Dinuclear Five-co-ordinate Zinc Complexes Bridged by a Phosphate Monoester or an Inorganic Phosphate Group

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Two dinuclear five-co-ordinated zinc complexes, derived from tris[(2-pyridyl)methyl]amine and bridged by a phosphate monoester or an inorganic phosphate group respectively, have been synthesized; the structure of the former has been established by X-ray crystallography and shows the bridging mode of the phosphate to be *syn–anti* whereas in alkaline phosphatase it is *syn–syn*.

Investigations into the use of multinuclear zinc complexes as biomimetic compounds has begun to attract attention.[1–5] This is because of the definition of a number of zinc enzymes containing two, or more, zinc atoms in co-catalytic, or co-active, sites.[6–8] Structural information concerning these active sites has been made available; one such enzyme is alkaline phosphatase the function of which is to catalyse the non-specific hydrolysis of phosphate monoesters.[9]

Alkaline phosphatase was the first zinc enzyme to be discovered in which there are three metal atoms (two zinc and one magnesium or in the absence of magnesium, a third zinc), at the active site. The crystal structure of alkaline phosphatase complexed with inorganic phosphate shows that the three metal atoms are in close proximity (Fig. 1); the intermetallic separations are d[Zn(1)···Zn(2)] = 3.94, d[Zn(2)–Mg] = 4.88 and d[Zn(1)–Mg] = 7.09 Å in one subunit and 4.18, 4.66 and 7.08 Å respectively in the second subunit.[9] The first zinc atom [Zn(1)] is five-co-ordinated; the co-ordination polyhedron at the metal is best described as pseudotetrahedral with both oxygen atoms of the chelating Asp-327 occupying one apex. The second zinc atom [Zn(2)] is tetrahedrally co-ordinated and the zinc atoms are bridged by the inorganic phosphate. The carboxyl group of Asp-51 forms a bridge between Zn(2) and the magnesium atoms, and the phosphate is also associated with the magnesium atom *via* one of the water molecules co-ordinated to that atom. The magnesium has a slightly distorted octahedral co-ordination environment.

In the absence of phosphate a water molecule completes the co-ordination at Zn(1) and the hydroxyl group of Ser-102 completes the co-ordination at Zn(2). The catalytic role of zinc in mononuclear zinc enzymes has been ascribed to the presence of co-ordinated water. Ionisation of this activated water or polarization by a base form of an amino acid can produce zinc hydroxide at neutral pH; alternatively ready displacement of the water can lead to Lewis acid catalysis by the metal.[7,8]

In order to try to imitate the bioinorganic chemistry involved it is important to use appropriate ligands that will (*i*) present co-ordination environments around zinc structurally similar to those that are found in the enzymes, (*ii*) form stable and discrete complexes with zinc at physiological pH (≈7) and (*iii*) leave catalytic sites open on zinc at which the deprotonation of co-ordinated water can lead to the generation of nucleophilic species effective at relatively low pH values.[10,11]

The properties of the tetradentate ligand tris[(2-pyridyl)-methyl]amine (tpa) suggest that it can act with considerable utility as it can form five-co-ordinate zinc complexes with additional ligation from one exogenous anionic or neutral (*e.g.* H_2O) ligand through which multinuclear zinc complexes can be constructed.[2] Furthermore aqueous solution studies have shown that (tpa)Zn–H_2O complexes form the corresponding (tpa)Zn–OH^- species at pH values of ≈8.[12]

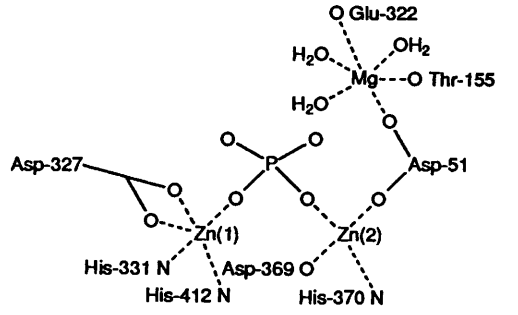
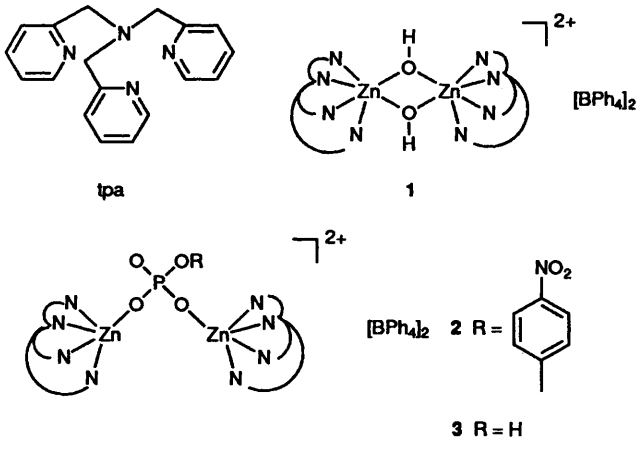


Fig. 1 Schematic of the trinuclear metal constellation in the *Escherichia coli* alkaline phosphatase–inorganic phosphate complex

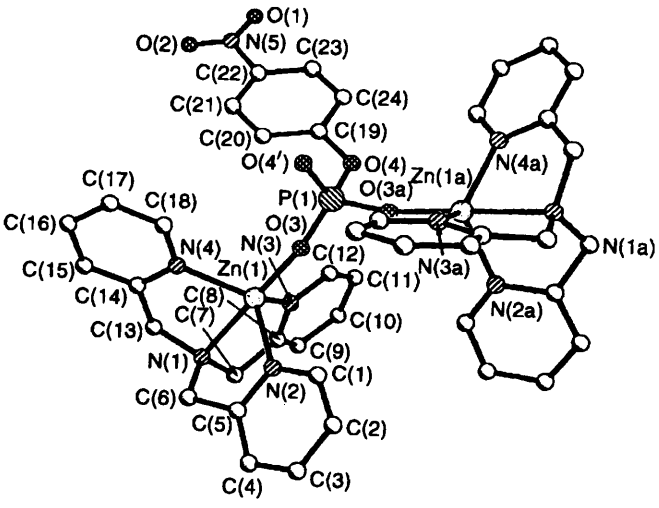


Treatment of $Zn(BF_4)_2 \cdot H_2O$ and tpa[13] with KOH in 95% methanol–water and under nitrogen, according to the literature procedure,[2] gave a clear solution which was allowed to stand at room temperature overnight to generate colourless crystals of $[(tpa)Zn(\mu\text{-}OH)_2Zn(tpa)][BF_4]_2$ **1** (68% yield). A methanolic solution containing 0.5 equivalents of mono(*p*-nitrophenyl) phosphate was added to a clear solution generated as above and

the mixture was treated, whilst hot, with an equimolar amount of sodium tetraphenylborate to yield the dinuclear complex $[(tpa)Zn\{OP(O)(OC_6H_4NO_2)O\}Zn(tpa)][BPh_4]_2$ **2**† as a crystalline solid (51% yield). The solid was recrystallized to give crystals suitable for a crystal-structure determination.‡

The structure of the dinuclear μ-phosphate is illustrated in Fig. 2. The cation possesses crystallographic C_2 symmetry, with symmetry related Zn(tpa) units, and the bridging phosphate lying across the symmetry axis with disordered *p*-nitrophenolate and terminal oxygen atoms. Four nitrogen donor atoms from each ligand co-ordinate to each zinc atom, and the phosphate bridges the two complex units such that each zinc atoms adopts a pentagonal-bipyramidal co-ordination geometry. The zinc–zinc separation is 5.97 Å which may be compared with the distance of 5.14 Å found in the related neutral dizinc complex $[LZn\{OP(O)(OC_6H_4NO_2)O\}ZnL]$ [L = tris(3,5-diisopropylpyrazol-1-yl)hydroborate].[3] In both of these complexes the inter-zinc distance is well in excess of the value of 3.94 Å found for the zinc pair in alkaline phosphatase. In the latter instance the co-ordination mode of the phosphate is *syn–syn* whereas in the synthetic analogues the phosphate bridges in a *syn–anti* mode. Furthermore in the small molecule models the metal environments are not subject to any constraints that might be imposed by the presetting of a metal-binding cleft by the protein.

The UV/VIS spectrum of complex **2** in 50% aqueous ethanolic solution containing **1** (20 mmol dm^{-3}) at pH 8 and 30 °C showed that no absorption band arising from hydrolysis of the monoester at 405 nm over a period of several hours. This indicated that complex **2** is rather stable and that the P–O bond of phosphate ester is not hydrolytically cleaved under the above conditions. Treatment of bis(*p*-nitrophenyl) phosphate with **1** under the same conditions gave complex **2** as main product; evidently, the species (tpa)Zn–OH has sufficient esterase-like activity to cleave one of the P–O bonds in bis(*p*-nitrophenyl) phosphate leading to the μ-phosphate monoester complex **2**.



Zn(1)–N(1) 2.252(9), Zn(1)–N(2) 2.082(10), Zn(1)–N(3) 2.034(12), Zn(1)–N(3) 2.056(9), P(1)–O(3) 1.467(11), P(1)–O(4) 1.57(4), P(1)–O(4') 1.57(4), P(1)–O(3a) 1.467(11), Zn(1) · · · Zn(1a) 5.97, Zn(1)–O(3)–P(1) 164.2(7), O(3)–P(1)–O(3a) 110.6(9), O(3)–P(1)–O(4) 107(2)

Treatment of complex **1** with 0.5 equivalents of Na_2HPO_4 in 90% methanol–water following the same reaction procedure as above gave the crystalline dinuclear μ-phosphate complex $[(tpa)Zn\{OP(O)(OH)O\}Zn(tpa)(H_2O)][BPh_4]_2$ **3** (44% yield).§ The ^{31}P NMR peak at δ 2.2, assigned to HPO_4^{2-}, confirmed the presence of an inorganic phosphate in the complex. Complex **3**, which can be presumed to be similar in structure to **2** as it has very similar spectroscopic properties, is interesting because of its more direct comparison with the dinuclear zinc site in the alkaline phosphatase–inorganic phosphate structure. Although some metal complexes[3,15–20] containing a bridging phosphate group are known, a dinuclear zinc complex bridged solely by an inorganic phosphate has not been reported; unfortunately we have not yet been able to grow crystals of **3** suitable for X-ray analysis. There has however been a recent report of a trinuclear zinc complex, derived from hydroxyethyl-1,4,7,10-tetraazacyclodecane, which contains a μ_3-phosphate group.[21] In relation to this latter report two crystalline trinuclear tpa complexes, one containing three zinc atoms, the second containing two zinc atoms and one copper(II) atom, both having an inorganic phosphate anion as a bridge, have been synthesized in the present study and will form the focus of further work.

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† Spectroscopic and analytical data for **2**. IR(KBr): 1609 (C=C), 1508 (NO), 1339 (NO), 1267 (PO), 1146 cm^{-1} (PO); ^1H NMR(CD_3CN): δ 4.05 (s, 12 H, CH_2), 6.85 (t, 6 H, H^4 py), 6.95–7.30 (m, 40 H, BPh_4), 7.45 (d, 6 H, H^3 py), 7.60 (d, 2 H, $H^{2,6}$ Ph), 7.90 (t, 6 H, H^5 py), 8.15 (d, 2 H, $H^{3,5}$ Ph), 9.25 (d, 6 H, H^6 py); ^{13}C NMR(CD_3CN): δ 57.6 (CH_2), 118.3 (BPh_4), 121.3 ($C^{2,6}$ Ph), 122.3 (C^4 py), 125.7 (C^4 Ph), 125.9 ($C^{3,5}$ Ph), 126.6 (C^5 py, C^5 Ph), 136.7 (C^3 py), 142.3 (C^4 Ph), 150.1 (C^6 Py), 156.3 (C^2 py), 164.5 (C^2 Ph); ^{31}P NMR(CD_3CN): δ −0.6; mass spectrum (positive ion FAB): *m/z* 1250 (M^+), 928 $[ZnL]_2(C_6H_4NO_2PO_4)$, 572 $[ZnL](C_6H_4NO_2PO_4)$; UV/VIS (MeCN): 258 (ε 29 585), 304 nm (9300 dm^3 mol^{-1} cm^{-1}) (Found: C, 67.20; H, 5.45; N, 8.05. Calc. for $C_{90}H_{80}B_2N_9O_6PZn_2$: C, 67.45; H, 5.30; N, 7.85%).

‡ Crystallographic data for **2**. $C_{90}H_{80}B_2N_9O_6PZn_2$, M = 1567.05, monoclinic, space group $C2/c$, a = 27.724(6), b = 11.488(2), c = 26.709(5) Å, β = 93.53(3)°, U = 8491(3) Å^3, $F(000)$ = 3264, Z = 4, D_c = 1.226 g cm^{-3}. Data were collected in the range 3.5 < 2θ < 45° on a Siemens P4 diffractometer by the ω scan method. 2540 Independent reflections (of 4051 measured) for which $|F|/\sigma(|F|) > 4.0$ were corrected for Lorentz and polarization effects, but not for absorption. The structure was solved by direct methods and refined by full-matrix least-squares methods. The *p*-nitrophenolate fragment (of which the phenyl ring was refined with constrained geometry) and the terminal oxygen atom were disordered (1:1) between the two terminal sites of the bridging phosphate group which lay across the crystallographic C_2 axis: two independent oxygen sites were located and refined. Hydrogen atoms were included in calculated positions and refined in riding mode. Refinement converged at a final R = 0.0982 ($wR2$ = 0.2621, for all 4051 reflections, 470 parameters, mean and maximum δ/σ 0.000, 0.100) with allowance for the thermal anisotropy of all non-hydrogen atoms. The minimum and maximum final electron densities were −0.788 and +1.487 e Å^{-3}. A weighting scheme $w = 1/[\sigma^2(F_o^2) + (0.1855P)^2 + 44.03P]$ where $P = (F_o^2 + 2F_c^2)/3$ was used in the latter stages of the refinement. Complex scattering factors were taken from the program package SHELXL93[14] as implemented on a Viglen 486dx computer. Atomic coordinates, thermal parameters and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1995, Issue 1, pp. xxv–xxx.

§ Spectroscopic and analytical data for **3**. IR(KBr): 1609 (C=C), 1269 (PO), 1127 cm^{-1} (PO); ^1H NMR(CD_3CN): δ 4.05 (s, 12 H, CH_2), 6.80 (t, 6 H, H^4 py), 6.90–7.30 (m, 40 H, BPh_4), 7.45 (d, 6 H, H^3 py), 7.75 (t, 6 H, H^5 py), 9.10 (d, 6 H, H^6 py); ^{13}C NMR(CD_3CN): δ 57.7 (CH_2), 118.3 (C^1 Ph), 122.8 (C^4 py), 125.5 (C^4 Ph), 126.6 (C^5 py, $C^{3,5}$ Ph), 136.7 (C^3 py), 150.5 (C^6 py), 156.2 (C^2 py); ^{31}P NMR(CD_3CN): δ 2.2; mass spectrum (positive ion FAB): *m/z* 1127 (M^+), 807 $[(ZnL)_2(HPO_4)]$, 451 $[ZnL(HPO_4)]$; UV/VIS (MeCN) λ 258 nm (ε 26 017 dm^3 mol^{-1} cm^{-1}) (Found: C, 69.65; H, 5.70; N, 7.70. Calc. for $C_{84}H_{79}B_2N_8O_5PZn_2$: C, 69.80; H, 5.35; N, 7.75%).

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