# General Acid-Promoted Hydrolysis of Phosphate Ester in [Zn$^{II}$(bnp)(tpa)](ClO$_4$) (tpa = tris(2-pyridylmethyl)amine, bnp = bis(*p*-nitro-phenyl) phosphate); Plausible Functional Model for P1 Nuclease

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The Zn$^{II}$ phosphate complex, [Zn$^{II}$(bnp)(tpa)](ClO$_4$) (**2**) was structurally characterized. The hydrolysis reaction of phosphate ester in **2** was promoted under acidic aqueous media efficiently. The relevancy of this system to P1 nuclease, which has a low optimal pH condition, is also discussed.

Hydrolytic enzymes such as phosphatases or nucleases are known to catalyze the hydrolysis of phosphate ester.[1,2] Many of these enzymes contain metals such as zinc at the active sites. The catalytic role of zinc is ascribed to the binding and activation of substrates, while deprotonation of coordinated water to produce a nucleophilic zinc hydroxide is also proposed as an essential catalytic hydrolysis function. It is well known that model complex system can mimic this enzymatic function under basic condition. However, one of these enzymes, such as P1 nuclease[2–7] have relatively low optimal pH condition (pH 4–6) for their catalytic reaction.[1,8] From inorganic point of view, it seems unlikely that the zinc(II)-bound water can deprotonate to form the hydroxo moiety in such low pH condition. Thus, nucleophilic zinc hydroxide may not be involved in their catalytic reaction. The possibility of other reaction mechanism cannot be neglected at the present stage.

In this report, we will describe hydrolysis reaction of phosphate diester ligated to Zn$^{II}$(tpa) (tpa = tris(2-pyridylmethyl)amine) complex. The pH-hydrolysis rate profile suggested that general acid-catalyzed hydrolysis reaction for ligated phosphate ester was efficient in our system.

The treatment of [Zn$^{II}$(H$_2$O)(tpa)](ClO$_4$)$_2$ (**1**)[9] with bis(*p*-nitro-phenyl) phosphate (bnpH) under the presence of 1 equiv. of triethylamine affords the complex, [Zn$^{II}$(bnp)(tpa)](ClO$_4$) (**2**).[10] The X-ray structure of **2** is shown in Figure 1.[11] The angle of O1-Zn-N4 (175.1 °) suggested that phosphate ester coordinates axially to the slightly distorted trigonal-bypiramidal complex **2** where amine ligand in tpa positions at the trans position to the phosphate oxygen and three pyridine nitrogen atoms form trigonal plane.

The potentiometric titration of **1** under the presence of bnpH in water gave stability constants for the formation of complex **2** at 30 °C ($I = 0.2$ with NaClO$_4$).[12,13] Theoretical species distribution diagram which is based on these measured stability constants (Figure 2) suggests that ligation of bnp anion to (tpa)Zn$^{II}$ complex is dominant in the whole range of pH condition in this system. The existence of free bnp(H) in this solution is negligible in every pH condition. The electron spray ionization mass (ESI-MS) spectrum of aqueous solution at pH 5.6 of complex **1** (10.0 mM (1 mM = 1 mmol dm$^{-3}$)) under the presence of bnpH (1.0 mM) presented major peak at m/z 693 corresponding to the [Zn$^{II}$(bnp)(tpa)]$^+$ in positive mode.[14] This result is consistent with the data provided by potentiometric titration. On the other hand, the existence of ion peak corresponding to the Zn$^{II}$(OH)(bnp)(tpa) which was proposed by
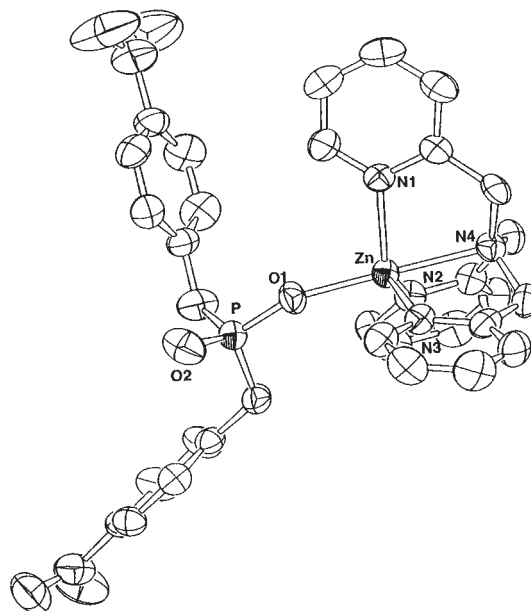


**Figure 1.** An ortep drawing (40% probability drawing) of complex **2**. All hydrogen atoms and ClO$_4$ anion is omitted for clarity. Selected bond legths (Å) and angles (deg): Zn-O1 1962(3); Zn-N1 2.075(3); Zn-N2 2.066(3); Zn-N3 2.059(3); Zn-N4 2.201(4); O1-P 1.477(3); O2-P 1.454(3); O1-Zn-N4 175.1(1): Zn-O1-P 156.7(2).
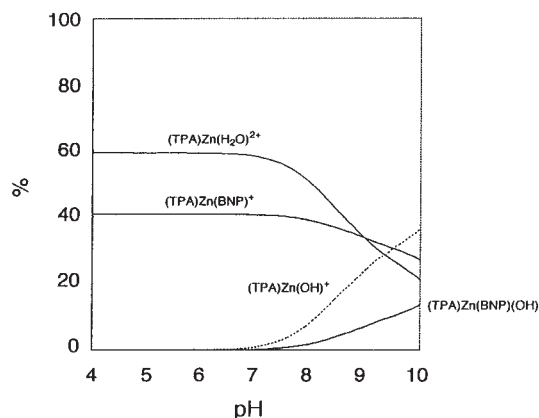


**Figure 2.** Distribution diagram for 1.2 mM ZN$^{II}$, 1.2 mM tpa(HClO$_4$)$_3$ and 0.5 mM bnp system as a function of pH at 30 °C and $I = 0.20$ (NaClO$_4$).

potentiometiric titration was not observed from aqueous solution of complex **1** (10.0 mM) with bnpH (1.0 mM) at pH 9.1 by ESI-MS, possibly due to the difficulty for the detection of neutral complex by ESI-MS, while the ESI-MS spectrum of this aqueous solution at pH 9.1 showed the peak at m/z 693 which was corresponded to the [Zn$^{II}$(bnp)(tpa)]$^+$ in positive mode. These observations suggest the stable ligation of bnp anion to (tpa)Zn(II) complex in the whole

range of pH conditions measured in present study.

The hydrolysis reactions of bnpH (0.5 mM) under the presence of complex **1** (5.0 mM) were studied in the pH range of 5.6–10 at 30 °C.[15] The reactions were followed by the increase in an absorption of the released *p*-nitro-phenolate ion. The pseudo-first order rate constant $k_{obs}$(bnp) was obtained by a log plot method at each pH condition. The pH versus rate constant ($k_{obs}$(bnp)) profile is shown in Figure 3. Interestingly the rate of hydrolysis reaction is dramatically increased in proportion to the decrease of pH in acidic condition. Indeed, the hydrolysis rate at the lowest pH condition measured in this system, (pH 5.6) is 10 fold faster than that of free bnp(H). In this pH condition, the slope of the linear plots of $k_{obs}$(bnp) against [complex 1] gave the second order rate constant, $k$(bnp) $(2.13 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1})$ (See eq. (1)).

$$v = k(\text{bnp})[\text{complex1}][\text{bnp}] \quad (1)$$

($v$ is the hydrolysis rate of bnp(H))

Since the result of potentiometric titration suggested that ligation of phosphate ester was dominant in this pH condition, monomeric Zn(II) complex, $[\text{Zn}^{II}(\text{bnp})(\text{tpa})]^+$ can be regarded as "reactive intermediate" for this hydrolysis reaction. Taken together, it is concluded that Lewis acidity of Zn(II) ion facilitates the general acid-promoted hydrolysis of ligated phosphate ester under acidic condition. On the other hand, the pseudo-first order rate constants of this system are comparable to those of free phosphate ion under basic conditions respectively. While potentiometric titration suggests that the ligation of phosphate to metal ion is dominant, it does not appear that $\text{Zn}^{II}$ complex affects the hydrolysis reaction under basic pH condition in our system. While we don't have a clear reason in this point, nucleophilic attack of hydroxide anion may not be effectual for the hydrolysis of bnp anion in our system, even though the phosphate diester seems to be ligated to zinc(II) complex.
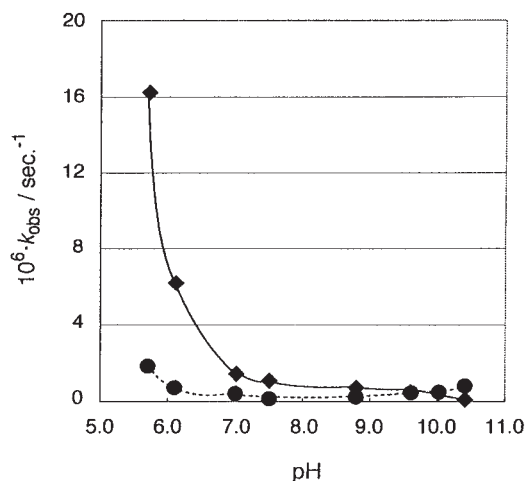


**Figure 3.** The pH-rate profile for the first-order rate contants of bnp ([bnp] = 0.5 mM) under the presence of complex **1** ([complex 1] = 5 mM) at 30 °C and $I = 0.20$ (NaClO$_4$) (solid circle) and the experimanatal results without complex **1** (solid square).

Since the present system shows that hydrolysis rate is accelerated dramatically in acidic pH condition and no acceleration of hydrolysis rate is observed in basic condition, the reaction mechanism of present system may have a relevancy to that of hydrolytic enzyme such as P1 nuclease, which has relatively low optimal pH condition. To elucidate how the geometry around the zinc ion in the phosphate ester complex relates to the hydrolysis reaction in such low pH condition, the effort to prepare other zinc phosphate complex by using other ligand systems is now in progress.

**References**
1 N. W. Lipscomb and N. Sträter, *Chem. Rev.*, **96**, 2375 (1996) and references therein.
2 D. E. Wilcox, *Chem. Rev.*, **96**, 2435 (1996).
3 M. J. Fraser and R. L. Low, "In nucleases," 2nd ed., ed. by S. M. Linn, R. S. Lloyd, and R. J. Roberts Cold Spring Harber Laboratory Press, Plainview, NY (1993), pp 171–207.
4 M. Fujimoto, A. Kuninaka, and H. Yoshino, *Agric. Biol. Chem.*, **39**, 1991 (1975).
5 B. V. L. Potter, B. A. Connolly, and F. Eckstein, *Biochemistry*, **22**, 1369 (1983).
6 A. Volbeda, A. Lahm, F. Sakiyama, and D. Suck, *EMBO J.*, **10**, 1607 (1991).
7 D. Suck, R. Dominguez, A. Lahm, and A. Volbeda, *J. Cell. Biochem.* Suppl. **17C**, 154 (1993).
8 M. Fujimoto, A. Kuninaka, and H. Yoshino, *Agric. Biol. Chem.*, **38**, 1555 (1974).
9 G. Anderegg, E. Hubmann, N. G. Podder, and F. Wenk, *Helv. Chem. Acta*, **60**, 123 (1977). The mononuclear structure of $[\text{Zn}^{II}(\text{H}_2\text{O})(\text{tpa})](\text{ClO}_4)_2$ was confirmed by Crystallography. M. Ito, unpublished results.
10 A solution of $[\text{Zn}^{II}(\text{H}_2\text{O})(\text{tpa})](\text{ClO}_4)_2$ (**1**) (0.20 mmol) and bis(*p*-nitro-phenyl) phosphate (bnpH)(0.20 mmol) in acetonitrile (10 ml) was treated with triethyla-mine (0.20 mmol) and stirred for 5 hours. The recrystallization by Et$_2$O vapour diffusiton of this mixture gave colorless crystals of $[\text{Zn}^{II}(\text{bnp})(\text{tpa})](\text{ClO}_4)$ (**2**) (Yield 80%) which was suitable for X-ray diffraction. **Caution!** *Perchlorate salts of transition metal complexes are potentially explosive. These complexes should be prepared only in small quantities and handled with care.* Anal. Found C,45.59; H, 3.43; N, 10.90; Cl, 4.29%. Calcd for **2** Zn$_1$C$_{30}$N$_6$H$_{26}$O$_8$P$_1$Cl$_1$: C. 45.47; H. 3.31; N. 10.61; Cl 4.47%. IR(KBr, cm$^{-1}$), $v$(C = C), 1608, $v$(P-O) 1215, $v$(ClO$_4$), 1110. $^1$H-NMR (CD$_3$CN, 300 MHz, 25 °C); 4.21 (s, 6H, C$H_2$), 7.56–7.60 (m, 10H, 3-$H_{py}$, 5-$H_{py}$ and *p*-NO$_2$-C$_6$H$_4$), 8.07 (**t**, 3H, J = 7.8 Hz, 4-$H_{py}$), 8.24 (d, 4H, J = 8.0 Hz, *p*-NO$_2$-C$_6$H$_4$), 8.89 (d, 3H, J = 5.4 Hz. 6$H_{py}$).
11 Crystal data for **2**, Mr = 697.7; **2** was crystallized in the triclinic space system with $P\bar{1}$, $a = 10.589(1)$ Å, $b = 17.708(3)$ Å, $c = 9.1498(8)$ Å, $\alpha = 100.81(1)°$, $\beta = 101.68(7)°$, $\gamma = 91.17(1)°$, $V = 1647.4(4)$ Å$^3$, $Z = 2$, $Dc = 1.60 \text{ g cm}^{-3}$, $\mu$(Mo-K) = 9.49 cm$^{-1}$. The R(Rw) value is 7.0 (10.4)% for 5547 reflections $(3° < 2\theta < 55°, Fo > 3\sigma(Fo))$. The X-ray data collection was carried out at room temperature. The structure was solved direct methods (SIR92) and the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with The Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-155611. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax:(+44)1223-336-0331; e-mail:deposit@ccdc. cam.ac.uk).
12 The formation constants of each product were determined by means of pH-titraton program BEST. ($a_{H^+}$ is the activity of H$^+$); p$K1 = 8.29$ ($K1 = [\text{Zn}^{II}(\text{H}_2\text{O})(\text{tpa})]/[\text{Zn}^{II}\text{OH}(\text{tpa})]a_{H^+}$), p$K2 = 8.82$ ($K2 = [\text{Zn}^{II}(\text{bnp})(\text{H}_2\text{O})(\text{tpa})]/[\text{Zn}^{II}(\text{bnp})(\text{OH})(\text{tpa})]a_{H^+}$), p$K3 = 10.10$ ($K3 = [\text{Zn}^{II}(\text{H}_2\text{O})(\text{tpa})]_2/[(\text{Zn}^{II}(\text{tpa})_2(\text{OH})]a_{H^+}$), p$K4 = 10.05$ ($K4 = [(\text{Zn}^{II}(\text{tpa})_2(\text{OH})_2]a_{H^+}/[\text{Zn}^{II}(\text{tpa})_2(\text{OH})]$), p$K5 = 13.59$ ($K5 = [(\text{tpa})\text{H}]/[\text{tpa}]a_{H^+}$), p$K6 = 5.37$, ($K6 = [(\text{tpa})\text{H}_2]/[(\text{tpa})\text{H}]a_{H^+}$), p$K7 = 10.61$ ($K7 = [(\text{tpa})\text{H}_2]a_{H^+}/[(\text{tpa})\text{H}_3]$), p$K8 = 2.94$ ($K8 = [\text{bnpH}]/[\text{bnp}][\text{H}]$), p$KL1 = 13.55$, ([tpa][$\text{Zn}^{II}]/[(\text{tpa})\text{Zn}^{II}]$), log $K$L2 = 6.27 ($K$L2 = [$\text{Zn}^{II}(\text{tpa})][\text{bnp}]/[\text{Zn}^{II}(\text{bnp})(\text{tpa})]$). While the dinuclear structure of $\text{Zn}^{II}$ $\mu$-OH complex, $[(\text{tpa})\text{Zn}^{II})_2(\mu\text{-OH})_2\text{Zn}^{II}(\text{tpa})](\text{ClO}_4)_2$ complex has been already reported, Distribution diagram based on potentiometiric titration shows that mononuclear $\text{Zn}^{II}$(tpa) species is dominant in every pH condition. See; N. N. Murthy, and K. D. Karlin, *J. C. S. Chem. Commun.*, **1993**, 1236.
13 E. Martell and R. J. Montekaitis, "Determination and Use of Stability Constants," 2nd ed., VCH, New York (1992).
14 In this pH condition, we also could observe the ion peaks at m/z 196 which is corresponding to the $[\text{Zn}^{II}(\text{H}_2\text{O})_2(\text{tpa})]$ as a most intense peak. The ratio of intensity of ion peaks at m/z 693 ($[\text{Zn}^{II}(\text{bnp})(\text{tpa})]^+$) toward 196 ($[\text{Zn}^{II}(\text{H}_2\text{O})_2(\text{tpa})]^{2+}$) was 0.92.
15 The cleavage reaction rates of bnpH (0.5 mM) under the presence of complex **1** (5 mM) (i.e. the rate of release of *p*-nitro-phenolate anion) were measured by following 392 nm absorption at 30 °C. In each experiment, buffer solution containing 50 mM Good buffer was used and the ionic strength was adjusted to 0.20 with NaClO$_4$. The complex **1** was dissolved in the buffer solution, the UV absorption increase recorded immediately and then followed the formation of *p*-nitro-phenolate ion generally until ca. 5% formation of *p*-nitro phenolate ion (and *p*-nitro-phenol), where log $\varepsilon$ values for *p*-nitro phenolate ion were 3.00 (pH 5.6), 3.32 (pH 6.2), 4.02 (pH 7.0), 4.10 (pH 7.5), 4.25 (pH 8.8) and 4.28 (pH 10) respectively. Every experiment was run for three times to check the reproduci-bility. Since the final conversion of hydrolysis for bnp(H) is ca. 40% (in comparison to the amount of added bnp(H) in each experiment, the initial slope method until 3% formation of *p*-nitro phenolate ion was applied to estimate the pseudo-first order rate constants, $k_{obs}$(bnp) which were obtained by a log plot method at each pH. From the slopes of linear plots of $k_{obs}$(bnp) against [complex **1**], second order rate constant, $k$(bnp) $(2.13 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1})$ was obtained at pH 5.6.