Hydrolytic Function of a Hydroxo-$Zn^{II}Pb^{II}$ Complex toward Tris-*p*-nitrophenyl Phosphate: A Functional Model of Heterobimetallic Phosphatases

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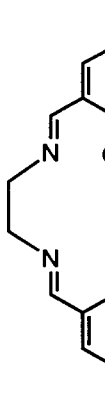
A $Zn^{II}Pb^{II}$ complex $ZnPb(L)(H_2O)_2(ClO_4)_2$ of a macrocyclic compartmental ligand L^{2-} is converted under alkaline conditions into a hydroxo complex $ZnPb(L)(OH)(H_2O)ClO_4$ that hydrolyzes tris-p-nitrophenyl phosphate (TNP) to bis-p-nitrophenyl phosphate (BNP^-) forming a BNP-bridged complex $[ZnPb(L)(BNP)]ClO_4$.

Bimetallic cores are versatile in biological systems[1] and model studies by the use of simple metal complexes are becoming increasingly important to understand biological functions of such bimetallic cores. Heterodinuclear cores were recently recognized at the active sites of purple acid phosphatase (FeZn),[2] human calcineurin (FeZn)[3] and human protein phosphatase 1 (MnFe)[4] and these findings have stimulated interest in hydrolytic function of heterodinuclear complexes. It is considered that these phosphatases employ a pair of dissimilar metal ions to facilitate the concerted incorporation of substrate at one metal center and nucleophilic attack of hydroxide or water on the other metal center. Here we report a functional model of the heterobimetallic phosphatases, using a $Zn^{II}Pb^{II}$ complex of the macrocyclic compartmental ligand L^{2-} having "salen"- and "saldien"-like entities sharing the phenolic moieties (see Scheme 1). The ZnPb metal ion pair is chosen by taking into consideration the nature of Zn^{II} to form Zn-OH bond and a high affinity of Pb^{II} toward phosphate oxygen.

The $Zn^{II}Pb^{II}$ complex, $ZnPb(L)(H_2O)_2(ClO_4)_2$ (**1**),[5] was prepared by a modification of the literature method.[6] FAB mass spectrum of **1** shows a molecular ion peak of M/Z 788 that corresponds to $\{ZnPb(L)(ClO_4)\}^+$. The ν_3 vibration mode of perchlorate group appears as unsplit band around 1100 cm^{-1}. One of the water molecules is probably bonded to the Zn ion affording a five-coordinate geometry about the metal ion.[7] Conductivity studies indicate that **1** behaves as 2:1 electrolyte in DMSO (Λ_M: 59 S cm^2 mol^{-1} at 25 °C).[8]

1 was readily converted into $ZnPb(L)(OH)(H_2O)ClO_4$ (**2**)[9] under alkaline conditions. **2** shows a molecular ion peak of M/Z 688 corresponding to $\{ZnPb(L)-H\}^+$ in FAB mass spectrometry. Our preliminary crystallographic studies for **2** have indicated that the hydroxo group is bonded to the Zn center and the Zn(OH)Pb core dimerizes through the Zn-OH--Pb linkage in the crystal. In DMSO solution **2** exists as the monomeric Zn(OH)Pb species judged from its 1:1 electrolytic nature (Λ_M: 28 Scm^2 mol^{-1} in DMSO). The corresponding $Cu^{II}Pb^{II}$ and $Ni^{II}Pb^{II}$ complexes of L^{2-}, $MPb(L)(ClO_4)_2 \cdot H_2O$, are known[6,10] but hydroxo complex analogous to **2** cannot be derived for the complexes in spite of our many efforts.

Hydrolytic activity of **1** and **2** toward tris-p-nitrophenyl phosphate (TNP) has been examined in DMSO. Based on ^{31}P NMR spectroscopy **1** exhibited only a low activity whereas **2** had a high activity to hydrolyze TNP to BNP in a nearly stoichiometric manner (BNP^- = bis-p-nitrophenyl phosphate ion). Further, the reaction of **2** and TNP in acetonitrile resulted in the precipitation of $[ZnPb(L)(BNP)]ClO_4$ (**3**).[11] The same



L^{2-}

Scheme 1. Chemical structure of $(L)^{2-}$.

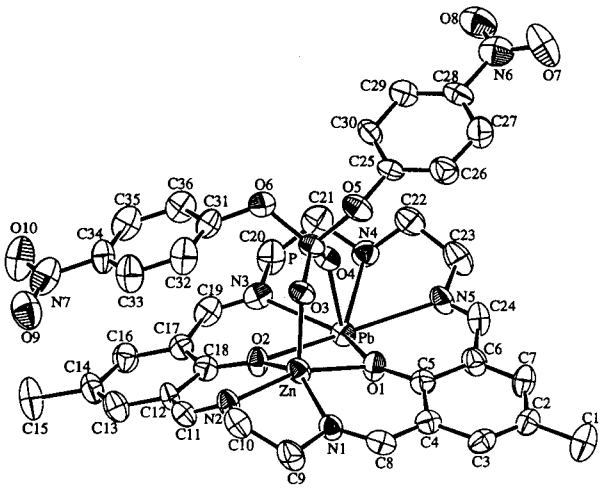


Figure 1. Prespective view of **3**. Selected bond distances and angles: Zn-O1 2.043(6), Zn-O2 2.035(5), Zn-O3 1.993(6), Zn-N1 2.046(7), Zn-N2 2.054(7), Pb-O1 2.519(5), Pb-O2 2.608(5), Pb-O4 2.360(5), Pb-N3 2.554(7), Pb-N4 2.549(7), Pb-N5 2.565(7), P-O3 1.492(6), P-O4 1.480(6), Zn--Pb 3.464(2) Å; O1-Zn-O2 93.4(2), O1-Zn-N1 88.1(3), O2-Zn-N2 88.5(2), N1-Zn-N2 79.4(3), O1-Pb-O2 70.8(2), O1-Pb-N5 71.7(2), O2-Pb-N3 70.5(2), N3-Pb-N4 68.9(2), N4-Pb-N5 68.5(2), Zn-O1-Pb 98.3(2), Zn-O2-Pb 95.7(2)°.

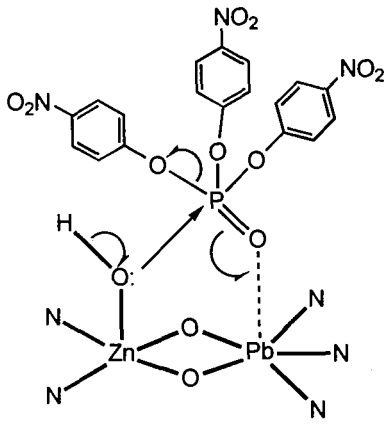
BNP complex was obtained by the reaction between **1** and TNP in the presence of triethylamine or by the reaction between **2** and BNP (free acid) in acetonitrile.

The crystal structure of **3** has been determined by X-ray crystallography.[12] An ORTEP view is given in Figure 1. The complex consists of the macrocyclic ligand $(L)^{2-}$, a Zn^{II} and a Pb^{II} cations, and a BNP^- and a perchlorate anions. The Zn and Pb ions are bridged by the phenolic oxygens O1 and O2 of the macrocyclic ligand and by the the BNP^- oxygens O3 and O4. The Zn--Pb intermetallic separation is 3.464(2) Å. The Zn resides in the "salen"-like N_2O_2 site and assumes a square-pyramidal geometry together with O3 of the bridging BNP^- (the parameter τ[13] discriminating square-pyramidal and trigonal-bipyramidal geometries is 0.20). The axial Zn-O3 bond (1.993(6) Å) is short relative to the equatorial Zn-to-L bonds (2.035(5)-2.054(7) Å). The Zn is 0.468 Å deviated from the basal least-squares plane towards O3.

The Pb ion in the "saldien" site has a six-coordinate geometry together with O4 of the bridging BNP^-. The geometry about the Pb can be depicted as a rare pentagonal-pyramid with O1, O2, N3, N4 and N5 of "saldien" on the base and the BNP O3 at the axial position. The sum of the angles O1-Pb-O2, O2-Pb-N3, N3-Pb-N4, N4-Pb-N5 and O1-Pb-N5 is 350.4°. The in-plane Pb-to-ligand bond distances range from 2.519(5) to 2.608(5) Å. The axial Pb-O4 distance is very short (2.360(5)Å). The Pb is 0.563 Å deviated from the basal least-squares plane towards the open face. Thus, the geometry about the Pb shows a distortion to "umbrella" shape.

The hydrolysis of TNP by **2** in DMSO was followed by visible spectroscopy (conditions: **2** 1×10^{-3} mol and TNP 3.3×10^{-5} mol; at 25 °C). An absorption characteristic of p-nitrophenolate anion appeared at 432 nm whose intensity increased with time and converged in 12 minutes. This absorption was barely observed when **1** was used in place of **2**. Further, we have confirmed that the $Ni^{II}Pb^{II}$ complex $NiPb(L)(ClO_4)_2\cdot H_2O$ cannot hydrolyze TNP.

From above discussions it is evident that the Zn(OH)Pb core with a hydroxo group on the Zn ion is essential for the efficient hydrolysis of TNP to BNP. The mechanistic scheme for the TNP hydrolysis is given in Scheme 2. TNP molecule is bound at the Pb center through its oxo oxygen and the hydroxo ion attached to the adjacent Zn ion nucleophilically attacks the phosphorus of TNP, leading to the formation of **3**. **2** illustrates a functional model of the heterobimetallic phosphatases and **3** can be regarded as an intermediate in the phosphatase catalysis. A significant stability of **3** is certainly due to a high affinity of Pb^{II} toward phosphate oxygen. TNP hydrolyses by analogous hydroxo-$Zn^{II}M^{II}$ complexes of L^{2-} with various M^{II} ions in the "saldien" site are in progress in this laboratory.



Scheme 2. Mechanistic scheme for TNP hydrolysis by **2**.

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5 Anal. Found for $ZnPb(L)(H_2O)_2(ClO_4)_2$: C, 30.97; H, 3.16; N, 7.91; Zn, 6.70%. Calcd for $C_{24}H_{31}N_5O_{12}Cl_2PbZn$: C, 31.16; H, 3.38; N, 7.57; Zn, 7.07%.

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9 Anal. Found for $ZnPb(L)(OH)(H_2O)ClO4$: C, 34.94; H, 3.44; N, 8.44; Zn, 7.65%. Calcd for $C_{24}H_{30}N_5O_8ClPbZn$: C, 34.95; H, 3.66; N, 8.49; Zn, 7.93%.

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11 Anal. Found for $[ZnPb(L)(BNP)]ClO_4$: C, 38.57; H, 3.24; N, 8.71; Zn, 6.05%. Calcd for $C_{36}H_{35}N_7O_{14}ClPPbZn$: C, 38.31; H, 3.13; N, 8.69; Zn, 5.79%.

12 Crystal data for $[ZnPb(L)(BNP)]ClO_4$: F.W. = 1128.72, triclinic, space group P1, with a = 13.494(9), b = 13.88(1), c = 12.765(8) Å, α = 94.71(6), β = 97.02(6), γ = 61.68(5)°, V = 2088(2) Å^3, Z = 2, D_c = 1.795 g cm^{-3}, $F(000)$ = 1112.0, μ(MoKα) = 47.84 cm^{-1}. Cell parameters were defined by 25 reflections with $29.83 < 2\theta < 30.04°$. 7346 unique reflections were measured with $2\theta_{max} = 50°$. All the measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Mo-Kα radiation (λ=0.71069) and a 12 kW rotation anode generator. The structure was solved, expanded and refined using the teXsan crystallographic software package from Molecular Structure Corporation. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement based on 6070 observed reflections ($I\geq3\sigma(I)$) converged with R = 0.036 and R_w = 0.047. Atomic coordinates, thermal parameters, bond lengths and angles, and hydrogen coordinates of **3** have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Information for Authors, Issue No. 1.

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