – $2.0 \times 10^{-4}$  M). FAB mass spectrometry in *m*-nitrobenzylalcohol matrix indicated the parent ion peaks centered around *m*/*z* 654.2 corresponding to {FeZn(L)(AcO)<sub>2</sub>}<sup>+</sup>.

Hydrolytic activity of the FeZn complex toward tri(*p*-nitrophenyl) phosphate (TNP) and hydrogen di(*p*-nitrophenyl) phosphate (HDNP) was examined in aqueous DMF (H<sub>2</sub>O : DMF = 2 : 98 in volume) at 25 °C by means of UV-visible spectroscopy. An aqueous DMF solution containing the FeZn complex  $(2.0 \times 10^{-4} \text{ M})$  and the substrate (TNP or HDNP;  $6.7 \times 10^{-5} \text{ M}$ ) was prepared and subjected to spectroscopic measurements, using a complex solution in aqueous DMF  $(2.0 \times 10^{-4} \text{ M})$  as reference.

Spectral changes in the hydrolysis of TNP by the FeZn complex are shown in Figure 3. The absorption band of TNP at 280 nm decreased with time with a concomitant increase at 304 nm due to the formation of DNP<sup>-</sup>. Another absorption band observed at 422 nm is characteristic of *p*-nitrophenolate ion. Based on the absorbance at 304 nm, the hydrolysis of TNP into DNP<sup>-</sup> must be completed in 100 min, but the absorbance at 304 nm, and that at 422 nm as well, showed a tendency to increase further. This fact suggests that the hydrolysis of TNP into MNP<sup>2-</sup> occurs after the completion of the hydrolysis of TNP into

 $BNP^-$ . It is worth noting that the FeCu complex has a high activity in the hydrolysis of TNP relative to analogous ZnZn complex  $[Zn_2(L)(AcO)_2]ClO_4$  when compared under the same conditions (see Insert).



**Figure 3.** Spectral changes in the hydrolysis of TNP by the FeZn complex (measured every 10 minutes). The insert is the spectral changes in the hdrolysis of TNP by  $[Zn_2(L)(AcO)_2]$ -ClO<sub>4</sub> (measured every 10 minutes).

The hydrolysis of HDNP by the FeZn complex was studied by a separate run (Figure 4). In this case spectral changes in the near UV region are small because the absorption band of HDNP and that of  $MNP^{2-}$  are located at close wavelength (304 and 308 nm, respectively). However, the hydrolysis of HDNP by the FeZn complex is evident from the absorption band of *p*-nitrophenolate ion appearing at 425 nm. The solution soon after dissolution gave spectrum **a** which changed to spectrum **b** after 100 min and then gradually to **c** after 700 min. The spectral feature of **b** showing 'negative absorption' around 370 nm implies that a FeZn-DNP adduct is formed at the initial stage and the bound DNP<sup>-</sup> is slowly hydrolyzed into MNP<sup>2-</sup>. The hydrolysis of DNP<sup>-</sup> into BNP<sup>2-</sup> is almost completed in 700 min judged from the time-course of the absorbance at 425 nm.



**Figure 4.** Spectral changes in the hydrolysis of DNP<sup>-</sup> by the FeZn complex (measured every 100 minutes).

It must be emphasized that analogous ZnZn complex has no activity to hydrolyze DNP<sup>-.15</sup> We have confirmed that the absorption bands at 340 and 480 nm of the FeZn complex in aq. DMF (H<sub>2</sub>O : DMF = 2 : 98) diminish their intensities upon high dilution ( $<2 \times 10^{-4}$  M). Furthermore, the molar conductance of the complex in aq. DMF increased upon dilution from 50 S cm<sup>2</sup>mol<sup>-1</sup> at 2 × 10<sup>-4</sup> M to 90 S cm<sup>2</sup>mol<sup>-1</sup> at 4 × 10<sup>-5</sup> M. These facts imply that one acetate bridge of [FeZn(L)(AcO)<sub>2</sub>]<sup>2+</sup>

is released more or less in a dilute solution, providing two vacant sites on the Fe center and one vacant site on the Zn center. The resulting  $[FeZn(L)(AcO)]^{3+}$  can accommodate DNP<sup>-</sup> in the chelating mode on the Fe center and OH<sup>-</sup> (or H<sub>2</sub>O) on the Zn center (Fig. 1, B), allowing the nucleophilic attack of the OH<sup>-</sup> (or H<sub>2</sub>O) to the phosphorus nucleus of DNP<sup>-</sup>.

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- 11 Found: C, 63.14; H, 5.95; N, 6.69; Fe, 5.48; Zn, 6.17%. Clcd for BC<sub>55</sub>FeH<sub>61</sub>N<sub>5</sub>O<sub>8</sub>Zn: C, 62.78; H, 5.84; N, 6.66; Fe, 5.31; Zn, 6.21%.
- 12 Crystal data: [FeZn(L)(AcO)<sub>3</sub>]BPh<sub>4</sub>·H<sub>2</sub>O, F.W. 1052.15; monoclinic space group  $P_{2_1}/n(#14)$ , a = 18.8620(4), b = 15.3948(4), c = 20.0449(4) Å,  $\beta = 117.222(1)^\circ$ , V = 5157.9(2) Å<sup>3</sup>, Z = 4,  $D_c = 1.350$  g/cm<sup>3</sup>. Intensity data were collected at  $-90^\circ$ C on a Rigaku RAXIS-RAPID Imaging Plate using graphite-monochromated Mo Kα radiation ( $\mu$ (Mo Kα) = 8.02 cm<sup>-1</sup>). No. of measured = 44600 and No. of unique reflections = 11633. R = 0.110 (all data), R<sub>W</sub> = 0.159, R1 = 0.065 ( $I > 2.00\sigma(I)$ ).
- 13 Fe–O1 1.970(3), Fe–O2 2.036(3), Fe–O4 1.949(3), Fe–O6 1.955(3), Fe–N1 2.086(4), Fe–N2 2.230(4), Zn–O1 2.092(3), Zn–O3 2.009(3), Zn–O5 2.144(3), Zn–N3 2.191(3), Zn–N4 2.131(3), Zn–N5 2.171(3) Å; Fe–O1–Zn 112.9(1), O1–Fe–O2 92.7(1), O1–Fe–O4 99.9(1), O1–Fe–O6 91.6(1), O1–Fe–N1 86.1(1), O1–Fe–N2 166.6(1), O2–Fe–O4 90.8(1), O2–Fe–O6 172.4(1), O2–Fe–N1 84.9(1), O2–Fe–N2 87.3(1), O2–Fe–O6 94.7(2), O4–Fe–N1 172.8(1), O4–Fe–N2 93.5(1), O6–Fe–N1 89.1(2), O6–Fe–N2 87.1(1), N1–Fe–N2 80.5(1), O1–Zn–O3 101.0(1), O1–Zn–O5 86.1(1), O1–Zn–N3 90.2(1), O1–Zn–N4 163.1(1), O1–Zn–N5 88.4(1), O3–Zn–O5 98.8(1),O3–Zn–N3 92.3(1), O5–Zn–N4 93.6(1), O3–Zn–N5 169.1(1), N3–Zn–N4 77.2(1), N3–Zn–N5 78.4(1), N4–Zn–N5 99.7(1) °.
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