Hydrolytic Activity of $Zn^{II}Zn^{II}$ and $Zn^{II}Ba^{II}$ Complexes toward Tri(*p*-nitrophenyl) Phosphate and Di(*p*-nitrophenyl) Phosphate: A Functional Model of Heterobimetallic Phosphodiesterase

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Dinuclear hydroxo $Zn^{II}Zn^{II}$ and $Zn^{II}Ba^{II}$ core complexes, derived from a macrocyclic compartmental ligand, are examined in their hydrolytic activity toward tri(*p*-nitrophenyl) phosphate (TNP) and sodium di(*p*-nitrophenyl) phosphate (NaDNP) in aqueous DMSO. TNP is hydrolyzed by both complexes. On the other hand, DNP^- is hydrolyzed by the ZnBa complex but not by the ZnZn complex.

Dinuclear Zn cores are found in phosphoesterases such as alkaline phosphatase, phospholipase C, P1 nuclease, phosphotriesterase, etc.[1–4] It is known that phosphotriesterase has only two Zn ions at the active site[5] whereas phospholipase C and P1 nuclease require an additional Zn ion to hydrolyze phosphodiesters[6,7] and alkaline phosphatase requires an additional Mg ion to hydrolyze phosphomonoesters.[8] Moreover, heterodinuclear cores were recognized at the active sites of purple acid phosphatase (FeZn),[9] human calcineurin (FeZn)[10] and human protein phosphatase 1 (FeMn)[11]. It appears that these enzymes employ a heterodinuclear core instead of trinuclear Zn core to facilitate the hydrolysis of phosphodiesters and phosphomonoesters. Here we report different hydrolytic activity of ZnZn and ZnBa complexes toward di(*p*-nitrophenyl) phosphate (DNP^-).

$[\{Zn(dien)\}\{ZnZn(L)(OH)_2\}](ClO_4)_2\cdot 2H_2O$ (**1**) and $[\{Ba(dien)\}\{ZnBa(L)(OH)_2\}](ClO_4)_2\cdot CH_3OH$ (**2**) of the macrocyclic compartmental ligand (L^{2-}) (Figure 1, A) were prepared as follows. To a suspension of *N*,*N*′-ethylenedi(3-formyl-5-methylsalicylideneaminato)zinc(II) (1.0 mmol) in methanol (10 cm^3) was added a solution of $M(ClO_4)_2\cdot 6H_2O$ (M = Zn, Ba) (1.0 mmol) and diethylenetriamine (dien, 1.0 mmol) in methanol (20 cm^3), and the mixture was stirred at room temperature for 1 h. Further addition of a methanol solution of $M(ClO_4)_2\cdot 6H_2O$ (1.0 mmol) and dien (1.0 mmol) resulted in the precipitation of yellow microcrystals (Yield: **1**, 43%; **2**, 33%).[12]

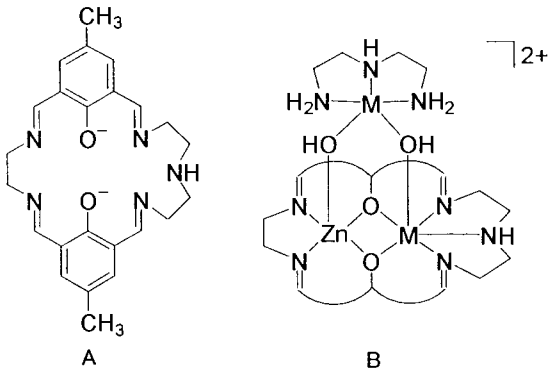

Figure 1. (A) Chemical structure of $(L)^{2-}$ and (B) a possible structure of $[\{M(dien)\}\{ZnM(L)(OH)_2\}](ClO_4)_2$ (M = Zn (**1**) and Ba (**2**)).

The analytical results of **1** and **2** suggest the involvement of the cation $\{M(dien)\}^{2+}$. Dien complexes of Zn^{II} and Ba^{II} ions are known[13,14] and the existence of $\{M(dien)\}^{2+}$ is inferred from the distinct ν_{as}(N–H) and ν_s(N–H) stretching bands at ~3350 and ~3300 cm^{-1}, respectively. An IR band near 3435 cm^{-1} can be ascribed to ν(OH) vibration of the hydroxo group. Hydroxo ligand generally function as a bridge in metal complexes and unidentate hydroxo ligation is rare. We have confirmed a Zn–OH–Pb bridge for analogous $[ZnPb(L)(OH)]_2(ClO_4)_2$ in a dimer-of-dimers structure.[15] Based on these facts, **1** and **2** are presumed to have the dinuclear core $\{ZnM(L)(OH)_2\}$ with one hydroxide group on each metal and $\{M(dien)\}^{2+}$ is bonded to the dinuclear core through the hydroxo bridges (Figure 1, B).

Hydrolytic activity of **1** and **2** toward tri(*p*-nitrophenyl) phosphate (TNP) and sodium di(*p*-nitrophenyl) phosphate (NaDNP) was examined in aqueous DMSO (H_2O : DMSO = 1 : 99 in volume) at 25 °C by means of UV–visible spectroscopy. A solution containing the ZnM complex (**1** or **2**; 2×10^{-4} M) and the substrate (TNP or NaDNP; 3.3×10^{-5} M) was prepared and subjected to spectroscopic studies, using a complex solution in aqueous DMSO (2×10^{-4} M) as the reference.

The spectral changes for the hydrolysis of TNP by **1** and **2** are shown in Figure 2. In the hydrolysis by **1** (Figure 2, top) the absorption at 280 nm due to TNP decreased with the pas-

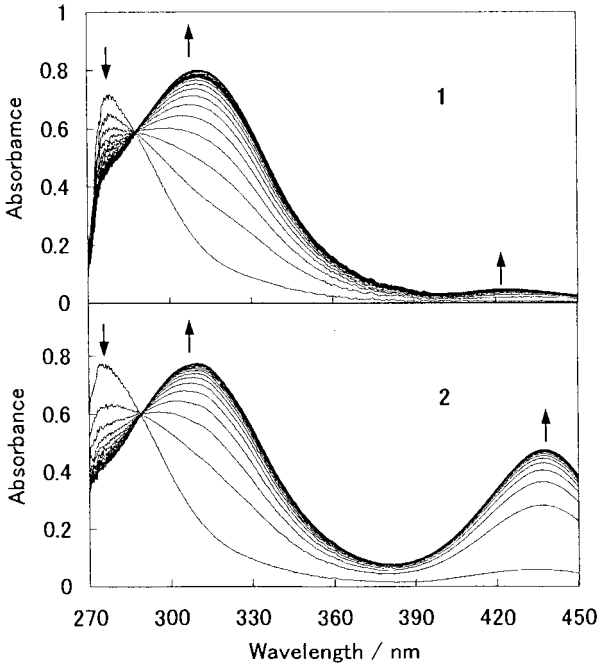

Figure 2. Spectral changes for the hydrolysis of TNP by the ZnZn complex (**1**) (top) and by the ZnBa complex (**2**) (bottom).

sage of time with a concomitant increase at 305 and 422 nm. The absorption at 305 nm is characteristic of DNP^- and that at 422 is attributable to *p*-nitrophenolate ion. The result indicates the hydrolysis of TNP by **1**. Similarly, **2** showed a hydrolytic activity toward TNP (Figure 2, bottom). In this case the absorption due to *p*-nitrophenolate is observed as an intense band at 432 nm. This is probably because the deprotonation of *p*-nitrophenol to *p*-nitrophenolate is promoted by Ba^{2+} ion arising from $\{Ba(dien)\}^{2+}$. We have confirmed that an aqueous DMSO solution of dien and $M(ClO_4)_2 \cdot 6H_2O$ (M = Zn, Ba) [1 : 1] has no activity to hydrolyze TNP, suggesting that the dinuclear $[ZnM(L)(OH)_2]$ moiety is concerned with the hydrolysis of TNP. It is likely that $[ZnM(L)(OH)_2]$ hydrolyzes TNP by concerted binding of the substrate on the M center and nucleophilic attack of the hydroxide provided at the Zn center.

The spectral changes for the hydrolysis of NaBNT by **1** and **2** are shown in Figure 3. No spectral change occurred in the reaction by **1**. On the other hand, a spectral change is obvious in the hydrolysis by **2**. Evidently, **1** has no hydrolytic activity whereas **2** has an activity to hydrolyze DNP^- to MNP^{2-}.

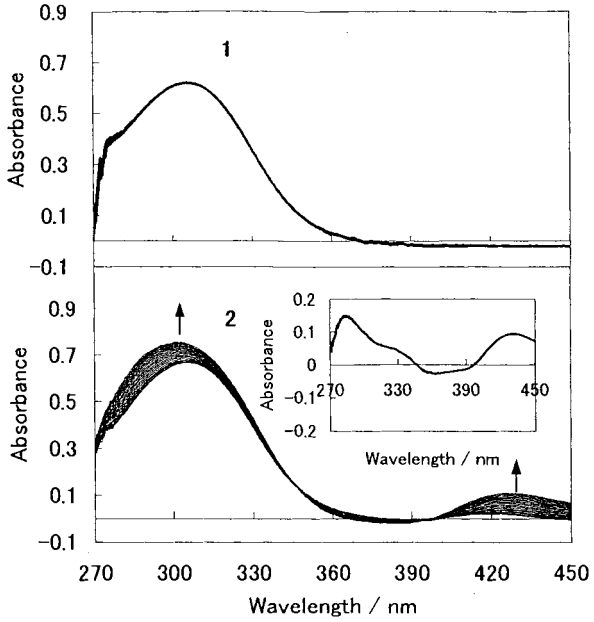

Figure 3. Spectral changes for the hydrolysis of DNP^- by the ZnZn complex (**1**) (top) and by the ZnBa complex (**2**) (bottom). The insert in the bottom is the difference spectrum between the final and the initial spectra.

We have found that TNP hydrolysis by $[ZnPb(L)(OH)]ClO_4$ is stoichiometric but not catalytic because the resulting DNP^- bridges the two metal ions affording a stable DNP complex $[ZnPb(L)(DNP)]^+$.[15] The formation of such a DNP-bridged complex must be the reason why **1** has no hydrolytic activity toward DNP^- (Figure 4, A). In the reaction with **2**, DNP^- might be bound to the Ba^{2+} center in a chelating mode allowing the nucleophilic attack of the hydroxide ion, attached to the Zn center, to the phosphorous nucleus (Figure 4, B).

In conclusion the ZnBa complex illustrates a significance of heterodinuclear core in the hydrolysis of phosphodiesters.

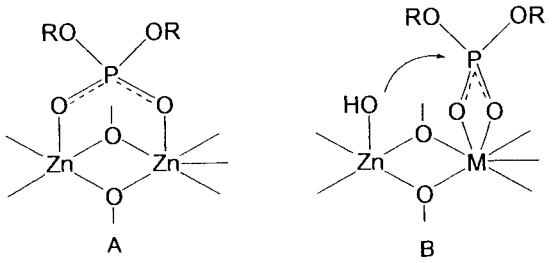

Figure 4. Likely structures of (A) a DNP^- adduct of **1** and (B) a DNP^- adduct of **2** (R = *p*-nitrophenyl).

Mechanistic studies for the DNP^- hydrolysis by **2** and analogous complexes are under way.

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