Synthesis and structure of [Zn(OMe)(L)]·[Zn(OH)(L)]·2(BPh$_4$), L = *cis,cis*-1,3,5-tris[(*E,E*)-3-(2-furyl)acrylideneamino]cyclohexane: structural models of carbonic anhydrase and liver alcohol dehydrogenase†

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Direct reaction of [Zn(OH)(L)]$^+$, L = *cis,cis*-1,3,5-3-(2-furyl)acrylideneamino]cyclohexane, with methanol gives a mixture of the starting material and [Zn(OMe)(L)]$^+$; structural analysis of the complexes shows that they are models of reactive intermediates in the catalytic cycles of the zinc enzymes carbonic anhydrase and liver alcohol dehydrogenase.

The catalytic activities of the zinc(II)-containing metalloenzymes, carbonic anhydrase (CA) and liver alcohol dehydrogenase (LADH) are known to be dependent on the ability of zinc–water or zinc–ethanol complexes respectively to deprotonate at physiological pH.[1] Important information has been obtained from small molecule model complex studies of the metalloenzyme active sites, which have demonstrated that the binding of either water or small alcohol molecules to zinc results in the formation of reactive zinc–hydroxide or zinc–alkoxide species.[2–5] Indeed, isolating such species in the laboratory has proved to be challenging due to their reactivity. For example, Berreau *et al.*[6] recently reported the formation of a five-coordinate zinc hydroxide complex that reacts with methanol to form the corresponding methoxide complex, where the alkoxide is stabilised by an intramolecular hydrogen bond. Moreover, there are only three known structures of four-coordinate monomeric zinc–hydroxide species and no known structures—other than that of LADH[7]—of monomeric, four-coordinate zinc–alkoxide species, where the alkoxide is a 'simple' unsubstituted alkoxide such as methoxide and ethoxide.[8–10]

Previously we have observed that monomeric cobalt(II)–ethoxide complexes of the tri-iminocyclohexane-based ligand, 1,3,5-tris[(*E,E*)-3-cinnamylideneamino]cyclohexane[11] and trimeric copper(II)–methoxide complexes of the related ligand, *cis,cis*-1,3,5-tris[(*E,E*)-3-(2-furyl)acrylideneamino]cyclohexane[12] (L) ligand could be isolated and structurally characterised. Both ligands provide a face-capping imine-N$_3$ coordination geometry around the metal ion and surround the metal ions' remaining coordination sites with a rigid hydrophobic cavity. The imines mimic histidine coordination and the cavity, which is an important part of the ligand design, affords a degree of stabilisation to reactive species which are bound to the metal ion—akin to the structure of the active sites seen in many metalloenzymes.

Herein we report the preparation of zinc complexes of L. We show that both the zinc–hydroxide and zinc–methoxide complexes can be prepared, and that subsequent structural analysis shows the complexes to be models of the reactive zinc–hydroxide and zinc–alkoxide intermediates seen in the catalytic cycles of carbonic anhydrase and liver alcohol dehydrogenase respectively.[13,14] A CSD search[9] shows that the methoxide complex is the first structural characterisation of a cationic four-coordinate monomeric zinc complex with a 'simple' unsubstituted alkoxide. It is also one of the few examples of a zinc–alkoxide complex that can be prepared directly from reaction of the hydroxide with alcohol.

The preparation is shown in Scheme 1. L[12] is reacted directly with zinc hydrate perchlorate (CAUTION) to give an intermediate complex that can be reacted with KOH to give [Zn(OH)L]ClO$_4$ in 73% yield.† Treatment of [Zn(OH)L]ClO$_4$ with NaBPh$_4$ gives [Zn(OH)L]BPh$_4$ (**1**). Attempts at crystallising **1** were unsuccessful, but the complex was characterised by NMR and IR spectroscopy, MS and elemental analysis. In particular the presence of the hydroxide moiety was confirmed by IR [v(O–H) 3650 cm^{-1}] and ^{1}H-NMR spectroscopy [δ(O–H) −0.08 ppm].[5]

A new complex, **2**, can be crystallised in 30% yield by cooling (4 °C, 48 h) of a MeOH–CH$_2$Cl$_2$ solution of **1**. Crystallographic analysis§ of **2** at 190 K shows the structure to have the composition [Zn(OMe)L]·[Zn(OH)L](BPh$_4$)$_2$·1.8 MeOH 0.8CH$_2$Cl$_2$ containing two discrete [Zn(X)L]BPh$_4$ units with X = OH and X = OMe (Scheme 1). The [Zn(OMe)L]$^+$ unit is well-defined with L coordinated in its expected face-capping mode, and with the three pendant 'arms' of the ligand creating an encapsulating environment around the methoxide ion (Fig. 1). The Zn–O distance is 1.879(7) Å, which is consistent with the distances reported by Vahrenkamp *et al.* for zinc–alkoxide species.[10] The methoxide is involved in a hydrogen-bonded interaction with a methanol solvent molecule [O(8)···O(9) 2.679(11) Å] which is also encapsulated within the cavity created by L. The hydrogen bond is similar to one that
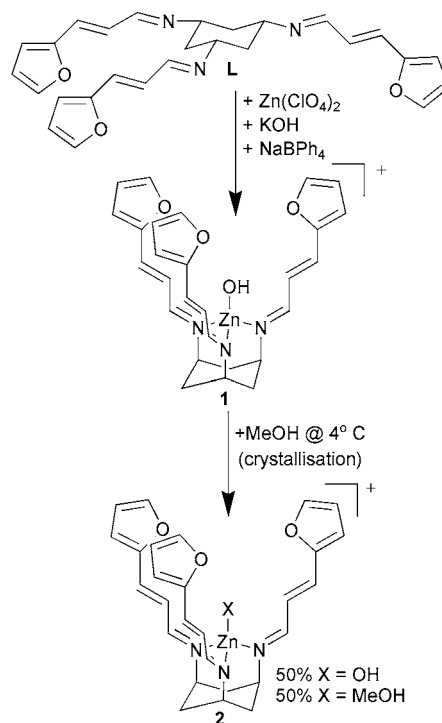

Scheme 1

appears in the active site of liver alcohol dehydrogenase, between the bound ethoxide and an amino acid side chain.

The structure of the [Zn(OH)L]+ cation (Fig. 1) is analogous to that of [Zn(OMe)L]+. The Zn–O distance is 1.896(10) Å, longer than the equivalent distance in [Zn(OMe)L]+, as expected for coordinated hydroxides when compared to methoxides.[10] A disordered methanol molecule [hydrogen-bonded to the hydroxide O(4)···O(1B) 2.578(15) Å] is present within the cavity defined by L and the charge of the complex is neutralised by a spectator BPh4– ion. Small differences exist between the two [Zn(X)L]+ complexes, which are easily visualised when the two complexes are superimposed on each other so that the N3ZnO moieties line up as far as possible (Fig. 2). The blue
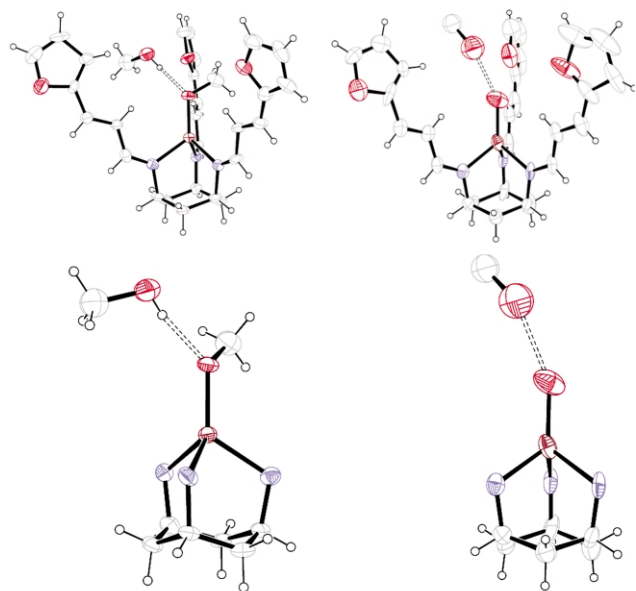
Fig. 1 ORTEP plots of right) [Zn(OH)L]+ and left) [Zn(OMe)L]+, 30% probability ellipsoids (colour code: the zinc ion is dark purple, nitrogen atoms blue, carbon atoms grey, oxygen atoms red and hydrogen atoms represented by open spheres)—selected distances (Å) and angles (°) [Zn(OH)L]+; Zn(1)–O(4) 1.896(10), Zn(1)–N(1) 2.016(12), Zn(1)–N(2) 2.032(10), Zn(1)–N(3) 2.070(9), O(4)–Zn(1)–N(1) 118.2(5), O(4)–Zn(1)–N(2) 125.2(5), O(4)–Zn(1)–N(3) 123.4(4), N(1)–Zn(1)–N(2) 95.8(4), N(1)–Zn(1)–N(3) 94.5(4), N(2)–Zn(1)–N(3) 92.1(4). [Zn(OMe)L]+; Zn(2)–O(8) 1.879(6), Zn(2)–N(4) 2.049(9), Zn(2)–N(5) 2.022(8), Zn(2)–N(6) 2.041(9), O(8)–Zn(2)–N(4) 122.8(3), O(8)–Zn(2)–N(5) 121.1(3), O(8)–Zn(2)–N(6) 122.3(3), N(4)–Zn(2)–N(5) 97.2(3), N(5)–Zn(2)–N(6) 94.6(3), N(4)–Zn(1)–N(6) 91.4(3). A dichloromethane solvent molecule is located outside the cavity and the charge on the complex is neutralised by a spectator BPh4– counter ion.
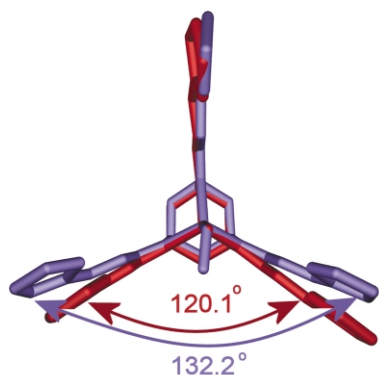
Fig. 2 [Zn(OMe)L]+ (blue) superimposed on [Zn(OH)L]+ (red) and the angles formed between the adjacent planes are given: the planes are defined such that each incorporates the central zinc ion and the atoms of a cinnamyl moiety of L.

outline represents the [Zn(OMe)L]+ complex and the red outline represents the [Zn(OH)L]+ complex. It can be seen that the extra steric bulk associated with the coordinated methoxide ion 'pushes' the two adjacent cinnamyl moieties apart when compared to the [Zn(OH)L]+ complex.

^{1}H-NMR spectroscopy solution studies of **2** did not give clear evidence of a 50 : 50 mixture of the methoxide and hydroxide complexes as seen in the structure of **2**. This observation presumably means that the 50 : 50 mixture is only stable in the solid state, which is not surprising considering the inherent reactivity of the complexes. It is possible that some oligomerisation or hydrolysis of the complexes occurs in solution, as seen in the copper(II) methoxide complexes of the same ligand.[12]

The direct reaction of the hydroxide complex with methanol to give the methoxide complex is unusual. This reaction is directly analogous to the displacement of water by ethanol in the active site of liver alcohol dehydrogenase. Such a reaction, which runs contrary to the relative acidities of water[6] and the alcohol, suggests that the hydrophobic cavity seen in the enzyme and in our complexes has an important role in stabilising the alkoxide complex. This observation, when seen alongside the alkoxide complexes prepared by Berreau et al.,[6] where hydrogen-bonded interactions between the ligand backbone and the coordinated alkoxide are important factors in the stabilisation of the alkoxide complex, suggests that *both* the hydrophobic cavity and the hydrogen-bond interactions in the active site of LADH are important in ethoxide stabilisation.

Notes and references

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