A New Pyrazolylborate Zinc Hydroxide Complex Capable of Cleaving Esters, Amides and Phosphates

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The L^2·Zn–OH complex of the new ligand L^2 = hydrotris(3-*p*-isopropylphenyl-5-methylpyrazolyl)borate is a strong nucleophile which effects cleavage of activated esters, amides, non-activated phosphorus acid esters and diphosphates, thereby providing stoichiometric models of esterase, peptidase and phosphatase enzyme activity.

It is thought that the catalytic activity of zinc compounds in technical or biological hydrolysis reactions rests in a strongly nucleophilic Zn–OH function.[1] It has turned out to be nontrivial, however, to prepare simple zinc complexes with OH ligands as molecular models for hydrolysis studies. For tetrahedral zinc just two such complexes have been completely characterized, Kimura's $[LZnOH]^+$ (L = $[12]aneN_3$)[2] and our $[L^1ZnOH]$ [L^1 = hydrotris(3-*tert*-butyl-5-methylpyrazolyl) borate].[3] While Kimura's complex,[2] similar to Brown's undefined LZn complexes [L = various tris(imidazolyl)phosphines][4] or Wooley's [LZnOH] complex (L = 14-membered N_4 cycle),[5] is a moderately good catalyst for CO_2 hydration and hydrolysis of activated esters or phosphate esters, our complex has allowed the construction of a complete stoichiometric carbonic anhydrase model, and a similar pyrazolylborate complex by Kitajima[6] was shown to effect stoichiometric cleavage of *p*-nitrophenyl phosphates. We present here the new pyrazolylborate ligand L^2 which creates an even more favourable steric and electronic situation around the metal in its zinc complex **1**, thus making **1** a versatile model compound for catalytic or stoichiometric reactions of metal-bound OH^-.

The potassium salt of ligand L^2 is obtained in 70% yield by heating neat 3-cumyl,5-methyl-pyrazole[7] and KBH_4 in a 3 : 1 molar ratio to 240 °C and subsequent precipitation from pentane. The ligand L^2 can stabilize a wide variety of co-ligands at the fourth coordination site of the zinc ion and clearly surpasses the stabilization ability of the previously reported bulky pyrazolylborate ligands.[3,6,8] Thus it readily forms the Zn–OH complex **1** in 65% yield by reaction of equimolar amounts of KL^2, $Zn(ClO_4)_2·6H_2O$, and KOH in methanol–CH_2Cl_2 (1 : 1) and precipitation from methanol after filtration and removal of CH_2Cl_2 *in vacuo*.†

Complex **1**, in addition to being quite stable, is a nucleophile of greater strength than that of the other L·Zn–OH complexes obtained before.[2,3,6] This has been demonstrated by cleavage reactions of hydrolysable substrates which bear relation to biological processes catalysed by zinc enzymes. One such process is ester hydrolysis which, among the zinc enzymes, is effected by many peptidases as well as by carbonic anhydrase.[1,9,10] Our stoichiometric equivalent for this catalytic process is given in eqn. (1). It proceeds for the activated esters shown, albeit not for simple esters like ethyl acetate. All three esters react smoothly at room temp. to form the crystalline products **2**.†‡ Of the HOX byproducts, *p*-nitrophenol consumes a second equivalent of **1** forming L^2·Zn–$OC_6H_4NO_2$ **3**, and the HOX function liberated from propiolactone becomes part of product **2c**.

$$\begin{array}{lll} \mathbf{1} + RCO\text{–}OX & \rightarrow L^2\text{·Zn–OC(O)R} + HOX & (1) \\ MeCO\text{–}OC_6H_4NO_2 & \rightarrow \mathbf{2a}, R = Me & \\ CF_3CO\text{–}OMe & \rightarrow \mathbf{2b}, R = CF_3 & \\ CH_2\text{—}CO & & \\ \quad\vert\quad\vert & \rightarrow \mathbf{2c}, R = CH_2CH_2OH & \\ CH_2\text{—}O & & \end{array}$$

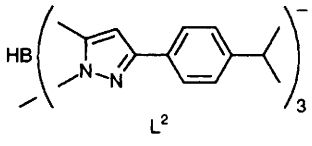
While a number of peptidase and esterase model studies exist involving ester hydrolysis catalysed by zinc complexes,[9] no such stoichiometric reaction has been reported before. The resulting carboxylate complexes **2** do, however, have precedents in zinc pyrazolylborate chemistry.[3,8] Reaction (1) and its products **2a–c**, although of limited biological significance owing to the rarity of metal containing esterases, give support to the mechanistic ideas put forth for the metal-assisted cleavage of esters by the OH^- ion.[9]

Unlike ester hydrolysis, peptide hydrolysis is of extreme importance in biology, featuring zinc enzymes like carboxypeptidase as the most prominent catalysts.[1,10] But model studies for peptide cleavage with metal containing catalysts are rarer than those for ester cleavage,[10,11] and again stoichiometric examples for peptide cleavage by metal containing bases have not been reported yet. We have now observed that complex **1** is nucleophilic enough to cleave the C–N bond of very reactive amides according to eqn. (2).

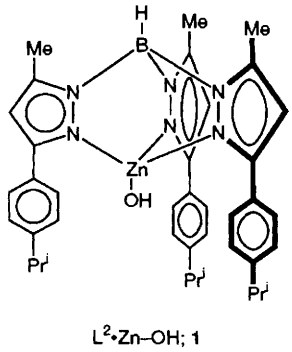
$$\begin{array}{lll} \mathbf{1} + RCO\text{–}NHX & \rightarrow L^2\text{·Zn–OC(O)R} + H_2NX & (2) \\ CF_3CO\text{–}NH_2 & \rightarrow \mathbf{2b}, R = CF_3 & \\ CH_2\text{—}CO & & \\ \quad\vert\quad\vert & \rightarrow \mathbf{2d}, R = CH_2CH_2NH_2 & \\ CH_2\text{—}NH & & \end{array}$$

The reactions proceed like the ester cleavages, and they are a bit faster owing to the highly activated amide substrates.‡ Of the crystalline reaction products,† **2b** was already obtained from CF_3CO–OMe, and **2d** again contains the liberated H_2NX function as part of the molecule. Many mechanistic proposals for peptidase activity involve Zn–OH and Zn–OC(O)R intermediates.[1,10] Complexes **1** and **2** are simple chemical realizations of these intermediates, and reaction (2) models their interrelation.

Among the third class of hydrolytic enzymes, the phosphatases catalysing phosphate transfer from phosphate esters or oligophosphates, zinc containing enzymes again play a dominating role.[1,10] The ease of hydrolytic P–O cleavage and the suitability of phosphate derivatives as ligands in metal complexes have allowed various model studies with catalytic and stoichiometric systems[1,10] including one with zinc pyrazolylborate complexes.[6] But these model studies have almost invariably used the highly activated *p*-nitrophenyl derivatives



L^2



L^2·Zn–OH; **1**

as phosphate esters, and a stoichiometric cleavage of an oligophosphate by a metal-containing base has, to our knowledge, not been observed yet.

Using complex **1**, Kitajima's *p*-nitrophenylphosphate cleavages[6] can readily be reproduced. In addition, however, simple aliphatic phosphorus acid esters of low steric bulk are easily hydrolysed, *cf.* eqn. (3). Furthermore, diphosphate esters are cleaved according to eqn. (4) in a 2 : 1 stoichiometry.

$$\mathbf{1} + HPO(OMe)_2 \rightarrow \underset{\mathbf{4}}{L^2{\cdot}Zn{-}OPHO(OMe)} + MeOH \quad (3)$$

$$2\,\mathbf{1} + (RO)_2PO{-}O{-}PO(OR)_2 \rightarrow 2\,L^2Zn{-}OPO(OR)_2 + H_2O \quad (4)$$

5a, R = Et
5b, R = Ph

The cleavage of $HPO(OMe)_2$ is as fast as that of *p*-nitrophenyl acetate, the cleavages of the diphosphates are about half as fast.‡ Complexes **4** and **5** are crystalline compounds again.† The fact that water as the byproduct of reaction (4) does not hydrolyse the Zn–O bonds of **5a** and **b** is in accord with the formation of such complexes from L·Zn–OH and the corresponding phosphorus acids.[6] Thus, while the stability of the phosphate complexes **4** and **5** was to be expected, their formation from nonactivated reagents serves to underline once more the nucleophilic strength of complex **1**.

In reactions (1)–(4) complex **1** is an enzyme model reduced to its central features: a zinc ion coordinated by a nucleophilic water constituent and a ligand L providing three heteroaromatic nitrogen donors. Just like in our carbonic anhydrase model based on a similar ligand,[3] however, it is the steric and electronic properties of the encapsulating ligand L, *i.e.* the substitute for the protein environment in the enzyme, which make this model function. As the increase in nucleophilicity of the L·Zn–OH complexes in going from the *tert*-butyl substituted ligand L^1 to the cumyl substituted ligand L^2 cannot be related to the electron releasing power of the substituents (*tert*-butyl > cumyl) it may be assumed that the higher degree of encapsulation by L^2, *i.e.* a more hydrophobic environment of the Zn–OH unit, accounts for the observed effects. We are trying to further increase the nucleophilic activity of such L·Zn–OH complexes thereby extending our stoichiometric model approach to less reactive substrates.

This work was supported by the Deutsche Forschungsgemeinschaft. We thank Dr W. Deck for measurements and Dr S. Trofimenko for stimulating discussions.

Received, 23rd August 1993, Com. 3/05101C

Footnotes

† All compounds in this paper have been characterized by C, H and N elemental analyses. The ^1H NMR shifts for ligand L^2 are similar in all complexes. Selected ^1H NMR data ($CDCl_3$, *J* in Hz): **1**, δ 7.78 (d, 8.2, 6H), 7.31 (d, 8.2, 6H), 6.28 (s, 3H), 2.94 (sept, 6.9, 3H), 2.55 (s, 9H), 1.27 (d, 6.9, 18H). **2a**: 1.49 (s, 3H). **2c**: 3.40 (m, 2H), 1.87 (t, 5.0, 2H). **2d**: 2.21 (t, 4.1, 2H), 1.66 (t, 4.1, 2H). **3**: 7.42 (d, 8.7, 2H), 5.75 (d, 8.7, 2H). **4**: 6.27 (d, 653.5, 1H, P–H), 3.19 (d, 11.8, 3H). **5a**: 3.44 (d, q, 7.0, 7.0, 4H), 0.85 (t, 7.0, 6H). **5b**: 6.66–7.06 (m, 10H). Further NMR data: **2b** (^{13}C): 160.1 (d, J_{CF} 37.4), 115.1 (d, J_{CF} 287.3). **4** (^{31}P): 1.1. **5a** (^{31}P): −2.0. **5b** (^{31}P): −16.6. Prominent IR bands (KBr, ν/cm^{-1}): **1**, 3647w (OH). **2a**: 1632s (C=O). **2b**: 1711vs (C=O). **2c**: 1590s (C=O). **2d**: 1697vs (C=O). **3**: 1584s, 1307vs (N=O). **4**: 2365m (PH), 1246s (P=O). **5a**: 1252s (P=O). **5b**: 1282vs (P=O). Preliminary crystal structure data, some of which were plagued by disorder problems due to the threefold symmetry of L^2, have been obtained for **2c**, **2d**, **3**, **4** and **5b**.

‡ All cleavage reactions with **1** were run at room temp. on a 0.5 mmole scale in *ca.* 10 ml of dichloromethane or benzene. The ester cleavages took 30 min (**2b**) to 120 min, (**2a**) to go to completion, the amide cleavages 20–30 min, the $HPO(OMe)_2$ cleavage 2 h, and the diphosphate cleavages 4–5 h. All reactions were finished by running overnight. After filtration the solvent was removed *in vacuo*, and the products were crystallized from ethanol or acetonitrile. While the reactions were practically quantitative in solution the isolated yields were 66–93%.

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