Comparing a mononuclear Zn(II) complex with hydrogen bond donors with a dinuclear Zn(II) complex for catalysing phosphate ester cleavage†

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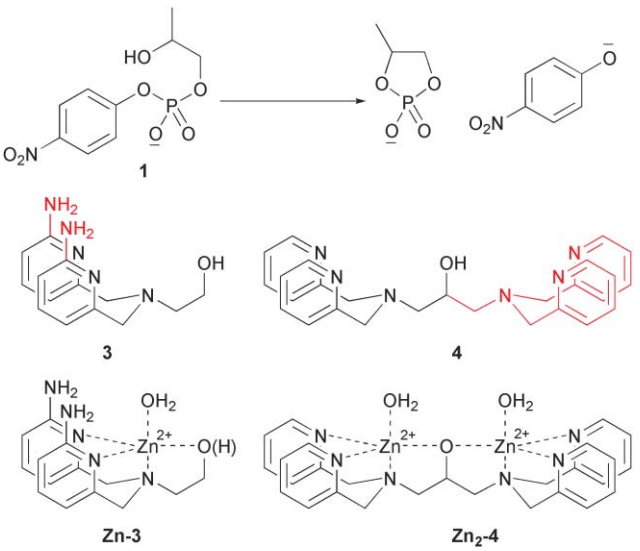
Introducing ligand based hydrogen bond donors to increase the activity of a mononuclear Zn(II) complex for catalysing phosphate ester cleavage can be a more effective strategy than making the dinuclear analogue.

In recent years, much attention has been given to the design of synthetic metallonucleases for the cleavage of RNA or DNA due to their potential applications as therapeutic agents, and as robust and versatile replacements for nucleases as laboratory tools.[1] More fundamentally, developing artificial systems both tests and expands our understanding of how catalysis works under biologically relevant conditions, although compared to enzymes, synthetic metallonucleases are still very inefficient. In most recent work, effort has focused on di- or polynuclear metal complexes, which are typically more reactive than the corresponding mononuclear metal complexes.[1e,2] In nature, many metalloenzymes that catalyse phosphate ester cleavage also use amino acid side chains to enhance activity compared to the metal ion by itself. This is beginning to be explored as a route to designing more powerful synthetic catalysts for phosphate ester cleavage, but so far there are only a few reports of applying this approach[3] although this strategy is becoming adopted more widely to influence the reactivity of metal ion complexes.[4]

We report a direct comparison between these two approaches, tested by catalysing the transesterification of 2-hydroxypropyl-4-nitrophenyl phosphate (HPNPP, **1**) to propylene phosphate and 4-nitrophenolate (Scheme 1). 2-[Bis-(pyridin-2-ylmethyl)-amino]-ethanol **2**,[5] which provides a tetradentate coordination sphere for Zn(II), forms the basis for our design and we compare the impact of introducing hydrogen bond donors to the ligand (**3**‡) with making a dinucleating analogue (**4**) (Scheme 1, modifications in red). The mononuclear Zn(II) complex of **3** (**Zn-3**) introduces aminopyridyl hydrogen bond donors that are preorganised to interact with a substrate coordinated to the Zn(II) ion, and the dinucleating structure of **4** (**Zn_2-4**) allows two Zn(II) ions to interact with the substrate simultaneously.



Scheme 1 Reaction, ligands used and proposed structures of **Zn-3** and **Zn_2-4**; **2** is the black portion of **3** and **4**.

To confirm that the active complexes are mononuclear and dinuclear as expected, $Zn(NO_3)_2$ was titrated into a 1 mM solution of each ligand (Fig. 1) and the observed rate constants for HPNPP cleavage measured.§ For ligand **3**, the rate of HPNPP transesterification increases to a plateau that is reached when one

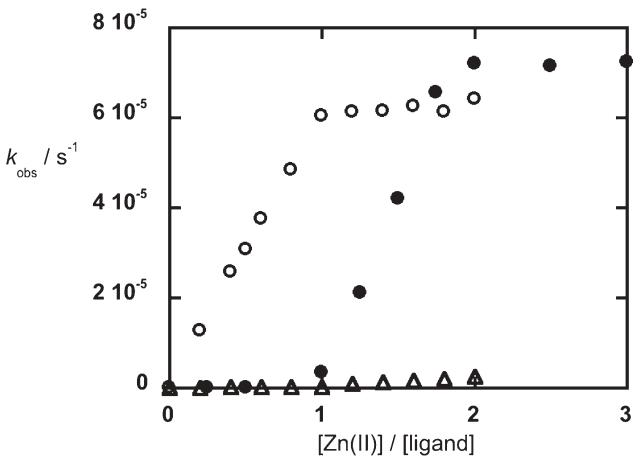


Fig. 1 Dependence of the rate of transesterification of HPNPP on $[Zn(NO_3)_2]$/[ligand] ratio at constant ligand concentration (1 mM) at 25 °C (50 mM HEPES). **2** (△,† pH 7.1); **3** (○, pH 7.3); **4** (●, pH 7.4).

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† Electronic supplementary information (ESI) available: Graphs showing expansion of Fig. 1 for data of **2** and first order dependence of HPNPP cleavage on free **Zn(II)**, **Zn-2**, **Zn-3** and **Zn_2-4** concentration; ^{31}P NMR spectra for the cleavage of HPNPP by **Zn-3** and **Zn_2-4**. See DOI: 10.1039/b514328d

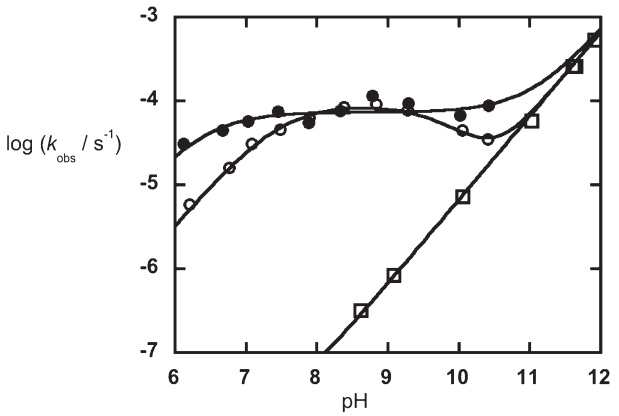

Fig. 2 pH–rate profiles for the transesterification of HPNPP at 25 °C (50 mM buffer): background reaction (□); in the presence of 1 mM **Zn-3** (○); in the presence of 1 mM **Zn_2-4** (●). The curve fit for the background reaction is for specific base catalysis, and the fits for the Zn complex catalysed reactions as described in the text.

equivalent of metal ion has been added, indicating that one metal ion per ligand is required for maximum activity; for ligand **4**, limiting reactivity is reached at two equivalents of Zn(II). In contrast, with ligand **2** present the system is less reactive than for catalysis by free Zn(II) ions.†

Fig. 2 shows the pH dependence for HPNPP cleavage for the reactions catalyzed by 1 mM **Zn-3** and **Zn_2-4** (formed *in situ* from the appropriate ratio of ligand and $Zn(NO_3)_2$). These data show that the maximal activities of complexes **Zn-3** and **Zn_2-4** are comparable. The data for **Zn-3** are fitted to two ionisations, assuming that the singly ionised species is the active ionic form (eqn. 1) and complex **Zn_2-4** is fitted to a single ionisation (eqn. 2).

$$k_{obs} = k_1[\mathbf{Zn-3}]\frac{K_a^1[H^+]}{\left(K_a^1K_a^2 + K_a^1[H^+] + [H^+]^2\right)} + k_3[HO^-] \quad (1)$$

$$k_{obs} = k_2[\mathbf{Zn_2-4}]\frac{K_a}{(K_a + [H^+])} + k_3[HO^-] \quad (2)$$

Both fits take into account the contribution from background hydrolysis at high pH ($k_3[HO^-]$; $k_3 = 0.065 \pm 0.002\ M^{-1}\ s^{-1}$) and give the parameters $k_1 = 9.2 \pm 0.5 \times 10^{-2}\ M^{-1}\ s^{-1}$ and $k_2 = 7.3 \pm 0.6 \times 10^{-2}\ M^{-1}\ s^{-1}$. The kinetic p$K_a$s obtained (7.4 ± 0.1 and 9.8 ± 0.2 for **Zn-3**, and 6.4 ± 0.4 for **Zn_2-4**) are consistent with previously reported pK_as from the titration of **Zn_2-4**,[6] and for the Zn(II) complex of a closely related analogue to **3** (with a 3-hydroxypropyl instead of 2-hydroxyethyl substituent[3g]). Both reactions are first order in the complex concentration up to 2 mM, and incubating **Zn-3** and **Zn_2-4** with **1** results in clean conversion to propylene phosphate as monitored by ^{31}P NMR at pH 7.1.† These catalysts undergo multiple turnovers, with complete turnover of 5 mM of HPNPP by 1 mM complex in each case, but no subsequent hydrolysis of propylene phosphate is observed over 3 days. Comparing the second order rate constants at pH 7.4, it is clear that both strategies are effective as both **Zn-3** ($4.6 \pm 0.4 \times 10^{-2}\ M^{-1}\ s^{-1}$) and **$Zn_2$-4** ($6.6 \pm 0.5 \times 10^{-2}\ M^{-1}\ s^{-1}$) are about 200-fold more effective than **Zn-2** ($3.0 \pm 0.3 \times 10^{-4}\ M^{-1}\ s^{-1}$) under physiological conditions.[7] It is striking that the maximal reactivities of the complexes are essentially identical, suggesting that the interactions with the hydrogen bond donors are as effective for enhancing catalytic activity as the introduction of a second Zn(II) ion.

To gain further insight into the observed reactivity of the complexes, we examined the effect of diester binding to the complex. The linear concentration dependence shows that both catalysts are being used under sub-saturating conditions; this means that the observed activity contains contributions from how effectively the catalyst recognises and binds the substrate from solution, and how reactive the catalyst–substrate (Michaelis–Menten) complex is. Fig. 3 shows a plot of normalized first order rate constant (k/k_0) for transesterification of HPNPP at pH 7.1 with increasing concentration of dimethyl phosphate (DMP). These data are fit to eqn. 3, which is derived for competitive inhibition by DMP.

$$\frac{k}{k_0} = \frac{K_i}{(K_i + [DMP])} \quad (3)$$

Weak inhibition is observed for the reaction catalysed by **Zn-3** ($K_i \sim 0.15$ M), but the reaction catalysed by **Zn_2-4** is strongly inhibited by DMP ($K_i \sim 0.009$ M). Assuming that binding of the substrate is comparable with DMP coordination, this suggests that **Zn_2-4** is more effective at forming the Michaelis–Menten complex, but that the reactivity of this complex is lower than the analogous complex formed with **Zn-3**. **Zn_2-4** shows comparable reactivity to **Zn-3** because it binds more substrate from solution, but once bound to the dinuclear complex of **Zn_2-4**, HPNPP is less reactive than when bound to the mononuclear centre of **Zn-3**.[8] It also means that **Zn_2-4** will be more affected by product inhibition than **Zn-3**, as the product of the cleavage reaction yields another phosphate diester which will bind comparably to the substrate. Most importantly, we note that **Zn-3** is expected to show a higher saturating rate, a property which is desirable for small catalysts that can be incorporated into artificial nucleases as well understood recognition processes can be utilised to enhance formation of a Michaelis–Menten complex, but not so readily used to enhance catalytic activity.

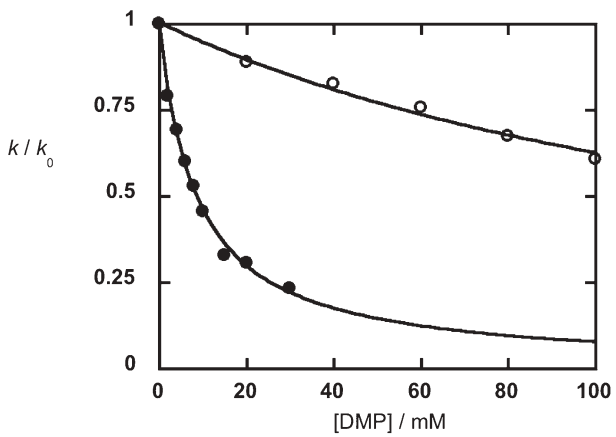

Fig. 3 Variation in the ratio of the rate constant for transesterification of HPNPP catalysed by 0.8 mM **Zn-3** (○) and 0.4 mM **Zn_2-4** (●) in the presence of DMP (k) to the rate in the absence of DMP (k_0) at pH 7.1 (50 mM HEPES, $I = 0.1$ M ($NaNO_3$)). The curve fits are for competitive inhibition (eqn. 3).

In summary, two common strategies used by nature to achieve the high catalytic activity of metallonucleases for catalysing the cleavage of phosphodiesters have been compared using small model systems. Overall, the rate of hydrolysis of phosphodiester bonds by a monometallic zinc(II) complex with hydrogen bonding groups is as fast as that of the analogous dizinc(II) complex. The monometallic catalyst, however, exhibits higher catalytic activity as the rate is the same despite having less bound substrate. We are continuing our studies to better understand and exploit the effects of hydrogen bonding environments for the development of more efficient artificial catalysts, and for a better understanding of the natural systems.

Notes and references

‡ 2-[Bis-(6-amino-pyridin-2-ylmethyl)-amino]-ethanol (**3**) was prepared from the reaction of ethanolamine with 2-bromomethyl-6-pivalamidopyridine followed by acidic hydrolysis.[3g] ^{1}H NMR (250 MHz, $CDCl_3$) δ/ppm 7.23 (dd, J = 7.9, 7.3 Hz, 2H, Py-H), 6.56 (d, J = 7.3 Hz, 2H, Py-H), 6.25 (d, J = 7.9 Hz, 2H, Py-H), 4.76 (br s, 5H, $2NH_2$ and OH), 3.61 (s, 4H, $2CH_2Py$), 3.54 (t, J = 5.0 Hz, 2H, CH_2OH), 2.69 (t, J = 5.0 Hz, 2H, CH_2). ^{13}C NMR (63 MHz, $CDCl_3$) δ/ppm 158.30 (Py), 157.58 (Py), 138.15 (Py), 112.81 (Py), 107.02 (Py), 59.93 (CH_2Py), 59.62 (CH_2OH), 56.40 (CH_2N). TOF MS (ES+) m/z 274 (M^+ + 1, 100%). HR-MS (ES+): calcd. for $C_{14}H_{20}N_5O$ (M^+ + 1): 274.1668; found: 274.1670.

§ Observed rate constants were obtained by initial rate (< 2% reaction) analysis using 1 mM HPNPP after confirming that good first order curves were obtained for complete cleavage of 0.05 mM HPNPP (which also gave the same rate constant); reactions were monitored at 400 nm to follow the release of 4-nitrophenolate.

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7 Note that the rate acceleration relative to free Zn(II) ions in solution is about 20 fold as the core ligand **2** reduces the activity of the ion.

8 This analysis assumes that the ground state binding mode is also the productive mode leading to reaction.