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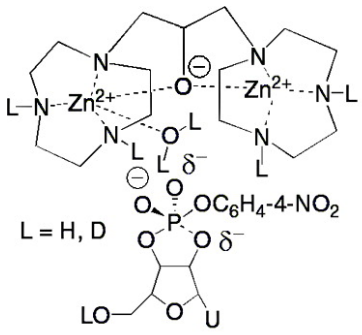

In water at 25 °C

Extraordinary catalytic activity is due solely to electrostatic interactions with tightly "packaged" zinc dications.

No concerted Brønsted acid/base catalysis!

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Solvent Deuterium Isotope Effects on Phosphodiester Cleavage Catalyzed by an Extraordinarily Active Zn(II) Complex

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We report that cleavage of **UpPNP** catalyzed by **Zn_2(1)** (Scheme 1) shows a normal equilibrium solvent deuterium isotope effect on the pK_a of an essential Zn^{2+}-bound water and no primary kinetic solvent isotope effect, so that concerted Brønsted acid−base catalysis does not contribute to the extraordinary rate acceleration observed for this catalyst.

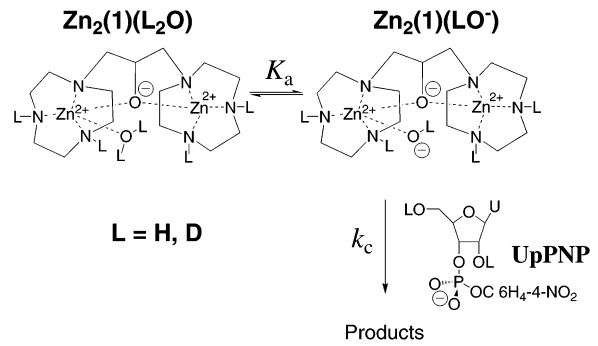
It is easier to understand catalysis by small compared to large molecules.[1] However, the rate acceleration for low molecular weight catalysts, such as buffers and metal ion complexes, is generally too small to account for the effective catalysis observed for larger proteins and ribozymes. By contrast, **Zn_2(1)(H_2O)** (Scheme 1) is a *small molecule* catalyst of phosphodiester cleavage which shows a catalytic rate acceleration that is a substantial fraction (ca 50%, 9.3 kcal/mol)[2,3] of that for larger protein and RNA catalysts. The extraordinary catalytic power of **Zn_2(1)(H_2O)** suggests that an examination of its origin should provide insight into the rate acceleration for protein-[4] and ribozyme-catalyzed cleavage of RNA.[5]

Cleavage of RNA proceeds with intramolecular displacement of an oxygen leaving group by the neighboring C-2 hydroxyl group.[6] The C-2 hydroxyl is weakly nucleophilic and may be activated by loss of a proton to form the alkoxide ion. The oxygen leaving group is strongly basic and may be activated by protonation. It is tempting to assume that concerted Brønsted acid−base catalysis which couples proton transfer to heavy-atom bond formation or cleavage contributes to efficient catalysis by large and small molecules, but this assumption must be verified by experiment. The observation of faster cleavage of phosphodiesters in H_2O than in D_2O provides evidence for concerted movement of a proton at the rate-determining transition state.[7] Significant solvent deuterium isotope effects (SDIE) have been reported for some protein-catalyzed[4] and ribozyme-catalyzed[5a,d,f] cleavage reactions of phosphate diesters. It is not known whether concerted Brønsted acid−base catalysis contributes to the catalytic rate acceleration of **Zn_2(1)(H_2O)**.

Potentiometric titration of the complex between free (**1**) (1 mM) and 2 equiv of Zn(II) in D_2O at 25 °C and I = 0.1 M ($NaNO_3$) shows two well-defined inflections.[8] A large inflection at low pD marks the release of six protons from the macrocycle nitrogens and of a seventh proton from the linker oxygen that occur upon binding of two Zn(II) to form **Zn_2(1)(D_2O)**, and a small inflection marks the release of one proton with an apparent pK_a of 8.5. The titrations in H_2O and D_2O are similar, except that in H_2O, the second inflection is at pH 8.0.[2] ^{1}H NMR and X-ray crystallographic data have shown that the linker-hydroxyl of **Zn_2(1)(L_2O)** is completely ionized at pH 7, so that the pK_a of 8.0 in H_2O and 8.5 in D_2O is for deprotonation of a metal-bound water molecule to form the monohydroxo complex **Zn_2(1)(LO^-)** (L = H or D, Scheme 1).[2] This is a normal perturbation by D_2O of the pK_a of a metal-bound water.[5g]

Transesterification of **UpPNP** (Scheme 1) to form the 2′,3′-cyclic phosphate diester and 4-nitrophenol was monitored as described in earlier work.[2] Plots of observed first-order rate constants, k_{obsd}, against catalyst concentration are linear through [**Zn_2(1)(L_2O)**] = 0.75 mM.[8] Second-order rate constants (k_{Zn}, M^{-1} s^{-1}) for transesterification catalyzed by **Zn_2(1)(L_2O)** were determined as the slopes of these linear correlations and are reported in Supporting Information. The effect of increasing pL on k_{Zn} (M^{-1} s^{-1}) for **Zn_2(1)(L_2O)**-catalyzed cleavage of **UpPNP** in H_2O and D_2O is shown in Figure 1. These data were fit to eq 1 derived for Scheme 1 to give $(pK_a)_{HOH}$ = 7.8 and $(k_c)_{HOH}$ = 200 M^{-1} s^{-1} for reaction in H_2O, and $(pK_a)_{DOD}$ = 8.4 and $(k_c)_{DOD}$ = 250 M^{-1} s^{-1} for reaction in D_2O. The pK_a values in H_2O and D_2O determined from the kinetic data are in agreement with pK_a values of 8.0 and 8.5, respectively, determined by potentiometric titration (see above). The ratio of values of k_c (eq 1) determined at high pH is $(k_c)_{HOH}/(k_c)_{DOD}$ = 0.80.[9a] We conclude that the larger values of k_{Zn} observed at low pL in H_2O than in D_2O reflect the lower pK_a for ionization of a critical catalytic residue in H_2O, and that there is no *primary* kinetic SDIE on the reaction catalyzed by the active form of the catalyst.

Scheme 1



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

The *shape* of the pL rate profiles (Figure 1) is consistent with either of the following catalytic reactions. (a) The active catalyst is **Zn_2(1)(LO^-)**, and this is protonated to give the inactive catalyst **Zn_2(1)(L_2O)**. (b) The active catalyst is **Zn_2(1)(L_2O)**, and this undergoes deprotonation to give inactive catalyst **Zn_2(1)(LO^-)**. In the first case, active catalyst **Zn_2(1)(LO^-)** is specific for cleavage of **UpPNP**, and in the second, **Zn_2(1)(L_2O)** is specific for cleavage of the O-2-ionized substrate. The rate laws for the two pathways are identical (eq 1) since they proceed through transition states of identical stoichiometry[9b] and differ only in the position of a proton.

The absence of a kinetic primary SDIE on the cleavage of **UpPNP** shows that the C-2 oxyanion is formed prior to rate-determining substrate cleavage, so that the active catalytic complex contains ionized substrate and protonated catalyst. The ionized substrate might bind from solution and then undergo cleavage, in which case $(k_c)'_{HOH} = [(K_a)_{cat}/(K_a)_{SH}](k_c)_{HOH}$ = 2 × 10^7 M^{-1} s^{-1}

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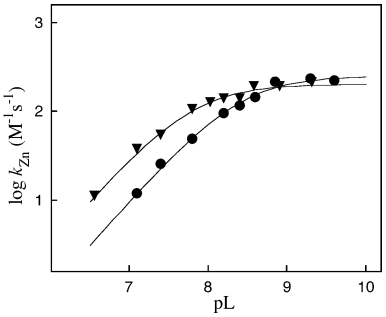
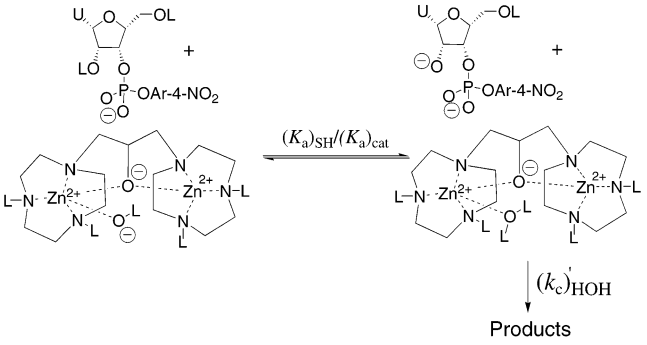


Figure 1. The pL rate profiles of second-order rate constants, k_{Zn}, for cleavage of **UpPNP** catalyzed by **Zn_2(1)(L_2O)** in H_2O (▼) and in D_2O (●). The solid lines show the theoretical fits of the data to eq 1 using values of pK_a = 7.8 and k_c = 200 M^{-1} s^{-1} for reactions in H_2O, and pK_a = 8.4 and k_c = 250 M^{-1} s^{-1} for reactions in D_2O.

Scheme 2



(Scheme 2), where $(K_a)_{cat} = 1 \times 10^{-7.8}$ M, $(k_c)_{HOH} = 200$ M^{-1} s^{-1} (above), and $(K_a)_{SH} \approx 1 \times 10^{-12.8}$ M for ionization of the C-2 substrate hydroxyl.[10] Alternatively, the neutral substrate might bind to the ionized catalyst and undergo intracomplex proton transfer. Deprotonation of **UpPNP** will be favored at the catalyst compared to solution if substrate binding to the catalyst perturbs the relative values of $(pK_a)_{cat}$ and $(pK_a)_{SH}$ and causes the difference to be <5 units, the difference for ionization in water.

The absence of a primary kinetic SDIE on cleavage of **UpPNP** shows that there is no *movement* of the proton at the rate-determining transition state for the cleavage reaction.[7] Therefore, there is no significant stabilization of this transition state from *concerted* Brønsted general acid−base catalysis of proton transfer. Further, there is minimal stabilization of the transition state through binding interactions with nonreacting portions of **UpPNP**,[3c] so that most or all of the rate acceleration for **Zn_2(1)(H_2O)**-catalyzed cleavage of phosphodiesters is from stabilization of the dianionic transition state by electrostatic interactions with the metal dications of **Zn_2(1)(H_2O)**. This shows that very large rate accelerations [9.8 $\times$ 10^6-fold for cleavage of 2-hydroxypropyl-4-nitrophenyl phosphate (**HpPNP**)[3c] and 1.8 $\times$ 10^5-fold for cleavage of **UpPNP**][2] may be obtained for low molecular weight catalysts *solely* by providing a compact densely charged catalytic core to interact with transition states of opposing charge. These results illustrate the power of simple electrostatics in small molecule catalysis of phosphate diester cleavage, and they suggest that electrostatics should be the most important element considered in the future design of low molecular weight catalysts of this reaction

A SDIE of 2 was recently reported for bifunctional catalysis of cleavage of **HpPNP** in which there is intramolecular general acid catalysis by an appended guanidinium cation and bimolecular general base catalysis by 2-methylpiperidine.[11] This primary kinetic SDIE shows that catalysis involves a component of proton transfer to the developing dianion at the oxyphosphorane intermediate or oxyphosphorane-like transition state. We now consider why, by contrast, no such Brønsted catalysis is observed for **Zn_2(1)**.

The metal ions of **Zn_2(1)(L_2O)** are drawn into a core by interaction with the ionized bridging alkoxy group,[2] with the result that the catalyst behaves as a compact "ball of charge" to provide a large electrostatic stabilization of the anionic transition state for phosphodiester cleavage. The relative barriers for catalysis of cleavage of **UpPNP** by competing stepwise and concerted (general base catalyzed) pathways will depend on (a) the electrostatic stabilization of the transition state for stepwise cleavage (Scheme 2) from interaction between opposing charges, and (b) the additional transition state stabilization from concerted general base catalysis. This stabilization by proton transfer at the C-2 hydroxyl has the effect of decreasing electrostatic stabilization of the transition state because of partial neutralization of negative charge at the partly protonated O-2 oxygen. In other words, the stepwise pathway is observed because of the dominant role played by electrostatics in transition-state stabilization.

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Supporting Information Available: Table of second-order rate constants for **Zn_2(1)(L_2O)**-catalyzed cleavage of **UpPNP** in H_2O and D_2O.

References

(1) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; Dover Publications: New York, 1987.
(2) Iranzo, O.; Kovalevsky, A. Y.; Morrow, J. R.; Richard, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 1988−1993.
(3) (a) Iranzo, O.; Richard, J. P.; Morrow, J. R. *Inorg. Chem.* **2004**, *43*, 1743−1750. (b) Iranzo, O.; Elmer, T.; Richard, J. P.; Morrow, J. R. *Inorg. Chem.* **2003**, *42*, 7737−7746. (c) Yang, M.-Y.; Richard, J. P.; Morrow, J. R. *J. Chem. Soc., Chem. Commun.* **2003**, 2832−2833.
(4) Park, C.; Raines, R. T. *Biochemistry* **2003**, *42*, 3509−3518.
(5) (a) Nakano, S.-I.; Chadalavada, D. M.; Bivilacqua, P. C. *Science* **2000**, *287*, 1493−1497. (b) Piccirilli, J. A.; Vyle, J. S.; Caruthers, M. H.; Cech, T. R. *Nature* **1993**, *361*, 85−88. (c) Pyle, A. M. *Science* **1993**, *261*, 709−714. (d) Shih, I.; Been, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1489−1494. (e) Sawata, S.; Komiyama, M.; Taira, K. *J. Am. Chem. Soc.* **1995**, *117*, 2357−2358. (f) Takagi, Y.; Taira, K. *J. Am. Chem. Soc.* **2002**, *124*, 3850−3852. (g) See footnotes 7 and 8 of ref 5e.
(6) (a) Zhou, D.-M.; Taira, K. *Chem. Rev.* **1998**, *98*, 991−1026. (b) Perreault, D. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 432−450.
(7) Schowen, R. L. *Prog. Phys. Org. Chem.* **1972**, *9*, 275−332.
(8) The procedures described in ref 2 were used for potentiometric titrations and in determining second-order rate constants for **Zn_2(1)(L_2O)**-catalyzed cleavage of **UpPNP**.
(9) (a) The uncertainty in this limiting isotope effect is estimated to be <±20% from the variation in the values of k_{Zn} at high pH (see Supporting Information). (b) Ref 1, pp 603−605.
(10) A pK_a of 12.8 has been determined by NMR titration for the C-2 hydroxyl of an analogue of **UpPNP** in which an ethyl group has been substituted for the nitrophenyl group: (a) Acharya, S.; Földesi, A.; Chattopadhyaya, J. *J. Org. Chem.* **2003**, *68*, 1906−1910. The same pK_a of 12.8 is used for **UpPNP** because the nitrophenyl for ethyl substitution at phosphorus is too distant (six atoms) from the site of ionization to have a large polar effect on acidity: (b) Hine, J. *Structural Effects on Equilibria in Organic Chemistry*; Wiley-Interscience: New York, 1975; pp 38−39.
(11) Piatek, A. M.; Gray, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 9878−9879.

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