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Relative importance of hydrogen bonding and coordinating groups in modulating the zinc–water acidity†

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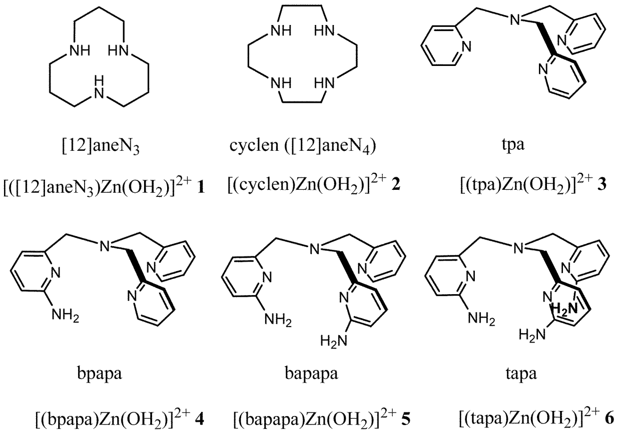
The presence of second-sphere –NH_2 groups in the proximity of a zinc(II)-bound water molecule enhances its acidity by *ca.* 2 pK_a units.

The effects and roles of metals and their first coordination sphere in regulating chemical processes in biology have been extensively studied.[1] It is known, however, that the microenvironment of the active site can also influence the function of metalloproteins through noncovalent interactions such as hydrogen bonding.[2] Thus, amino acid residues capable of hydrogen bonding to external ligands that are covalently bonded to a metal centre are found in the active sites of many metalloproteins.[2,3] The precise roles and magnitude of the effects associated with the second coordination sphere, however, are not well understood. As a result the development of synthetic systems that resemble more faithfully the microenvironments of metalloproteins has been attracting much current attention.[4]

Hydrolases are important examples of metalloproteins in which the metal, typically zinc, its primary coordination sphere and the active site microenvironment are used in conjunction to regulate the chemistry at the metal site.[5] A key property in the function of catalytic zinc sites is the acidity of the zinc-bound water molecule.[6] The nucleophilic zinc-bound water is activated by ligation to a zinc(II) ion. The identity of the ligands, typically three, coordinated to the zinc(II) ion is known to play a critical role in the extent of polarization and ionization of the water molecule, which ensures the generation of a hydroxide ion at neutral pH. Site-directed mutagenesis studies, however, indicate that hydrogen bonding interactions between the zinc-bound water and XH groups ($X = N$, O) of arginine, lysine, histidine, tyrosine and/or serine residues can also have a large effect in the zinc–water acidity.[7] The extent to which the identity, number and position of these amino acid residues affects the acidity of the zinc–water unit is not known. Moreover, the relative contribution or importance of the first and second coordination sphere of the zinc(II) ion towards determining the pK_a of the zinc-bound water remains also to be elucidated.

Herein we assess and compare the effects of the first and second coordination sphere of zinc(II)-aqua complexes (**1–6**) towards the acidity of a zinc–water unit by using ligands that offer different coordinating and hydrogen bonding groups (Scheme 1).

From potentiometric pH titrations† of the ligands (1 mM) in the absence and presence of 1 mM Zn^{2+} at 25 °C with $I = 0.1$ ($NaNO_3$), the deprotonation constants for each of the acid species of each ligand, as well as the complexation constants (log K_{LZn}) and deprotonation constants (pK_a for $Zn–OH_2 \rightleftharpoons Zn–OH$) of the zinc(II)-aqua complexes **3–6** were determined by the program HYPERQUAD[8] (Table 1). This data together with the corresponding literature values for **1**[9] and **2**[10] show that as the coordination number increases ($\mathbf{1} < \mathbf{2} = \mathbf{3}$) the charge on the zinc(II) centre is reduced, thereby its capacity to polarize/ionize the bound water molecule is reduced (pK_a of the zinc-bound water molecule of $\mathbf{1} < \mathbf{2} \approx \mathbf{3}$), which demonstrates the importance of the first coordination sphere. Given that amino groups are electron-donating it may have been reasonable to expect the pK_a of the zinc-bound water molecule to follow the trend $\mathbf{3} < \mathbf{4} < \mathbf{5} < \mathbf{6}$. The observed trend, however, was $\mathbf{3} > \mathbf{4} > \mathbf{5} > \mathbf{6}$ (Table 1, Fig. 1), which can be rationalized as resulting from the effect of intramolecular $N–H\cdots O(H)_n–Zn$ hydrogen bonding.[11,12] Thus, we have recently shown that the ligand unit (6-amino-2-pyridylmethyl)amine ideally positions an N–H for intramolecular hydrogen bonding to other metal-bound ligands both in the solid state and solution.[13] In principle, amino groups acting as hydrogen bond donors could cooperate with the Lewis acidic zinc(II) centre towards polarizing the O–H bond. In addition, hydrogen bond donors should provide greater stabilisation of the zinc–hydroxide relative to the zinc–water unit as the former is a better hydrogen bond acceptor. Here we demonstrate that the presence of additional hydrogen bond donors around the $Zn–O(H)_n$ ($n = 1, 2$) unit substantially augments the magnitude of the overall effect (water acidity lowered by *ca.* 1.5 pK_a units), presumably due to their ability to provide additional stabilization of the zinc–hydroxide species as these can act as double hydrogen bond acceptors. Remarkably, the magnitude of the changes observed suggest that the second coordination sphere can be *at least* as important as the first coordination sphere in determining the zinc–water acidity. Thus, whereas the zinc–water acidity changes by less than a pK_a unit upon changing the coordination number of the zinc(II) centre and/or the nature of the coordinating groups, it



Scheme 1

Table 1 Protonation and Zn(II) complexation constants determined by potentiometric pH titrations at 25 °C

	LH_3			$[(L)Zn(OH_2)]^{2+}$	
	log K_1	log K_2	log K_3	log K_{ZnL}	pK_a
1[a]	(12.6 ± 0.1)	(7.5 ± 0.2)	(2.4 ± 0.1)	(8.41 ± 0.02)	(7.30 ± 0.02)
2[b]	(10.7)	(9.7)	—	(16.2 ± 0.2)	(8.02 ± 0.03)
3	6.28 ± 0.05	4.49 ± 0.05	2.73 ± 0.1	11.06 ± 0.04	8.08 ± 0.04
4	7.25 ± 0.01	4.94 ± 0.02	3.25 ± 0.02	10.36 ± 0.02	7.62 ± 0.09
5	7.52 ± 0.02	5.76 ± 0.03	3.61 ± 0.01	8.82 ± 0.01	6.68 ± 0.02
6	8.00 ± 0.08	6.33 ± 0.07	4.94 ± 0.03	8.09 ± 0.05	5.99 ± 0.05

[a] $I = 0.1$ ($NaClO_4$), from ref 9 [b] $I = 0.2$ ($NaClO_4$), from ref 9 and 10.

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† Electronic supplementary information (ESI) available: experimental details (synthesis and pH titrations), pH titration figures and speciation plots for **3–6**, ^1H NMR spectra of **3–6** and zinc–chloride analogues. See http://www.rsc.org/suppdata/cc/b3/b310956a/

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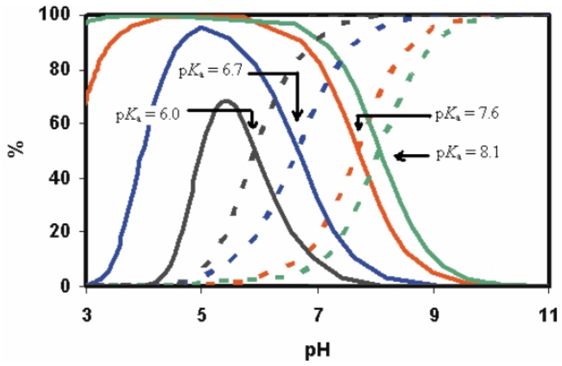

Fig. 1 Distribution of $LZnOH_2$ (solid lines) and LZnOH species (dashed lines) for L = tpa (green), bpapa (red), bapapa (blue) and tapa (black).

changes by *ca*. 1 pK_a unit *per* hydrogen bond donor. Recently the effect of various coordination environments on the acidity of the zinc-bound water in model complexes of tetradentate tripodal ligands was systematically evaluated.[14] From this work it was concluded that although the zinc–water acidity is significantly influenced (1–2 pK_a units) by both the charge of the ligand and the binding energy of the tripod, environmental effects may have a modulating or even dominating effect. Herein we provide experimental evidence of the latter. The effect that hydrophobic groups in the proximity of a zinc-bound water molecule exert on its acidity was also investigated using model zinc(II) complexes and was shown to be quite significant (changes of 1–2 pK_a units).[15]

There is much current interest in metal complexes with internal bonding. Recent important studies have highlighted the beneficial effects of incorporating such interactions in synthetic models of metalloenzymes.[4] Here we show that the acidity of a zinc–water unit can be increased with hydrogen bond donors by as much as one pK_a unit *per* hydrogen bond donor. As a result the zinc–water unit of **6** has the very low pK_a of 6.0. Moreover, the zinc–water acidity of **3–6** is as low as that achieved by zinc(II) hydrolases despite the use of a tetradentate rather than tridentate ligand environment. The implication of this result for metalloenzymes is that although features of the primary coordination sphere such as a low coordination number and moderately donating ligands play a role in lowering the zinc–water acidity, the active site microenvironment, if capable of hydrogen bonding to the zinc–water unit, could exert the dominant effect. We are currently exploring if the strategic positioning of hydrogen bonding groups in synthetic hydrolases can improve the catalytic properties of metal complexes by further activating the ground state and/or preferentially stabilizing the more anionic transition states of these reactions.

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12 The ^1H NMR of **3–6** in D_2O is very similar to that of the corresponding chlorides (see ESI†), which suggests that the zinc–aqua and zinc–chloride complexes adopt a similar structure in solution. The X-ray structure of the $[(bpapa)Zn(Cl)]^+$ cation revealed a trigonal bipyramidal geometry for the Zn^{2+} and internal N–H···Cl–Zn hydrogen bonding.[13].

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