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Multiple functional group cooperation in phosphate diester cleavage promoted by Zn(II) complexes†

Monica Livieri,[a] Fabrizio Mancin,*[a] Umberto Tonellato[a] and Jik Chin*[b]

[a] *Dipartimento di Scienze Chimiche, Università di Padova, via Marzolo 1, I-35131 Padova, Italy. E-mail: fabrizio.mancin@unipd.it; Fax: +39 0498275239; Tel: +39 0498275666*

[b] *Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Canada M5S 3H6. E-mail: jchin@chem.utoronto.ca; Fax: 946 7335; Tel: 946 7335*

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Zn(II) complexes bearing multiple auxiliary organic groups greatly accelerate the cleavage of a phosphate diester.

Enzyme catalyzed hydrolysis of nucleic acids is a fundamental reaction which allows the DNA manipulations essential to life. Nucleases are currently used also as laboratory tools and there is an increasing interest in the realisation of small and robust artificial DNA hydrolytic agents for their potential applications not only in molecular biology but also in the development of new drugs.[1] Many nucleases are metalloenzymes. Several examples of synthetic systems, based on metal complexes, which promote the hydrolysis of nucleic acids or of model phosphate esters have been reported in the last years, but their activity is still much lower than that of the corresponding enzymes.[2]

The high reactivity of nucleases is due to the cooperation of the prosthetic metal ions with several functional groups of the amino acid side chains present in the active site. One of us has previously shown that metal complexes bearing either an alcoholic group, acting as a nucleophile,[3] or a H-bond donating group, remarkably increase the ability of Cu(II) complexes to act as catalysts for phosphate ester cleavage.[4,5]

Bis-*p*-nitrophenyl phosphate (BNP) is employed in most studies on phosphate ester hydrolytic catalysts as a DNA model substrate. In this communication we report the reactivity toward BNP (Fig. 1) of the Zn(II) complexes of ligands **L1**–**L3**. The results obtained indicate that, as in the case of enzymes, cooperation of different functional organic groups with the metal centre may lead to a superior activity increase. In fact, **L2**·Zn(II) ranks among the most reactive Zn(II)-based monometallic complexes so far reported.

Potentiometric titrations of ligands **L1**–**L3** (see ESI†) in the absence and in the presence of one equivalent of $Zn(NO_3)_2$ allowed the determination (Table 1) of the pK_a of the protonated tertiary amino (K^1) and pyridino (K^2 and K^3) groups, of the complex formation constants (K_f) and of the deprotonation constant (K^n_a) of two metal-bound species, reasonably water molecules and/or the pendant hydroxyl group, as in the case of **L1** and **L2**. Two main pieces of information may be drawn from the data of Table 1: (i) ligands **L1**–**L3** strongly bind Zn(II) ions; and (ii) the pK_a values of the metal-bound species, either the hydroxyl or water molecules, decrease by 0.6–0.7 units on going from **L1**·Zn(II) to **L2** and **L3** complexes. The last two ligands feature two primary amino groups linked to the pyridino moieties, and the pK_a decrease observed may be taken as evidence of the formation of intracomplex hydrogen bonds with such amino groups. A similar effect, albeit larger (ΔpK_a of approximately 1.5 units) probably because of the greater rigidity of that system, was observed also in the case of the Zn(II) complex with tris-(6-amino-2-pyridylmethyl)amine and taken as the result of intracomplex hydrogen bonds.[6]

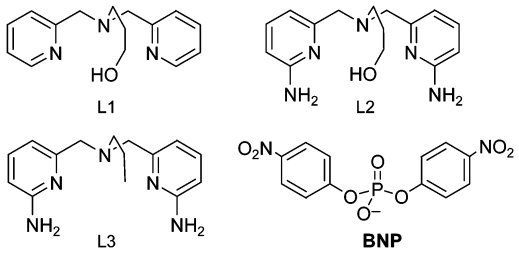

Fig. 1 Ligands and BNP.

Table 1 Deprotonation and Zn(II) complexation constants for ligands **L1**–**L3** determined from potentiometric titrations[a]

	$H3L^{3+}$			$[(L)Zn]^{2+}$		
Ligand	pK^1	pK^2	pK^3	log K_f	pK^1_a	pK^2_a
L1	6.57	3.06	<2	7.64	8.57	10.70
L2	7.72	5.33	1.95	6.68	7.94	9.96
L3	8.14	5.04	2.01	6.92	8.01	10.18

[a] 0.1 M NaCl, 25 °C.

Incubation of the Zn(II) complexes of **L1**–**L3** with BNP results in the substrate cleavage. Besides the different reaction rates, ^{1}H and ^{31}P NMR experiments showed that during the earlier stages of the reaction the cleavage of the BNP led to different products, depending on the presence of the hydroxyl group in the ligands (see ESI†). In the case of **L3**·Zn(II), only *p*-nitrophenol and *p*-nitrophenyl phosphate monoester were detected as reaction products, as expected from a clean hydrolytic cleavage. In the case of the complexes with **L1** and **L2** the reaction products were *p*-nitrophenol and the *O*-phosphorylated ligand resulting from a transesterification reaction.[7] Taken together, these results indicate that different nucleophiles are involved in the cleavage reaction according to the ligand structure: the alcoholic group in the case of **L1** and **L2** and a water molecule in the case of **L3**. Prolonged experiments showed that neither the *p*-nitrophenyl phosphate produced in the reaction with the **L3** complex, neither the phosphorylated ligands formed by **L1** and **L2** undergo any detectable further cleavage.

Kinetic studies were performed by monitoring the increase of the *p*-nitrophenolate absorbance at 400 nm, using the initial rates method. In each case the BNP cleavage rate increases linearly both with the concentration of metal complex and substrate. The pH dependences of the apparent second order rate constants are reported in Fig. 2. The pH profiles highlight, on one hand, the remarkable reactivity of the complex of **L2**, which includes both the alcoholic nucleophile and the H-bond donating amino groups and, on the other hand, a bell-shaped pattern with a maximum reactivity around pH 9–9.5. Fitting of the pH profiles with a kinetic model involving two deprotonation equilibria allowed the determination of the pK_a values of the reacting species and of the second order rate constants for the reactive mono-deprotonated metal complex (Table 2). The kinetic pK_a values thus evaluated are in good agreement with those of the metal-bound acidic species obtained from potentiometric titrations.

† Electronic supplementary information (ESI) available: synthesis of **L2** and **L3**, potentiometric titrations and NMR experiments. See http://www.rsc.org/suppdata/cc/b4/b412111b/

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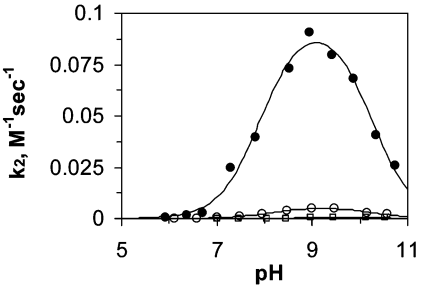


Fig. 2 pH dependence of the second order rate constant for the reaction between BNP and Zn(II) complexes of ligands **L1** (□), **L2** (●), **L3** (○) at 25 °C ([buffer] = 5.0×10^{-2} M). See ESI† for the magnification of the profiles for the complexes of **L1** and **L3**.

Table 2 Kinetic acid dissociation constants of the coordinated species (pK^n_a) and second order rate constants (k_2) for the BNP cleavage by Zn(II) complexes of **L1**–**L3**, in water at 25 °C[a]

Entry	Ligand	pK^1_a	pK^2_a	$k_2/M^{-1}\ s^{-1}$
1	**L1**	8.3	10.9	4.2×10^{-4}
2	**L2**	7.9	10.2	9.7×10^{-2}
3	**L3**	8.1	10.3	5.6×10^{-3}

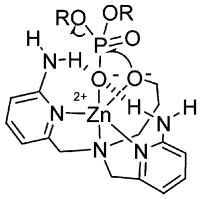
[a] [Buffer] = 5.0×10^{-2} M.

Bell-shaped pH reactivity profiles have been previously observed in similar systems[2a] and may indicate that the deprotonation of the two acidic metal-bound functions of the complex play opposite effects, the first one leading to a reactivity increase and the second to retardation. According to the commonly proposed mechanism, the first deprotonation leads to the formation of the active nucleophile (a metal-bound hydroxide or alkoxide) while the second deprotonation forms an anionic species which binds to the metal ion more tightly than the conjugated acidic precursor. As a consequence, the available coordination sites on the metal ion are saturated leaving little or no chance for the coordination of the substrate. Anyway, alternative reaction pathways cannot be completely ruled out and further studies aimed to explore in detail the reaction mechanism are under course.[7]

Analysis of the data reported in Table 2 confirms the indications obtained by inspection of the pH profiles: the complexes of **L2** are far more reactive than those of **L1** and **L3**. When reference is made to the rate of spontaneous hydrolysis of BNP at pH 7.0 and 25 °C, which can be estimated to be $1.6 \times 10^{-11}\ s^{-1}$ on the basis of the activation parameters determined at high temperatures,[8] BNP is cleaved by 1 mM **L2**·Zn(II) at a rate of $1.1 \times 10^{-5}\ s^{-1}$: an acceleration of about six orders of magnitude.

Comparison of the second order rate constants of Table 2 allows also the dissection of the contribution of the organic groups introduced in the structure of **L2**.[3] The alkoxide nucleophile is 17-fold more reactive than the metal-bound hydroxide (entries 2 and 3). A comparable effect was previously observed with Cu(II) complexes.[3] On the other hand, a much more important effect stems from the presence of the H-bond donating primary amino groups, resulting in a 230-fold reactivity increase (entries 1 and 2). We also investigated, as a reference, the Zn(II) complex of *N*-bis(pyridylmethyl)propylamine, lacking both the alcoholic and the aromatic amino groups. This turned out to be virtually inert in the BNP cleavage at 25 °C and, thus, a direct evaluation of the benefit due to the combined action of the two organic functional groups is not possible. However, at least a three orders of magnitude (17 × 230) reactivity increase can be estimated on the basis of the effects of the single groups as previously evaluated.

On the basis of the experimental evidences so far reported, we suggest (Scheme 1) that the BNP cleavage promoted by the Zn(II) complexes of **L2** involves an intracomplex nucleophilic attack of the metal-bound alkoxide on the metal coordinated phosphate diester. The two primary amino groups of the ligand are in the right position to form hydrogen bonds with the metal coordinated substrate thus providing additional Lewis acid activation.[9] Here, the overall result of the different acceleration effects which are at play: alkoxide nucleophile activation, double H-bond and Zn(II) Lewis acid activation, make the system capable of promoting the BNP cleavage with a reactivity increase, at pH 7, of six orders of magnitude. Such an activity is much higher than most mononuclear Zn(II)-based systems[10] and is close to that of the highly reactive lanthanide ions.[11]



Scheme 1 Proposed mechanism for the BNP cleavage by **L2**·Zn(II).

A similar mechanism operates also in the case of **L3** complexes, with a metal-bound water molecule being the active nucleophile instead of the ligand alkoxy group.[12] In this case, the acceleration is smaller but it is still remarkable (10^5) and the system is truly catalytic.

In conclusion, the realisation of simple and robust artificial phosphate ester hydrolytic agents, exploiting the cooperation between multiple organic functional groups and metal ion activation, is a promising strategy to obtain really efficient systems. In particular, the implementation of the structure of the reactive complex with groups capable of forming intramolecular hydrogen bonds with the substrate results in important reactivity increments.

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7 As pointed out by one of the referees, the possibility of the presence of major and minor ionization states for the mono-deprotonated species will need to be investigated. If the observed reactivity arises for a minor state, true second order rate constants could be substantially greater that those reported in Table 2.

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