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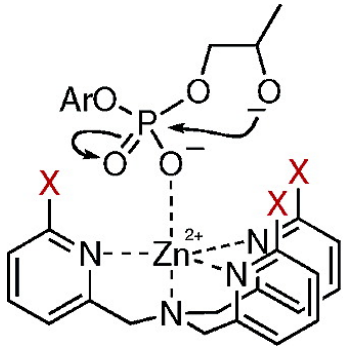
J. Am. Chem. Soc., **2005**, 127 (39), 13470-13471• DOI: 10.1021/ja054003t • Publication Date (Web): 08 September 2005



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A Highly Reactive Mononuclear Zn(II) Complex for Phosphodiester Cleavage

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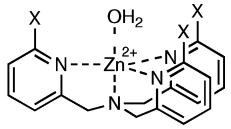
We report that the effect of introducing ligand-based hydrogen bond donors to enhance the activity of a Zn(II) complex for catalyzing phosphodiester cleavage can be dramatic. A detailed mechanistic study shows that the effect is comparable to the rate acceleration provided by the metal ion itself and generates a mononuclear complex more active than the most reactive dinuclear Zn(II) complex reported to date.

Enzymes and ribozymes that catalyze the hydrolysis of phosphate esters frequently utilize metal ion cofactors in a central catalytic role, and the most effective artificial systems have been based on mimicking this activity using simple metal ion complexes. In return, as it is easier to understand catalysis by small compared to large molecules, establishing how these catalysts work provides insight into the plausible catalytic roles of metal ions in biological catalysts. In many cases, additional interactions in the active site supplement the effect of the metal ion center and influence the properties of metal ion complexes,[1] but there are only few reports of applying this approach to the corresponding model complexes for phosphate ester cleavage.[2]

1, X = NH_2
2, X = H



We have used the structurally homologous[3,4] Zn(II) complexes **1** and **2** to examine the effect of introducing three aminopyridyl hydrogen bond donors that are rigidly preorganized to interact with a substrate coordinated to the Zn(II) ion. The reaction we selected is the cleavage of 2-hydroxypropyl 4-nitrophenyl phosphate **3** (Ar = 4-nitrophenyl), which undergoes intramolecular transesterification to produce propylene phosphate **4**, and has been used as a convenient model for RNA cleavage.

The cleavage reactions show a first-order dependence on increasing complex concentrations (0.3−3 mM for **1**, and 1−8 mM for **2**, with no indication of saturation) but are not accelerated by doubling the buffer concentration and so are not subject to general acid or base catalysis. In each case, the reaction catalyzed is the cyclization to produce **4**, and the complex undergoes multiple turnovers as monitored by ^{31}P NMR at pH 7. No hydrolysis of **4** is observed over 7 days, and no reaction was observed when methyl 4-nitrophenyl phosphate, lacking an intramolecular nucleophile, was used as the substrate. Figure 1 shows the pH dependence of the cyclization of **3** in aqueous solution, and of the second-order rate constant for catalysis by **1** and **2** (I = 0.1 M ($NaNO_3$), 50 mM buffer at 25 °C). These reflect the concentration of the Zn-hydroxo form of the free complex and nonlinear least-squares fitting of these data yield kinetic pK_a's of 5.66 ± 0.05 (**1**) and 7.63 ± 0.05 (**2**), in reasonable agreement with previously determined titration data.[3] The limiting second-order rate constants are 7.9 ± 0.1 × 10^{-2} M^{-1} s^{-1} and 1.0 ± 0.1 × 10^{-3} M^{-1} s^{-1} for **1** and **2** respectively. The background reaction is fit to specific base catalysis with a second-order rate constant of 7.0 ± 0.4 × 10^{-2} M^{-1} s^{-1} (k_b in Scheme 1, blue).

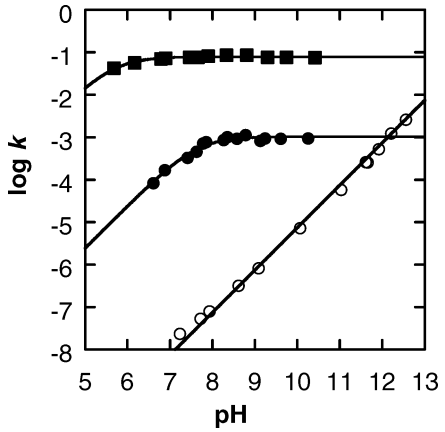
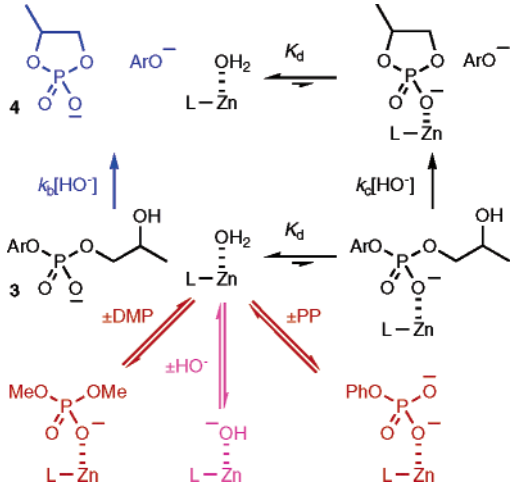


Figure 1. pH rate profiles for the transesterification of **3**: background reaction (○, s^{-1}); cleavage catalyzed by **1** (■, M^{-1} s^{-1}); cleavage catalyzed by **2** (●, M^{-1} s^{-1}).

Scheme 1



To establish whether the differences observed are due to ground-state or transition-state binding, the binding constants between the substrate and phosphate esters must be evaluated. We estimated this by measuring how effectively dimethyl phosphate (DMP) and phenyl phosphate (PP) inhibit the cleavage reaction. Figure 2 shows the normalized rate constants for the cleavage reaction catalyzed by **1** (data for **2** not shown) with increasing inhibitor at different pHs. These data are fit to eq 1, derived for competitive inhibition

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10.1021/ja054003t CCC: $30.25

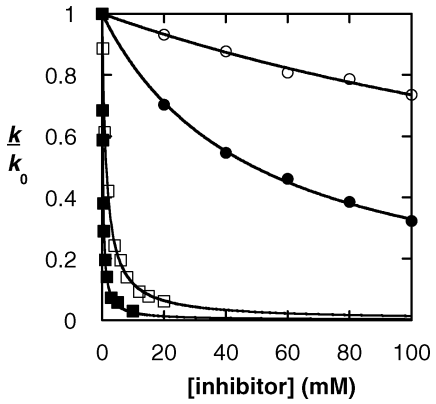

Figure 2. Relative rate constants for the transesterification of **3** catalyzed by **1** (0.3 mM) in the presence of phenyl phosphate (PP) and dimethyl phosphate (DMP). ○: DMP at pH 7.1; ●: DMP at pH 6.1; □: PP at pH 8.1; ■: PP at pH 7.1. The lines are curve fits to eq 1.

of the complex by inhibitor (I) where K_i^{obs} is the observed dissociation constant of I to the catalyst.

$$\frac{k}{k_0} = \frac{K_i^{obs}}{(K_i^{obs} + [I])} \quad (1)$$

Significantly, K_i^{obs} for both DMP and PP increases as the pH is raised. If the Zn-hydroxo form of the complex is the catalytically active form, which is the simplest interpretation of the pH rate profile, K_i^{obs} should be pH independent above the kinetic pK_a. Clearly, the esters inhibit the catalyzed reaction by competing for the Zn-*aqua* form of the complex (Scheme 1, red). The mechanism shown in Scheme 1 (black), where the substrate only binds to the Zn-aqua form of the complex then undergoes specific base-catalyzed transesterification,[5] is consistent with all the data. This is kinetically equivalent to catalysis by the Zn-hydroxo form of the complexes, but mechanistically the limiting rate at high pH is explained by deprotonation of the complex (Scheme 1, purple) reducing the concentration of the active form in precise compensation for the increasing hydroxide concentration.

$$K_i = \frac{[H^+]}{([H^+] + K_a)} K_i^{obs} \quad (2)$$

Combining eqs 1 and 2 (which shows the relation between K_i^{obs}, K_a for the complex and the association constant, K_i, of the inhibitor to the aqua form of the complex; full details available in Supporting Information) gives $K_i = 10 \pm 2$ mM for **1** and 130 ± 30 mM for **2** for the DMP anion,[6] and of 5 ± 1 μM for **1** and 60 ± 20 μM for **2** for the PP dianion.[4]

The rate acceleration achieved by coordinating **3** to **1** or **2** is given by the ratio of k_c to k_b (i.e. when the catalyst is saturated with substrate); assuming that the binding constant for DMP is a good approximation for substrate binding (K_d in Scheme 1), $k_c = 2 \times 10^5$ M^{-1} s^{-1} (**1**) and 3×10^2 M^{-1} s^{-1} (**2**), and thus the cyclization of **3** is accelerated (3×10^6)-fold by **1**, and (4×10^3)-fold by **2**. Remarkably, the enhancement brought about by the three amino groups provides a contribution to catalysis comparable to the core Zn(II) ion in **2**, giving an additional 750-fold acceleration in the catalyst−substrate complex. These rate-limiting accelerations are constant, but above the kinetic pK_a increasing catalyst (or substrate) concentrations are required to achieve the saturating conditions to which they apply. An analogous analysis of the dinuclear Zn(II) complex of Morrow and Richard,[7] previously the most effective Zn(II) complex reported to date for catalyzing the transesterification of **3**,[8] leads to a rate acceleration of 1.6×10^5, ~20-fold less effective than that of **1**. The mononuclear control gives a figure of 5.2×10^3, very similar to that of **2**.

If we consider the rate acceleration under subsaturating conditions, these figures become 3×10^8 M^{-1} for **1** and 3×10^4 M^{-1} for **2**, a 10^4-fold difference;[9] these accelerations are equivalent to the formal dissociation constant of the transition state ($K_{TS} = (k_{cat}/K_M)/k_{uncat} \equiv (k_c/K_d)/k_b$ in Scheme 1) from the catalyst.[10] The catalytic effect of **2** can be accounted for by considering the PP dianion as a crude mimic of the dianionic intermediate which forms in the rate-limiting step;[11] the binding constant for PP agrees well with K_{TS} and is presumably electrostatic stabilization of the developing negative charge by the Zn ion. However, for **1** the same comparison leaves a factor of (1.5×10^3)-fold which must be a consequence of more specific stabilization of the transition state for cyclization, presumably through enhanced hydrogen bonding between the ligand amino groups and the transition state leading to the dianionic intermediate.

Our results demonstrate that the benefits of tighter metal ion coordination through tetradentate tripodal ligands can be retained without sacrificing activity; in fact, this approach compares favorably with the strategy of introducing a second metal ion center. We are continuing our studies to characterize and exploit the effects of these types of complexes in catalyzing phosphoryl transfer.

Supporting Information Available: Derivation of equations and procedures for curve fitting for inhibition data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA054003T