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Substrate specificity for catalysis of phosphodiester cleavage by a dinuclear Zn(II) complex

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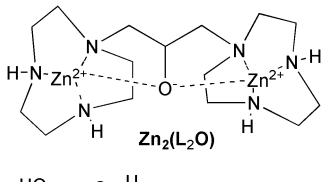
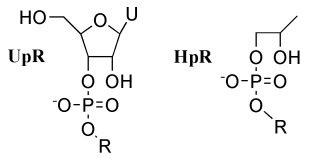
The 2.4 kcal mol^{-1} greater stabilization of the transition state for cleavage of the minimal substrate HpPNP compared to the nucleoside substrate UpPNP by the efficient dinuclear metal ion catalyst $Zn_2(L_2O)$ provides evidence that access to the cationic core of $Zn_2(L_2O)$ is sterically blocked for the bulkier nucleoside substrates, a flaw that will need to be dealt with in later generations of metal ion catalysts of RNA cleavage.

Much effort has been directed towards the design of metal ion complexes that catalyse the cleavage of RNA,[1,2] but much less work has been carried out to rationalize the mechanism by which these catalysts stabilize the transition state for nucleophilic addition to phosphodiesters. These analyses expand our understanding of the catalytic action of metal ions, and they may provide insight into strategies for the design of catalysts with the high activity and specificity required for inactivation of RNA targets in solution[1,3,4] or in cell cultures.[5]





R = PNP (-C_6H_4-4-NO_2), Ph (-C_6H_5)

The dinuclear complex **$Zn_2(L_2O)$** has two tightly bound Zn(II) ions and a hydroxide ligand at near neutral pH and is an effective catalyst for the hydrolytic cleavage of RNA analogs as well as oligoribonucleotides.[6,7] We report here a comparison of the pH rate profiles for the catalytic hydrolysis of **HpPNP** and the nucleoside substrate **UpPNP** (Fig. 1).† The data from this figure, and the derived kinetic parameters (Table 1) show the following:

(1) The apparent second-order rate constant for hydroxide-ion catalyzed cleavage of **UpPNP** (k_{HO} = 1570 M^{-1} s^{-1}) is more than 10^4-fold larger than for cleavage of **HpPNP** (k_{HO} = 0.099 M^{-1} s^{-1}). A similarly large difference has been noted for the second-order rate constants for hydroxide-ion catalyzed cleavage of **UpPh** (12 M^{-1} s^{-1}) and **HpPh** (9.8 × 10^{-4} M^{-1} s^{-1}),[8,9] and shown to be due to the additive effects of moving

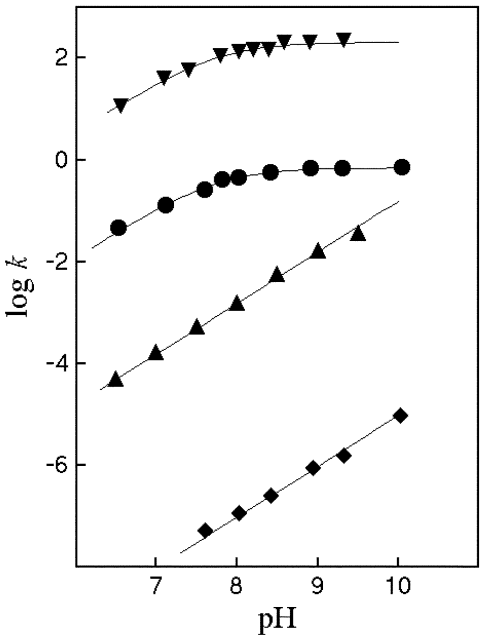


Fig. 1 pH rate profiles of second order rate constants k_{HO} and $(k_{Zn})_{obs}$ for cleavage of phosphodiester substrates **HpPNP** and **UpPNP** catalysed by hydroxide-ion and **$Zn_2(L_2O)$**. **HpPNP**: (●), k_{Zn}, (◆), k_{HO}; **UpPNP**: (▼), k_{Zn}, (▲), k_{HO}. The solid lines through values for k_{Zn} show the theoretical fits of the data to eqn. (1) using values of K_a = 10$^{-7.8}$ and the values of k_c from Table 1.

Table 1 Kinetic parameters and transition state stabilization for hydroxide-ion and **$Zn_2(L_2O)$**-catalysed cleavage of **UpPNP** and **HpPNP**[a]

	Catalyst					
	Hydroxide ion		**$Zn_2(L_2O)$**			
Substrate	k_{HO}/M^{-1} s^{-1}[d]	(k_{obsd})/s^{-1}[e]	k_c/M^{-1} s^{-1}[f]	k_{Zn}/M^{-1} s^{-1}[g]	$(k_{Zn}/k_{obsd})^b$/M^{-1}	$\Delta\Delta G^{\dagger c}$/kcal mol^{-1}
UpPNP	1570[h]	1.57 × 10^{-4}	203	28	1.8 × 10^5	−7.1
HpPNP	0.099	9.9 × 10^{-9}	0.71	0.097	9.8 × 10^6	−9.5
k_U/k_H[i]	16000		300			

[a] For reactions at 25 °C and I = 0.1 M ($NaNO_3$). [b] The relative rates of spontaneous cleavage and the cleavage reaction catalysed by 1.0 M catalyst at pH 7.0. [c] The stabilization of the transition state for cleavage catalysed by 1.0 M catalyst (Scheme 1). [d] Determined from the slope of a plot of the observed first-order rate constants against [HO$^-$]. [e] The observed rate constant for substrate cleavage at pH 7.0. [f] The limiting value of k_{Zn} at high pH. [g] The observed value of k_{Zn} at pH 7.0. [h] A value of k_{HO} = 840 M^{-1} s^{-1} has been reported in earlier work for a reaction at 25 °C and I = 0.25 M (Na_2SO_4).[9] [i] The ratio of rate constants for the reactions of **UpPNP** and **HpPNP**.

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from an acyclic to a cyclic cyclopentane diol backbone (30-fold effect), insertion of an oxygen in the cyclopentane ring (70-fold effect) and addition of a uracil group to the furanose ring (6-fold effect).

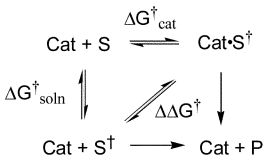
(2) The pH rate profiles of the observed second-order rate constants for cleavage of **UpPNP** and **HpPNP** catalyzed by **$Zn_2(L_2O)$** show a good fit to eqn. (1) derived for a simple scheme where the catalytic activity depends upon deprotonation of a critical group of pK_a 7.8. The limiting second-order rate constant for the reaction of **UpPNP** (k_c = 203 M^{-1} s^{-1}) at high pH is 300-fold larger than for the reaction of **HpPNP** (k_c = 0.71 M^{-1} s^{-1}).

$$k_{Zn} = \left(\frac{k_c K_a}{K_a + [H^+]} \right) \quad (1)$$

(3) A comparison of the observed first-order rate constants for hydroxide ion-catalysed cleavage ($k_{obs} = k_{HO}[HO^-]$, Table 1) and the observed second-order rate constant for **$Zn_2(L_2O)$**-catalysed cleavage [k_{Zn}, eqn. (1)] at the common pH of 7.0 shows that the rate acceleration from catalysis by 1 M of **$Zn_2(L_2O)$** (k_{Zn}/k_{obs}, Table 1) is 50-fold larger for cleavage of **HpPNP** (9.8×10^6-fold) than for cleavage of **UpPNP** (1.8×10^5-fold). This corresponds to a 9.5 kcal mol^{-1} stabilization of the transition state for cleavage of the minimal substrate **HpPNP** by interaction with **$Zn_2(L_2O)$** ($\Delta G^{\dagger}_{cat} - \Delta G^{\dagger}_{soln} = \Delta\Delta G^{\dagger}$, Scheme 1)‡ and a smaller 7.1 kcal mol^{-1} stabilization of the transition state for cleavage of the nucleoside substrate **UpPNP**.

The observation that the transition state for cleavage of **HpPNP** is more strongly stabilized (tightly bound) by **$Zn_2(L_2O)$** than the transition state for cleavage of **UpPNP** is surprising and revealing because, while the *opportunity* for development of binding interactions to the nucleoside substrate **UpPNP** transition state is greater than that for the minimal substrate **HpPNP**, the observed interactions are significantly weaker. Intramolecular tethering of the metal ions at the macrocyclic ligands across the bridging alkoxide ion of **$Zn_2(L_2O)$** has the effect of generating a highly charged core of unusual catalytic activity.[6,7] Our results provide evidence for the notion that a significant drawback of this array is that access to the catalytic core is restricted, so that **HpPNP** may bind closely to the cationic core to achieve stabilization of the anionic transition state, while interaction of **UpPNP** with the catalyst is not as effective, perhaps due to steric interactions between the catalyst and non-reacting portions of this substrate.§

The high catalytic efficiency of **$Zn_2(L_2O)$** for cleavage of **HpPNP** and the enzyme-like property of this catalyst of showing strong specific recognition of the *transition state* for substrate cleavage are due to the strong electrostatic interactions between substrate and the densely charged catalyst core that develop on approaching the reaction transition state.[6] These interactions facilitate deprotonation of the C-2 hydroxyl and, most importantly, provide stabilization of the developing phosphorane dianion at the rate-determining transition state for substrate cleavage.[6]



Scheme 1

The design of related complexes with higher catalytic activity for cleavage of larger molecular weight substrates such as **UpPNP** and RNA will require that the cationic core of the dinuclear catalyst be made accessible to these bulkier substrates. In principle, this might be accomplished by the use of larger and more flexible ligands for the metal cation. This plan is easily formulated, but difficult to implement in the absence of simple and rapid methods for the synthesis and characterization of a large variety of ligands and their complexes to metal ions.

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Notes and references

† The substrate **UpPNP** was prepared by a published procedure.[10] The buffers and specific reaction conditions used in obtaining the pH rate profile for the reaction of this substrate are the same as reporter earlier for cleavage of the minimal substrate **HpPNP**.[6] The transesterification of **UpPNP** was monitored by following the increase in absorbance at 400 nm due to the release of 4-nitrophenolate ion. Pseudo-first-order rate constants k_{obs} (s^{-1}) for transesterification of **HpPNP** were determined from the slopes of semi-logarithmic plots of reaction progress against time, which were linear for at least three reaction halftimes. Second-order rate constants k_{Zn} (M^{-1} s^{-1}) for the catalyzed reactions were determined as the slope of linear plots of k_{obs} against catalyst concentration.

‡ Scheme 1 is a thermodynamic cycle which shows that the total binding energy between catalyst and transition state for the catalyzed reaction ($\Delta\Delta G^{\dagger}$) is equal to the difference in the activation barriers for formation of the transition states for the catalyzed ($\Delta G^{\dagger}_{cat}$) and uncatalyzed ($\Delta G^{\dagger}_{soln}$) reactions.

§ The larger rate acceleration for **$Zn_2(L_2O)$**-catalysed cleavage of **HpPNP** compared with **UpPNP** cannot easily be attributed to a difference in the structures of the transition states for these reactions that allows for more effective electrostatic stabilization of the transition state for cleavage of **HpPNP**, because the observation of similar Brønsted parameters β_{lg} = 0.56 and 0.54, respectively, for HO^--catalysed cleavage of **HpPNP**[11] and **UpPNP**,[9] provides good evidence that these substrates undergo cleavage in solution through transition states of similar structure.

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