Hydrolysis of Phosphotriesters Promoted by a Zinc(II) Complex bearing an Alcohol Pendant†

Ismail O. Kady,* Biao Tan, Zhanying Ho and Todd Scarborough

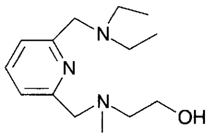
Department of Chemistry, East Tennessee State University, Johnson City, TN 37614, USA

A polyamine–zinc(II) complex with an alcohol pendant promotes the hydrolysis of diethyl(4-nitrophenyl)phosphate *via* transfer of the diethylphosphate group to its alkoxy pendant.

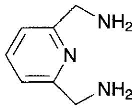
In recent years there has been considerable interest in the metal ion-promoted hydrolysis of phosphate esters as model systems for phosphatase enzymes.[1–6] Most of the enzymes responsible for the metabolism of phosphate compounds require divalent metal ions such as Zn^{2+}, Mg^{2+} or Mn^{2+} for activation.[7] For instance, alkaline phosphatase is known to contain at least one Zn^{II} ion at the active site during the catalytic cycle of the enzyme.[8] It is believed that the substrate is initially attacked by the deprotonated serine at the active site to yield a transient phosphoseryl intermediate, which is then attacked by the adjacent Zn^{II}-bound hydroxide to complete the hydrolysis.[9] However, the precise roles of the Zn^{II} and the serine OH group in the mechanism of the enzyme catalysis are not very clear and need to be displayed in reactive model systems where the route is well defined.

To elucidate possible participation of neighbouring alcohol groups of metal complexes in the catalysis of phosphate ester hydrolysis, we designed a novel model system involving a Zn^{II} complex bearing an alcohol pendant. **L**$_1$–Zn^{II} was found to be more than 10^4 fold more effective in promoting the release of 4-nitrophenol from diethyl(4-nitrophenyl)phosphate than **L**$_2$–Zn^{II} which lacks such an alcohol pendant. This strong rate enhancement by **L**$_1$–Zn^{II} indicates that the adjacent alcohol group could be involved directly in the reaction. Interestingly, **L**$_1$–Zn^{II} was also found to promote the transfer of the phosphate group from the substrate to the alcohol group of the ligand.

Ligand **L**$_1$ was prepared by the successive treatment of 2,6-bis(bromomethyl)pyridine with 1 equiv. of *N*-methyl-2-aminoethanol and 1 equiv. of diethylamine in anhydrous acetonitrile.‡ **L**$_2$ was prepared from pyridine-2,6-dicarboxaldehyde according to literature procedures.[10] Both ligands were further purified as their crystalline 3HBr salts. The Zn^{II} complexes were prepared by stirring an aqueous methanolic solution of the ligand·3HBr and 1 equiv. of $Zn(ClO_4)_2{\cdot}6H_2O$, after adjusting the pH to 9 with aq. 1 mol dm^{-3} NaOH. Evaporation of solvents and recrystallization from aqueous methanol provided the Zn^{II} complexes in analytically pure form. Elemental analyses showed that both complexes are in the dimeric form. A pH titration of an aqueous solution of **L**$_1$·3HBr in the presence of an equimolar amount of Zn^{II} showed complex formation at pH 6–9, with simultaneous deprotonation of the pendant alcohol OH when 4 equiv. of the base were added. It is of interest to note that both the alcoholic OH of **L**$_1$–Zn^{II} and the water of **L**$_1$–Zn^{II} at the fourth coordination sites of the metal deprotonate readily at 25 °C with pK_a values of 8.6 and 8.7, respectively. At 25 °C and pH 6–9, the dimerization constants of **L**$_1$–Zn^{II} and **L**$_2$–Zn^{II} are 0.8 and 1.1 dm^3 mol^{-1}, respectively. These small dimerisation constants mean that in aqueous solution the metal complexes exist mainly in monomeric form. Such results are consistent with the characteristics of other polyamine–Zn^{II} complexes.[11,12] The lower dimerization constant of **L**$_1$–Zn^{II} relative to **L**$_2$–Zn^{II} may be attributed partially to steric hindrance caused by the two ethyl and the hydroxyethyl groups.



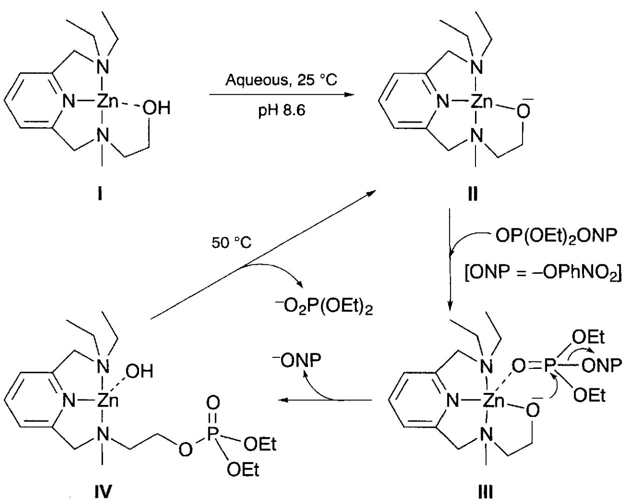
L$_1$



L$_2$

Reactions of **L**$_1$–Zn^{II} and **L**$_2$–Zn^{II} with diethyl(4-nitrophenyl)phosphate were performed at 25 °C and pH 8.6. A typical reaction procedure involved immediate monitoring of the changes in absorbance at 405 nm (owing to the release of 4-nitrophenolate) after rapid injection of 30 μl of 1.0 mmol dm^{-3} aqueous solution of the phosphotriester into 3.0 ml of 10 mmol dm^{-3} TAPS buffer solution (pH 8.6) containing 2.0 mmol dm^{-3} of the Zn^{II} complex. The final solution contained 0.01 mmol dm^{-3} of phosphotriester and 2 mmol dm^{-3} of the Zn^{II} complex. The ionic strength was maintained at 0.2 with $NaClO_4$. Reference experiments were carried out under identical conditions but in the absence of Zn^{II} complexes. Reactions were generally followed to more than 90% completion and showed good pseudo first order kinetics. Under these conditions, subsequent hydrolysis of the produced phosphodiester was not detected as confirmed by ^{31}P NMR spectroscopy. The pseudo first order rate constants were obtained by a log plot method (correlation coefficients > 0.99). The average observed rate constants (k_{obs}) for the release of 4-nitrophenolate based on three trials are 4.5×10^{-2} s^{-1} for **L**$_1$–Zn^{II} and 1.5×10^{-6} s^{-1} for **L**$_2$–Zn^{II}. This large difference in rate enhancement ($> 10^4$-fold) may be attributed in part to the higher nucleophilicity of the metal-bound alkoxy relative to the metal-bound hydroxy group.[11,12] Another major factor that contributes to the lower reactivity of **L**$_2$–Zn^{II} is its inhibition by the phosphodiester product. A significant reduction in the reaction rate of **L**$_2$–Zn^{II}, but not of **L**$_1$–Zn^{II}, was observed when hydrolysis was conducted in the presence of 1 equiv. of diphenylphosphate.

The pH dependence of the reactions was studied over a pH range of 6.0–9.0. Both reactions displayed sigmoidal pH rate profiles with inflection points around the pK_a values of the complexes (Fig. 1). Such pH profiles were observed in a number of phosphate ester hydrolyses promoted by Cu^{II},[13,14] Co^{III}[15] and Zn^{II}[2] complexes, and are indicative of the involvement of metal–hydroxy (or metal–alkoxy) intermediates. Data suggest



Scheme 1

that an intermediate of type **II** (Scheme 1) is probably involved in the reaction of **L$_1$**–Zn^{II} which may explain the higher reactivity of **L$_1$**–Zn^{II} compared to that of **L$_2$**–Zn^{II}.

End-product analysis of the reaction mixture by ^{31}P NMR (Fig. 2) clearly showed that the reaction of **L$_2$**–Zn^{II} with diethyl(4-nitrophenyl)phosphate produced the expected diethyl phosphate (δ 3.8). Reaction of **L$_1$**–Zn^{II}, on the other hand, produced a new phosphotriester **IV** (δ −1.9). ^{1}H–^{31}P coupling experiments on the reaction product of **L$_1$**–Zn^{II} showed that phosphorus is coupled to two sets of methylene protons (J_1 8.3, J_2 7.4 Hz). When the reaction mixture of **L$_1$**–Zn^{II} is allowed to stand at a higher temperature (50 °C) for 1 h, the transient phosphotriester **IV** hydrolyses to give diethyl phosphate and regenerate **II** (Scheme 1).

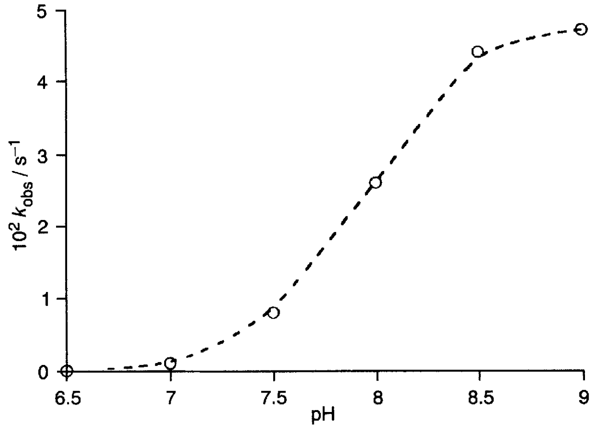


Fig. 1 The pH rate profile for the release of 4-nitrophenolate ion during **L$_1$**–Zn^{II}-promoted hydrolysis of diethyl(4-nitrophenyl)phosphate at 25 °C, I = 0.2 (NaClO$_4$)

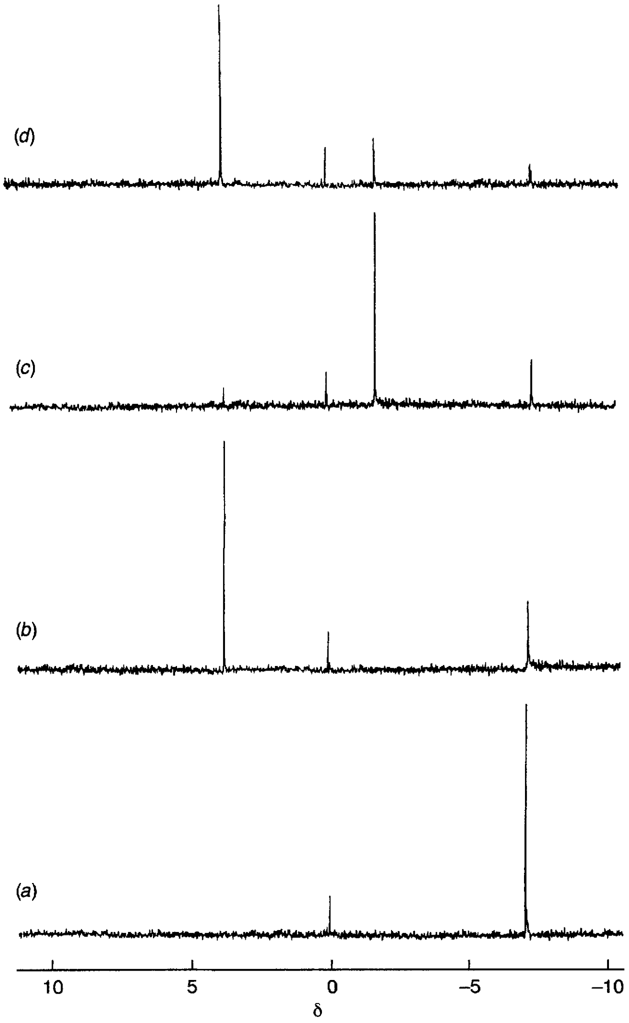


Fig. 2 ^{31}P NMR spectra for the hydrolysis of diethyl(4-nitrophenyl)-phosphate, see text for reaction conditions. (*a*) In the absence of metal complexes at 25 °C; (*b*) in the presence of **L$_2$**–Zn^{II} at 25 °C; (*c*) in the presence of **L$_1$**–Zn^{II} at 25 °C; (*d*) in the presence of **L$_1$**–Zn^{II} at 50 °C. Aqueous H$_3$PO$_4$ was used as the external reference. The solution contained 20% D$_2$O as an internal lock.

In summary, two well-defined Zn^{II} complexes have been synthesized and found to hydrolyse neutral phosphotriesters at 25 °C and pH 8.6. **L$_1$**–Zn^{II} with an alcohol pendant also promotes transfer of the diethylphosphate group from diethyl(4-nitrophenyl)phosphate substrate to the alcoholic OH group of the ligand. Therefore, this complex may serve as a good model for phosphate transferase enzymes, as well as to illustrate the ability of Zn^{II} to deprotonate the serine alcohol group at the active site of alkaline phosphatase. Further studies are presently under way to verify the mechanism of the involved reactions by separation and identification of potential intermediates. We are also evaluating potential applications of these complexes in the hydrolysis of biological phosphodiesters.

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Footnotes

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‡ *Selected analytical data* for **L$_1$**: MS *m/z* 251 (M$^+$), 233, 204, 163. ^{1}H NMR (CDCl$_3$) δ 1.10 (6H, t), 2.42 (4H, q), 2.55 (3H, s), 2.90 (2H, t), 3.35 (1H, s), 3.51 (2H, s), 3.64 (2H, s), 3.90 (2H, t), 7.21–7.40 (3H, m). ^{13}C NMR δ 14.0, 42.2, 48.6, 58.7, 62.3, 62.7, 74.2, 121.0, 134.2, 136.0, 150.6, 155.7. For **L$_1$**·3HBr Anal. Calc. (Found) for C$_{14}$Br$_3$H$_{28}$N$_3$O: C, 34.00 (33.93); H, 5.67 (5.68); N, 8.50 (8.48). For **L$_1$**–Zn^{II} dimer: Anal. Calc. (Found) for C$_{28}$Cl$_2$H$_{22}$N$_6$O$_{10}$Zn$_2$: C, 41.84 (41.69); H, 2.74 (2.75), N, 10.46 (10.48). For **L$_2$**–Zn^{II} dimer: Anal. Calc. (Found) for C$_{14}$Cl$_2$H$_{24}$N$_6$O$_{10}$Zn$_2$: C, 26.37 (26.25); H, 3.77 (3.79); N, 13.19 (13.22).

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