COMMUNICATION

ChemComm
www.rsc.org/chemcomm

The affinity of phosphates to zinc(II) complexes can be increased with hydrogen bond donors

Juan C. Mareque-Rivas,* Rafael Torres Martín de Rosales and Simon Parsons
School of Chemistry, The University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, UK EH9 3JJ. E-mail: juan.mareque@ed.ac.uk; Fax: 44 (0)131 650 4743; Tel: 44 (0)131 650 4761

Received (in Cambridge, UK) 11th November 2003, Accepted 8th January 2004
First published as an Advance Article on the web 9th February 2004

Amino H-bond donors adjacent to a zinc(II) centre increase the affinity of phosphates to the zinc(II) centre.

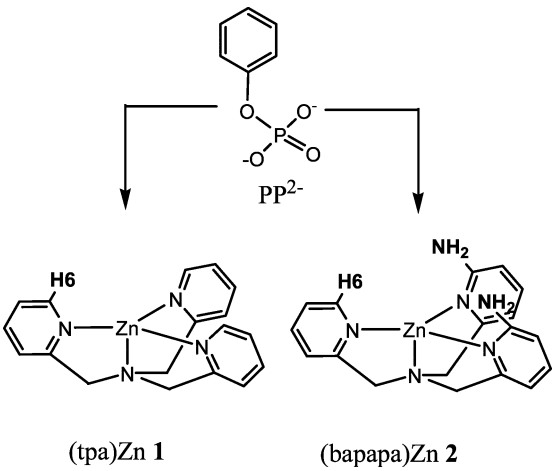
The design of efficient host molecules to achieve phosphate anion binding in water with high affinity is a major current goal in supramolecular chemistry.[1] Despite the current impetus in pursuing this challenging goal there is not an unified general strategy to create effective receptors for phosphate anion guests in water. Organic receptors equipped with acidic hydrogens have been proved to be efficient hosts for phosphate anions due to strong complementary H-bonding in non-aqueous solutions.[2] These hydrogen bonds, however, are typically disrupted in water and as a result it is difficult to achieve strong binding of phosphates with organic hosts.[3] As a result, the use of dinuclear and trinuclear metal ions has been conceived as a suitable strategy to improve binding of phosphate ions to artificial host molecules in water.[4] Some of these metal complexes have already found important applications in biotechnology.[5] Recently, the combination of metal binding, shape and H-bonding complementarity appeared to improve the affinity of phosphate anions to the host molecule.[6]

The design of agents capable of effecting the hydrolytic cleavage of the phosphate backbone of nucleic acids has also been attracting much current attention.[7] Organic receptors equipped with acidic hydrogens have been used to electrostatically activate phosphate esters toward nucleophilic attack.[8] In Nature, however, the hydrolytic cleavage of the phosphate backbone of nucleic acids is effected by metalloenzymes and it is facilitated by the cooperation of metal ions, typically Zn^{2+}, and the second coordination sphere.[9] Interestingly, it has been recently shown that binding of a phosphate ester to two Cu^{2+} ions with simultaneous H-bonding to one ammonium group can result in a remarkable acceleration, *ca.* 10^7-fold, of the hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP).[10] The excellent reactivity of this dicopper(II) complex was explained in terms of the additional electrostatic activation provided by the N–H···O–P H-bonding.

Thus, the desirable co-operation of metal ions and H-bonding groups could emerge as a powerful strategy to improve both the binding of phosphates and the efficacy of their chemical transformations at bio-inspired metallohosts in water. In addition, these metal complexes can also provide important new insights into the enzyme chemistry.

The pK_a of the zinc(II)-aqua complexes of tpa (**1**·H_2O) and bapapa (**2**·H_2O) (Scheme 1) were recently determined to be 8.1 and 6.7 respectively.[11] This result is consistent with the occurrence of intramolecular N–H···$O(H)_{1,2}$–Zn H-bonding.

Herein we report the effect that amine H-bond donors have on the affinity of phenyl phosphate PP^{2-} to **2** relative to **1**. NMR titrations of 1 mM **1** and **2** with PP (0–8 mM) in D_2O at 25 °C and pH 7 (50 mM HEPES) were carried out. The proton resonance of **1** and **2** experiencing the largest shifts upon addition of PP^{2-} is H6, presumably because it is the proton closest to the binding pocket formed by the preorganization of the ligands upon zinc(II)-binding. This proton resonance experiences downfield shifts upon addition of PP^{2-} (Fig. 1). NMR titration studies revealed that **1** and **2** bind to PP^{2-} in water at pH 7 to form 1:1 guest:host complexes. The apparent complexation constants, log K_{app}(LZn-PP), calculated by using the program HYPNMR 2000[12] are 3.6 ± 0.1 for **1** and 4.4 ± 0.1 for **2** at pH 7. It is remarkable that PP^{2-} binds more strongly to **2** than to **1**. Thus, electronically, the 6-amino groups are electron donating, which presumably should make the zinc(II) centre of **2** more electron rich and therefore lower its affinity for phosphates. In addition, the 6-amino groups should sterically hinder the approach of the external phosphate ligand. Moreover, the amino groups of bapapa increase the acidity of the zinc-water unit of **2** relative to that of **1** to such an extent that the percentage of the zinc-hydroxo form of **2** is *ca.* 4 times higher than that of **1** at pH 7.[11,13] In principle, the zinc-bound hydroxide should be more difficult to replace by the external phosphate than the zinc(II)-bound water molecule. It has been recently proposed, however, that N–H···O–P H-bonding was responsible for the dramatic increase in the binding affinity of dimethyl phosphate to a monometallic Co(III) complex.[14] Thus, the preference of PP^{2-} for **2** over **1** could be explained if the amino groups of bapapa are acting as H-bond donors to the zinc-bound phosphate. The X-ray crystal structure of [(bapapa)Zn-(NO_3)](NO_3) was determined,† and shows that the zinc(II) centre is



Scheme 1

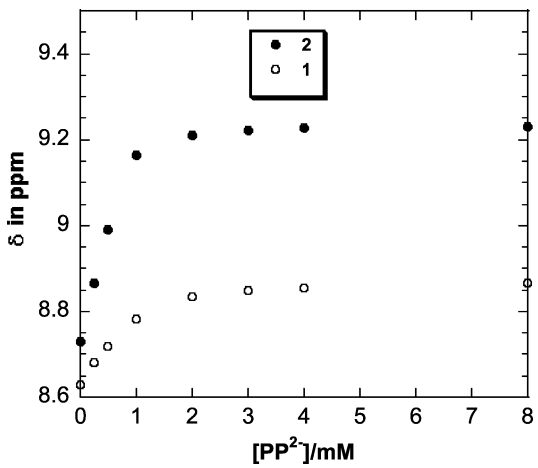


Fig. 1 Changes of the proton chemical shift of H6 of **1** (1 mM in D_2O) (○) and **2** (1 mM in D_2O) (●) upon addition of increasing amounts of PP at pH 7 ± 0.1 (50 mM HEPES).

DOI: 10.1039/b314616b

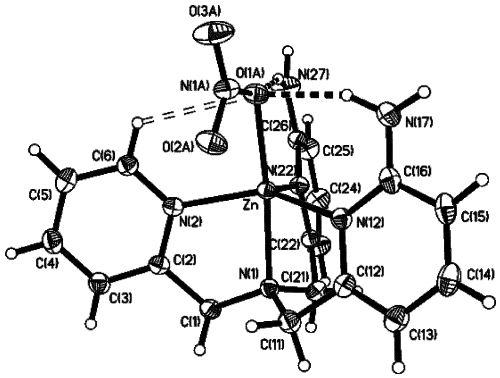


Fig. 2 Thermal ellipsoid plot (50% probability) showing the zinc(II) coordination environment of the $[(bapapa)Zn(NO_3)]^+$ cation. Selected bond lengths (Å) and angles (°): Zn(1)–N(1) 2.163(2), Zn(1)–N(2) 2.090(2), Zn–N(12) 2.093(2), Zn–N(22) 2.076(2), Zn–O(1A) 2.0993(19), N(22)–Zn–N(12) 109.71(8), N(22)–Zn–N(2) 101.33(8), N(12)–Zn–N(2) 140.10(8), N(22)–Zn–O(1A) 103.19(8), N(12)–Zn–O(1A) 100.51(8), N(2)–Zn–O(1A) 96.01(8), N(22)–Zn–N(1) 82.43(8), N(12)–Zn–N(1) 79.85(8), N(2)–Zn–N(1) 80.08(8), O(1A)–Zn–N(1) 173.77(8).

in a trigonal bipyramidal geometry with the two amino groups engaged in internal hydrogen bonds with the zinc(II)-bound oxygen of the coordinated NO_3^- anion (O(1A)···N(27) 2.926(3) Å, O(1A)···N(17) 2.943(3) Å, Fig. 2). This structure demonstrates that the positioning of the amino groups is suitable for simultaneous H-bonding to the bound PP^{2-}. Thus, we propose that the increased binding affinity of PP^{2-} to **2** may be due to the possibility of forming internal N–H···O–P H-bonding. It is remarkable that the improved affinity of PP^{2-} for zinc(II) receptors with H-bond donors is comparable in magnitude to that of dizinc(II) receptors relative to monozinc(II) receptors.[4] Thus, this result highlights the great potential of exploiting the second coordination sphere as strategy to improve the affinity of phosphates to zinc(II) complexes.

Recently, it has been shown that dizinc(II) complexes of tpa-based ligands that bind tightly to phosphates can be used for the detection of apoptotic cells.[5a,15] We aim to pursue the cooperation of several zinc(II) ions and H-bond donors to improve both the binding of phosphates and the efficacy of their chemical transformations at bio-inspired metallohosts in water.

We gratefully acknowledge the EPSRC (GR/R25743/01), the Royal Society (RSRG: 22702), the Nuffield Foundation (NAL/00286/G) and The University of Edinburgh for funding.

Notes and references

† *Crystal data* for $[(bapapa)Zn(NO_3)](NO_3)$: Intensity data were collected on colourless crystals of dimensions 0.5 × 0.5 × 0.33 mm at 150(1) K using a Bruker-AXS SMART APEX area detector diffractometer with Mo–K_α (λ = 0.71073 Å) radiation. The structure was solved by direct methods and refined to convergence against F^2 data using the SHELXTL-97 suite of programs. Data were corrected for absoption applying empirical methods using the program SADABS. $C_{18}H_{20}N_8O_6Zn$, M = 509.79, monoclinic, space group $I2/a$, a = 14.7313(12), b = 13.7642(11), c = 21.372(2) Å, α = 90, β = 104.670(4), γ = 90 °, U = 4192.1(6) Å^3, Z = 8, D_C = 1.615 g cm^{-3}, μ = 1.227 mm^{-1}, 24789 reflections measured, 5146 unique, R_{int} = 0.0430 (all data), $R1$ = 0.0512 (all data), $wR2$ = 0.1275 (all data), S = 1.080 (all data), largest difference peak, hole 1.997, −0.613 e Å^{-3}. CCDC 224743. See http://www.rsc.org/suppdata/cc/b3/b314616b/ for crystallographic data in .cif or other electronic format.

1 (*a*) P. D. Beer and P. A. Gale, *Angew. Chem. Int. Ed.*, 2001, **40**, 486; (*b*) P. D. Beer, *Acc. Chem. Res.*, 1998, **31**, 71.
2 F. P. Schmidtchen and M. Berger, *Chem. Rev.*, 1997, **97**, 1609.
3 (*a*) E. Kimura, *Top. Curr. Chem.*, 1985, **128**, 113; (*b*) B. Dietrich, *Pure Appl. Chem.*, 1993, **65**, 1457; (*c*) C. Bazzicalupi, A. Bencini, A. Bianchi, M. Cecchi, B. Escuder, V. Fusi, E. Garcia-España, C. Giorgi, S. V. Luis, G. Maccagni, V. Marcelino, P. Paoletti and B. Valtancoli, *J. Am. Chem. Soc.*, 1999, **121**, 6807.
4 E. Kimura, S. Aoki, T. Koike and M. Shiro, *J. Am. Chem. Soc.*, 1997, **119**, 3068.
5 (*a*) A. V. Koulov, K. a. Stucker, C. Lakshmi, J. P. Robinson and B. D. Smith, *Cell Death Diff.*, 2003, **10**, 1357; (*b*) E. Kimura, S. Aoki, E. Kikuta and T. Koike, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 3731.
6 S. L. Tobey, B. D. Jones and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 4026.
7 (*a*) P. Molenvend, J. F. Engbersen, H. Kooijam, A. L. Spek and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 1998, **120**, 6726; (*b*) N. H. Williams, B. Takasaki, M. Wall and J. Chin, *Acc. Chem. Res.*, 1999, **32**, 485; (*c*) J. K. Bashkin, *Curr. Opin. Chem. Biol.*, 1999, **3**, 752; (*d*) M. Komiyama and J. Sumaoka, *Curr. Opin. Chem. Biol.*, 1998, **2**, 751.
8 (*a*) V. Jubian, A. Veronese, R. P. Dixon and A. D. Hamilton, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1237; (*b*) D. A. Bell and E. V. Anslyn, *Comprehensive Supramolecular Chemistry. Hydrogen-bonding Receptors: Open-chain Catalytic Systems*; Ed. F. Vögtle, Pergamon: New York, 1996, **vol. 2**, ch. 14, 439.
9 (*a*) W. N. Lipscomb and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375; (*b*) D. E. Wilcox, *Chem. Rev.*, 1996, **96**, 2435; (*c*) R. Krämer, *Coord. Chem. Rev.*, 1999, **182**, 243.
10 E. Kövári and R. Krämer, *J. Am. Chem. Soc.*, 1996, **118**, 12704.
11 J. C. Mareque Rivas, R. Prabaharan and R. Torres Martin de Rosales, *Chem. Commun.*, 2004, 76.
12 (*a*) P. Gans, *HYPNMR 2000* (University of Leeds, Leeds, U.K.), 2000; (*b*) C. Frassineti, S. Ghelli, P. Gans, A. Sabatini, M. S. Moruzzi and A. Vacca, *Anal. Biochem.*, 1995, **231**, 374.
13 For **1** (1 mM in water), the concentration of the zinc-water and zinc-hydroxo forms are 0.77 and 0.15 mM, respectively. For **2** (1 mM in water), however, the concentration of the zinc-water and zinc-hydroxo forms are 0.28 mM and 0.63 mM, respectively.
14 J. Chin, S. Chung and D. H. Kim, *J. Am. Chem. Soc.*, 2002, **124**, 10948.
15 A. Ojida, Y. Mito-oka, M. Inoue and I. Hamachi, *J. Am. Chem. Soc.*, 2002, **124**, 6256.