

Note

The synthesis, structure and hydrolytic activity of a functional pentacoordinate zinc complex

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(Received October 25, 1993; revised December 20, 1993)

Abstract

The synthesis and structure of a functional pentacoordinate zinc(II) complex derived from *N,N'*-(salicylideneimino)-ethyl)-2-hydroxy-5-nitro-benzylamine are reported together with its ability to hydrolyse bis- and tris-nitrophenylphosphate.

Key words: Crystal structures; Hydrolytic activity; Zinc complexes; Polydentate ligand complexes; Pentacoordinate complexes

Structural studies on metalloenzymes have shown that coordination geometries around the metal ions can be distorted and it has been proposed that this may be related to the catalytic efficiency of the enzyme in that the protein can fold to generate special stereochemistries which help the metal to adapt to a geometry closer to that in the transition state of the reaction being catalysed than to a more normal ground state geometry. There is "a presetting of the geometry such that there is a catalytically poised state intrinsic to the active site" and this has been termed the 'entatic state' [1]. In many zinc-containing enzymes the metal centre acts as a strong Lewis acid and participates in solvolysis and hydrolysis reactions [2–4]. The d^{10} electron configuration of zinc rules out ligand field stabilizing effects for zinc complexes and leads to a considerable flexibility of coordination numbers and strongly distorted coordination polyhedra [5]. This ready achievement of an 'entatic state' is observed at active zinc sites in

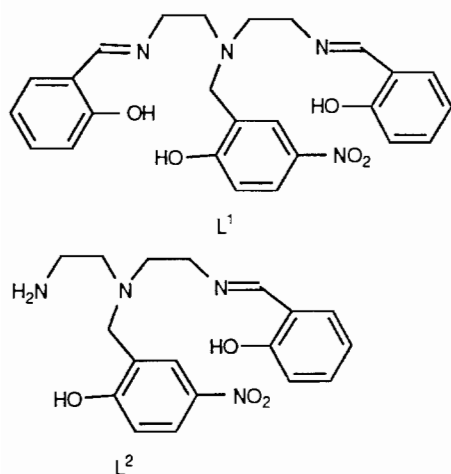
enzymes such as alkaline phosphatase (AP), a non-specific phosphomonoesterase, in which there is a trinuclear constellation of metal atoms present, two zinc (one catalytic) and one magnesium [6]. In the crystal structure of AP complexed with inorganic phosphate one zinc atom is five-coordinated by the imidazole nitrogens of two histidines, both carboxylate oxygens of an aspartate residue and a phosphate oxygen atom and the second zinc is tetrahedrally coordinated to one carboxylate oxygen from each of two aspartates, one imidazole nitrogen from a histidine and one of the phosphate oxygen atoms. The phosphate acts as a bridge and the metal-metal separation is 3.94 Å. In AP it is likely that a water molecule is present instead of phosphate at the five-coordinate zinc and so this site would resemble those of the catalytic sites in mononuclear zinc enzymes at which a water molecule is present. The deprotonation of coordinated water to produce a nucleophilic zinc hydroxide is proposed as an essential feature of the catalytic role of zinc [7–9].

Several small molecule model zinc complexes have been developed to study the hydrolysis of phosphate esters [7–13]. In these models relatively rigid ligands providing three sites were used as such ligands leave Zn(II) coordinatively unsaturated and so enable coordination of the water that is believed to play an essential role in the mechanism of hydrolysis. The complexes were also stable under alkaline conditions. The importance of five-coordinate trigonal bipyramidal zinc-ligand bonding in the reaction intermediates during the catalytic action of zinc in enzymes has been stressed [14–16]. Herein we report the synthesis and crystal structure of a novel pentacoordinate zinc(II) complex together with preliminary studies on its ability to enhance the rate of hydrolysis of bis-nitrophenyl phosphate (BNP) and tris-nitrophenyl phosphate (TNP).

The ligand (L^1) was prepared by the following route. Salicylaldehyde and diethylenetriamine were stirred together in absolute ethanol and then 2-chloromethyl-4-nitrophenol and Na_2CO_3 were added to the solution, and the mixture refluxed overnight. After cooling, the suspension was filtered and the filtrate evaporated to remove the solvent. The product was extracted with THF and this solvent removed to leave an oil. The oil was dissolved in CH_2Cl_2 :methanol (9:1) and chromatographed using a silica gel column to yield ligand L^1 . Treatment of L^1 with an equimolar amount of $Zn(ClO_4)_2 \cdot 4DMSO$ in methanol in the presence of triethylamine yielded a yellow powder. Recrystallization

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of the powder in methanol led to yellow crystals suitable for X-ray analysis.

The structural study reveals that the complex is the neutral pentacoordinate species $ZnL^2 \cdot CH_3OH$. The molecular structure*, with atom labelling, is illustrated in Fig. 1 together with relevant bond lengths and angles. The asymmetric unit comprises the mononuclear zinc(II) complex molecule and a methanol solvent molecule. One of the two imine bonds in the parent ligand has hydrolysed on reaction with $Zn(ClO_4)_2$. The resultant primary amine nitrogen is coordinated to the metal atom together with the remaining imino nitrogen atom and the tertiary amino-nitrogen atom. The other two coordinating atoms are phenolate oxygens. The coordination geometry shown by the zinc(II) ion is approximately trigonal bipyramidal (N(1) N(3) O(2) plane, with Zn(1) 0.221, O(1) 2.199 and N(2) -2.023 Å out of plane), with two different Zn–N distances, two of

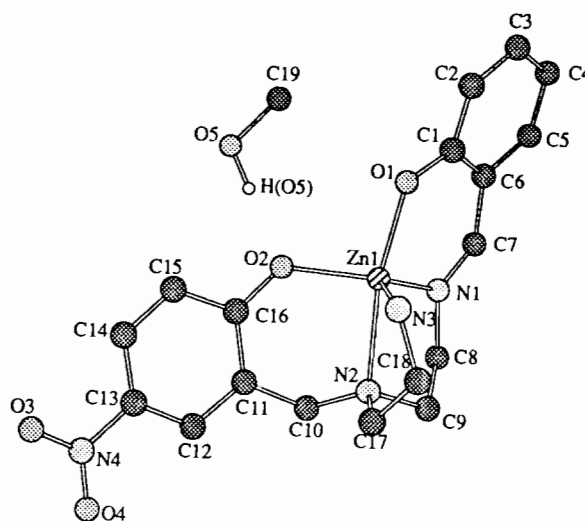


Fig. 1. The molecular geometry of the complex. The relevant bond lengths and bond angles are: Zn(1)–O(1) 1.986(5), Zn(1)–O(2) 1.979(7), Zn(1)–N(1) 2.045(6), Zn(1)–N(2) 2.260(5), Zn(1)–N(3) 2.061(8), H(O5)...O(2) 1.807 Å; O(1)–Zn(1)–O(2) 100.3(2), O(1)–Zn(1)–N(1) 91.1(2), O(1)–Zn(1)–N(2) 168.6(2), O(1)–Zn(1)–N(3) 98.0(2), O(2)–Zn(1)–N(1) 116.4(3), O(2)–Zn(1)–N(2) 90.3(2), O(2)–Zn(1)–N(3) 112.4(3), N(1)–Zn(1)–N(2) 80.5(2), N(1)–Zn(1)–N(3) 127.6(3), N(2)–Zn(1)–N(3) 81.3(2)°.

2.045, 2.061 Å in the equatorial plane, and 2.260 Å in an axial position; and two almost equal Zn–O distances of 1.979 and 1.986 Å. The methanol solvent molecule is involved in hydrogen bonding to the phenolate oxygen (O(5)...O(2) 2.807, H(O5)...O(2) 1.807 Å). It is worth noting that the formation of the hydrogen bond and the existence of the electron withdrawing group ($-NO_2$) at the position *para* to the phenolic oxygen does not weaken the Zn(1)–O(2) bond. This bond is marginally shorter than the second more normal zinc–oxygen bond (1.988 Å) and is the shortest bond within the zinc coordination sphere. The Zn(1)–N(2) bond (2.260 Å) formed between metal and tertiary nitrogen is the longest bond and is significantly longer than the averaged Zn–N distances. This may be related to the induction of stress from the presence of the two five-membered rings. The bond angles at the zinc atom are further indicative of *tbp* symmetry.

The phenyl rings are planar (r.m.s deviations 0.019, 0.011 Å); deviations of oxygens and nitrogen substituent atoms from planarity are small (less than 0.053 Å), but (imine)C and (amine)C, show 0.157 and -0.141 Å out of planarity. The nitro group is asymmetrically twisted by 8.4° from the plane of the phenyl ring. The angle between the phenyl rings is 35.3°. Torsion angles of both N.C.C.N fragments are +53°. The zinc O.C.C.C.N(imine) chelate ring is approximately planar (r.m.s. deviation 0.024 Å) and inclined at 5.7° to the phenyl ring.

*Crystal data for $ZnL \cdot CH_3OH$, $C_{19}H_{24}O_5N_4Zn$; $M_r = 453.78$. Crystallizes from methanol as prismatic yellow crystals; crystal dimensions $1.0 \times 0.085 \times 0.575$ mm. Triclinic, $a = 10.286(6)$, $b = 10.364(7)$, $c = 10.619(6)$ Å, $\alpha = 68.17(4)$, $\beta = 82.69(4)$, $\gamma = 71.34(5)$, $U = 995(1)$ Å³, $Z = 2$, $D_c = 1.520$ g cm⁻³, space group $P\bar{1}$ (C_1 , No. 2), Mo K α radiation ($\lambda = 0.71069$ Å), μ (Mo K α) = 12.99 cm⁻¹, $F(000) = 471.89$. Three-dimensional, room temperature X-ray data were collected in the range $3.5 < 2\theta < 50.0^\circ$ on a Nicolet R3 4-circle diffractometer by the omega scan method. The 2218 independent reflections (of 3531 measured) for which $|F|/\sigma(|F|) > 5.0$ were corrected for Lorentz and polarisation effects, and for absorption by analysis of 8 azimuthal scans (minimum and maximum transmission coefficients 0.449 and 0.598). The structure was solved by standard Patterson and Fourier techniques and refined by blocked cascade least-squares methods. Hydrogen atoms were included in calculated positions with isotropic thermal parameters related to those of the supporting atom and refined in riding model. Refinement converged at a final $R = 0.0620$ for 262 parameters with allowance for the thermal anisotropy of all non-hydrogen atoms, mean and maximum δ/σ 0.001 and 0.010. Minimum and maximum final electron density -0.59 and 1.04 e Å⁻³ located around the zinc atom. Complex scattering factors were taken from the program package SHELXTL [17] as implemented on the Data General DG30 computer.

In addition to the hydrogen bonding involving the methanol solvent molecule there is a pair of longer, symmetry related hydrogen bonds between the primary amine nitrogens and the other phenolate oxygens of a centrosymmetrically related molecule (N(3)...O(1)^a 2.950, H(N3A)...O(1)^a 2.018 Å; ^a(1-x, -y, -z)). The second hydrogen atom of the amine nitrogen points approximately to a symmetry related O(4) of the nitro group (N(3)...O(4)^b 3.130, H(N3B)...O(4)^b 2.612 Å; ^b(x, y, 1+z)).

Preliminary kinetic studies, using the UV-Vis spectroscopic method of Koike and Kimura [8], indicate that the zinc complex can enhance the hydrolysis of the monoanionic phosphodiester BNP and neutral phosphotriester TNP. The observed rate constant of TNP hydrolysis enhanced by the complex under the following reaction conditions: 25 °C, HEPES buffer (10 mM, 90% (vol./vol.) EtOH and pH 7.37), ionic strength $I=0.2$ [7-11] is $3.95 \times 10^{-4} \text{ s}^{-1}$ (based on a pseudo-first-order reaction) compared with the rate constant found in the absence of the complex ($3.60 \times 10^{-5} \text{ s}^{-1}$). A much diminished enhancement is noted for BNP hydrolysis where the observed rate constant is $2.69 \times 10^{-7} \text{ s}^{-1}$ compared with a rate constant of $6.73 \times 10^{-8} \text{ s}^{-1}$ in the absence of the complex. The conditions used were: 35 °C, TAPS buffer (10 mM, 90% (vol./vol.) EtOH and pH 8.05), ionic strength $I=0.2$. This diminution in activity has previously been noted [8, 12].

The pH was measured using a pH meter which was calibrated with standard buffers (pH 4.01 and 7.0) immediately before use. The hydrolysis of TNP and of BNP was followed by the increase in absorption maximum at 405 nm (assigned to the 4-nitrophenolate anion). In the former case the absorption increase was recorded until 90% hydrolysis was complete and in the latter case the absorption increase giving a straight line was recorded until 1% hydrolysis. The concentration of the zinc complex was 2 mM and those of TNP 25 μM and BNP 4 mM. Potentiometric titrations on solutions (90% (vol./vol.) ethanol) of the ligand (1.0 mM) and an equal molar amount of zinc perchlorate showed that the complex has essentially formed by pH 6.7, therefore at the concentrations and pH values employed in this study it is likely that the complex is fully formed and so the accelerations noted can be attributed to the influence of the complex.

This activity may be compared to that of the zinc complexes derived from three- and four-coordination ligands [7-10]. It was thought that only those rigid, tri- or tetra-dentate ligands that bind zinc ions firmly under alkaline conditions and for which the zinc complexes are coordinatively unsaturated and/or sterically more open for phosphate substrate approach can catalyse the hydrolysis of phosphate esters [9]. The above results show that a flexible five-coordinate zinc complex also

has the propensity for inducing catalytic hydrolysis of a phosphate ester. Mechanistic investigations have revealed that a Zn(II)-OH intermediate formed from the deprotonation of coordinated water is the essential active species in phosphate hydrolysis [7-10]. In the present case it is possible that in solution a water molecule is added to the zinc leading to the formation of an L^2ZnOH intermediate; it is also possible that one or both of the phenolic groups could be displaced to provide a position at which a water or substrate molecule can bind [8, 13]. Further studies are in progress in order to try and elucidate the mechanism of the reaction.

Supplementary material

Tables of bond lengths and angles, anisotropic temperature factors, atom coordinates and temperature factors, hydrogen atom coordinates and temperature factors and observed and calculated structure factors are available from the authors on request.

Acknowledgements

We thank Mr Simon Thorpe for assistance in the kinetic measurement experiments, the University of Sheffield for a Scholarship (to Q.H.), and the SERC and Royal Society for funds towards the purchase of the diffractometer. We would also like to thank the Universidad Autónoma Nuevo León for leave of absence to C.O.R de B.

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