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Article

A Reductionist Biomimetic Model System That Demonstrates Highly Effective Zn(II)-Catalyzed Cleavage of an RNA Model

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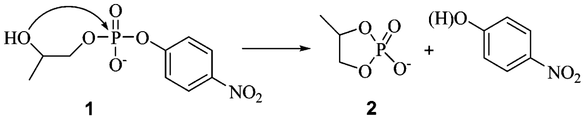
The cyclization of the RNA model 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNPP, **1**) promoted by Zn^{2+} alone and the 1,5,9-triazacyclododecane complex of Zn^{2+} (Zn^{2+}:[12]aneN_3) is studied in ethanol in the presence of 0.5 equiv of –OEt/Zn^{2+} to investigate the effect of a low polarity/dielectric medium on a metal-catalyzed reaction of biological relevance. Ethanol exerts a medium effect that promotes strong binding of HPNPP to Zn^{2+}, followed by a dimerization to form a catalytically active complex (HPNPP:Zn^{2+})$_2$ in which the phosphate undergoes cyclization with a rate constant of $k_{cat} = 2.9$ s^{-1} at ${}^s_s$pH 7.1. In the presence of the triaza ligand:Zn^{2+} complex, the change from water to methanol and then to ethanol brings about a mechanism where two molecules of the complex, suggested as EtOH:Zn^{2+}:[12]aneN_3 and its basic form, EtO–:Zn^{2+}:[12]aneN_3, bind to HPNPP and catalyze its decomposition with a rate constant of k_{cat} of 0.13 s^{-1} at ${}^s_s$pH 7.1. Overall, the acceleration exhibited in these two situations is 4×10^{14}-fold and 1.7×10^{12}-fold relative to the background ethoxide-promoted reactions at the respective ${}^s_s$pH values. The implications of these findings are discussed within the context of the idea that enzymatic catalysis is enhanced by a reduced effective dielectric constant within the active site.

Introduction

Many enzymes that catalyze phosphoryl transfer from a substrate to water or an alcohol OH group require two or more metal ions for maximum activity.[1] Due to the biological relevance of storage of genetic information in DNA and RNA, much attention has been focused on hydrolysis of phosphate diesters mediated by metal ions.[2] An actively investigated class of phosphate diester models for RNA is one containing a 2′-hydroxy group adjacent to the phosphate, some studied examples being various dinucleotides and the simple substrate 2-hydroxypropyl *p*-nitrophenyl phosphate (**1**).[3] Numerous studies have been reported where mono- and dinuclear M(II) complexes, particularly those of Zn(II),[4−6] facilitate the cleavage of these. The cleavage of **1** proceeds via intramolecular participation of the 2-hydroxypropyl group to form the cyclic phosphate (**2**) and provides about a 3000-fold acceleration of the expulsion of the *p*-nitrophenol(ate) leaving group relative to the hydrolytic reactions of phosphate diesters such as methyl *p*-nitrophenyl phosphate that do not contain the intramolecular OH.



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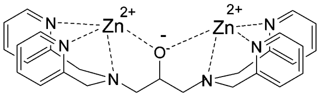
Metal ions alone in water can promote the cleavage of phosphate diesters, including **1**, but at pH values above the pK_a of the $M^{x+}(H_2O)_n \rightleftharpoons M^{x+}(^{-}OH)(H_2O)_{n-1} + H_3O^+$ ionization, the metal hydroxo species exist as gels or precipitates which complicate mechanistic analysis. In many cases the use of various ligands to form active complexes[4−6] of the metal−hydroxo forms circumvents the latter problem. The great bulk of the reported studies employ aqueous or largely aqueous solvents where the metal ions are complexed by various ligands to ameliorate their precipitation at elevated pH and, in the case of dinuclear complexes, to hold the metal ions close enough together that they may act cooperatively. Where comparison can be made, the dinuclear complexes are often more effective than a mononuclear complex, although this is not universally so.[5a,7] In some of these cases the catalysis afforded by the models is very good relative to the background reaction promoted by hydroxide at a comparable pH, although very few of the complexes are seen to have second-order rate constants for promoting the cyclization of **1** which greatly exceeds that of the hydroxide reaction.[8] For example, two of the most active dinuclear metal complexes reported, namely, the di-Zn^{2+} complex of 1,3-bis[(pyridine-2-ylmethyl)amino]propan-2-ol (**3**)[6] or di-Zn^{2+} complex of 1,3-bis-N_1(1,3,7-triazacyclononyl)propan-2-ol (**4**)[5d], react with HPNPP with second-order rate constants of 7.3×10^{-2} and 0.71 M^{-1} s^{-1}, respectively, whereas the second-order rate constant for the HO^- reaction is 6.5×10^{-2} M^{-1} s^{-1}.



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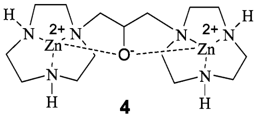

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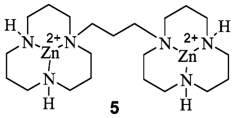

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Recently, we reported that the di-Zn^{2+} complex **5** exhibits a spectacular catalysis of the cleavage of **1** in methanol solution.[9] For example, a 1 mM solution of **5** in the presence of 1 equiv of added $^{-}OCH_3$ accelerates the reaction by 10^{12}-fold relative to the methoxide background reaction at a $^{s}_{s}$pH[10] of 9.5,[11] $T = 25$ °C. Remarkably, the second-order rate constant at 275 000 M^{-1} s^{-1} is 10^8-fold larger than the methoxide reaction (2.56×10^{-3} M^{-1} s^{-1}) and so is far larger than anything seen heretofore in water, underscoring a kinetically beneficial situation of a simple dinuclear Zn^{2+}-catalyst promoting the reaction of an anionic RNA model in a medium of reduced polarity and dielectric constant. In this report we deal with a continuation of the "reductionism"[12] methodology for simplifying the modeling of metal ion catalysis of the cleavage of **1** promoted by Zn^{2+} in ethanol. The use of the lower polarity/dielectric constant media is based on an attractive premise that "active sites of enzymes are nonaqueous, and the effective dielectric constants resemble those in organic solvents rather than that in

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(4) (a) Baykal, U.; Akkaya, E. U. *Tetrahedron Lett.* **1998**, *39*, 5861. (b) Baykal, U.; Akkaya, M. S.; Akkaya, E. U. *J. Inclusion Phenom. Macrocyclic Chem.* **1999**, *35*, 311. (c) Molenveld, P.; Kapsabelis, S.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1997**, *119*, 2948. (d) Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Org. Chem.* **1999**, *64*, 6337. (e) Moss, R. A.; Ragunathan, K. G. *Langmuir* **1999**, *15*, 107. (f) Moss, R. A.; Jiang, W. *Langmuir* **2000**, *16*, 49. (g) Williams, N. H.; Cheung, W.; Chin, J. *J. Am. Chem. Soc.* **1998**, *120*, 8079. (h) Wang, C.; Choudhary, S.; Vink, C. B.; Secord, E. A.; Morrow, J. R. *J. Chem. Soc., Chem. Commun.* **2000**, 2509. (i) Amin, S.; Voss, D. A.; Horrocks, W. DeW., Jr.; Morrow, J. R. *Inorg. Chem.* **1996**, *35*, 7466. (j) Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. *Biopolymers* **2000**, *55*, 496. (k) Leivers, M.; Breslow, R. *Bioorg. Chem.* **2001**, *29*, 345. (l) Gajda, T.; Krämer, R.; Jancsó, A. *Eur. J. Inorg. Chem.* **2000**, 1635. (m) Suh, J.; Hong, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 12545 and references therein. (n) Wall, M.; Hynes, R. C.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1633. (o) Wahnon, D.; Hynes, R. C.; Chin, J. *J. Chem. Soc., Chem. Commun.* **1994**, 1441. (p) Breslow, R.; Huang, D.-L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4080. (q) Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Chem. Commun.* **2005**, 2540. (r) Yamada, K.; Takahashi, Y.-i.; Yamamura, H.; Araki, A.; Saito, K.; Kawai. M. *Chem. Commun.* **2000**, 1315. (s) Mancin, F.; Rampazzo, E.; Tecilla, P.; Tonellato, U. *Chem.—Eur. J.* **2004**, 281. (t) Yashiro, M.; Kaneiwa, H.; Onaka, K.; Komiyama, M. *J. Chem. Soc., Dalton Trans.* **2004**, 605. (u) Jancsó, A.; Mikkola, S.; Lönnberg, H.; Hegetschweiler, K.; Gadja, T. *Chem.—Eur. J.* **2003**, *9*, 5404. (v) Gadja, T.; Düpre, T.; Török, I.; Harmer, J.; Schweiger, A.; Sander, J.; Kuppert, D.; Hegetschweiler, K. *Inorg. Chem.* **2001**, *40*, 4918.

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(6) Feng, G.; Mareque-Rivas, J. C.; Williams N. H. *Chem. Commun.* **2006**, 1845.

(7) Kim, J.; Lim, H. *Bull. Korean Chem. Soc.* **1999**, *20*, 491. Authors report that $Zn_2([12]aneN_3)_2$ is no more effective in promoting the hydrolysis of bis(2,4-dinitrophenyl) phosphate than is $Zn[12]aneN_3$.

(8) A criterion for a bifunctional behavior of the metal-bound hydroxide catalyst is that the second-order rate constant for a given process exceeds that of HO^- itself. Whereas there are some reports where the dinuclear Zn^{2+} or Cu^{2+} complexes can be more effective than hydroxide in promoting the hydrolysis of phosphates diesters such as bis(*p*-nitrophenyl) phosphate[4v] or 2′, 3′-cyclicAMP,[4u] there are few where the complexes effect the cyclization of **1** much better than HO^-.

(9) Neverov, A. A.; Lu, Z. L.; Maxwell, C. I.; Mohamed, M. F.; White, C. J.; Tsang. J. S. W.; Brown, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 16398−16405. .

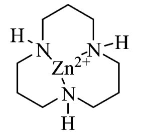
(10) For the designation of pH in nonaqueous solvents we use the forms recommended by the IUPAC, *Compendium of Analytical Nomenclature. Definitive Rules 1997*, 3rd ed.; Blackwell: Oxford, U.K., 1998. If one calibrates the measuring electrode with aqueous buffers and then measures the pH of an aqueous buffer solution, the term $^{w}_{w}$pH is used; if the electrode is calibrated in water and the pH of the neat buffered methanol solution then measured, the term $^{s}_{w}$pH is used; and if the electrode is calibrated in the same solvent and the "pH" reading is made, then the term $^{s}_{s}$pH is used.

(11) In the presence of 1 equiv of added methoxide, **5** exhibits a second-order rate constant of 2.56×10^5 M^{-1} s^{-1} for the reaction with **1** at $^{s}_{s}$pH 9.5, this being 10^8-fold larger than the k_2 value for the CH_3O^--promoted reaction ($k_{OCH_3} = (2.56 \pm 0.16) \times 10^{-3}$ M^{-1} s^{-1}).

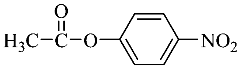
(12) Although it can be equally applied to science, reductionism is a term coined in the art world where it is believed by some that "Most significant painting... has been reductive, [for the artist's] analysis breaks the original complete scene into parts or separate dimensions of visual experience; reductionism is the concentration on or the preoccupation with the refinement of one dimension or aspect", see: Vitz, P. C.; Glimcher, A. B. *Modern Art and Modern Science, The Parallel Analysis of Vision*; Praeger: New York, 1984; pp 6−29.

water".[13,14] Zn^{2+} alone in water accelerates the cleavage of **1** only about 23-fold when present in excess of substrate,[15] but there are reports of a dramatic increases in the catalytic prowess of Zn(II) and Cu(II) in promoting the cyclization of **1** in acetonitrile[16a] as well as a Zr(IV) complex in benzene−methanol solution.[16b] Our own work[17] showed that La^{3+} binds **1** 10^4 times better in methanol than it does in water[2f] and, once bound, the intramolecular cleavage reaction of **1**:La^{3+} in methanol is 3×10^6 times faster than it is in water. However, La^{3+} is not a biologically relevant ion, so it is of interest to see whether Zn^{2+} exhibits any unusual behavior in reduced dielectric media such as methanol and ethanol, where the solution $^{s}_{s}\mathrm{pH}$ and $^{s}_{s}\mathrm{p}K_a$values for various catalytically important species can be controlled and measured.[17,18]

Herein we report that the change from water to ethanol increases the rate of cyclization of **1** when bound to Zn^{2+} by an unprecedented 4×10^{14} times relative to the background reaction at a $^{s}_{s}\mathrm{pH}$ of ∼7.1. In view of the fact that our earlier potentiometric titration studies indicated that the speciation of Zn^{2+} in methanol[18a] and ethanol[18b] comprises higher-order aggregates such as $(Zn^{II}_2(^-OCH_3)_2)^{2+}$, $(Zn^{II}_3(^-OEt)_2)^{4+}$, and $(Zn^{II}_3(^-OEt)_5)^+$ in the $^{s}_{s}\mathrm{pH}$ region of interest for catalysis of the cleavage of **1**, we compare the kinetics with Zn^{2+} alone with those for the 1,5,9-triazacyclododecane:Zn(II) complex, which holds the Zn(II) ion as an equilibrium distribution of two mononuclear complexes, (RO^-:Zn(II):[12]aneN_3) and (Zn(II):[12]aneN_3)[19] in these two solvents. We have also looked at the methanolysis and ethanolysis of a neutral carboxylate ester (*p*-nitrophenyl acetate, **6**) and determined the binding constant to Zn^{2+} of two other phosphate diesters **7** and **8**.



Zn^{2+}:[12]aneN_3



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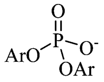

7 Ar = phenyl
8 Ar =*p*-nitrophenyl

Experimental Section

Materials. Methanol (99.8% anhydrous), sodium methoxide (0.5 M solution in methanol), sodium ethoxide (21 wt % solution in ethanol, titrated against N/50 Fisher Certified standard aqueous HCl solution and found to be 2.68 M), 1,5,9-triazacyclododecane ([12]aneN_3, 98%), tetrabutylammonium hydroxide in methanol (1 M, titrated against N/50 Fisher certified standard aqueous HCl solution and found to be 1.087 M), *p*-nitrophenyl acetate (**6**), and diphenyl phosphate (**7**) and bis(*p*-nitrophenyl) phosphate (**8**) were purchased from Aldrich and used without further purification. Diphenyl phosphate was used as the acid form in kinetic studies and when used for spectrophotometric studies was treated with 1 equiv of NaOEt to produce the salt. Anhydrous ethanol was purchased from Commercial Alcohols, Inc., and was degassed by bubbling Ar through it for 1 h and stored under Ar before use. $Cu(CF_3SO_3)_2$, $Zn(CF_3SO_3)_2$, and $HClO_4$ (70%, titrated to be 11.46 M) were purchased from Acros Organics and used without any further purification. The sodium salt of 2-hydroxylpropyl *p*-nitrophenyl phosphate (**1**) was prepared according to a slight modification[17] of the literature procedure.[3]

Methods. ^1H NMR and ^{31}P NMR spectra were determined at 400 and 162.04 MHz using a Bruker Avance-400 NMR spectrometer. The $CH_3OH_2^+$ and $CH_3CH_2OH_2^+$ concentrations were determined using a Radiometer pHC4000-8 or an Accumet model 13-620-292 combination glass electrode calibrated with Fisher certified standard aqueous buffers (pH = 4.00 and 10.00) as described in previous papers.[17,18] The $^{s}_{s}\mathrm{pH}$ values in methanol and in ethanol were determined by subtracting the respective correction constants of −2.24[17,18a] and −2.54[18b], respectively, from the readings obtained from the electrode, whereas the autoprotolysis constants are $10^{-16.77}$ and $10^{-19.1}$, respectively.

Kinetics. The rates of transesterification of substrates **1** and **6** in anhydrous methanol and in degassed anhydrous ethanol were followed by observing the rate of appearance of *p*-nitrophenol at 316 or 320 nm ($^{s}_{s}\mathrm{p}K_a$ of *p*-nitrophenol = 11.79 in ethanol[18b]) using either a Cary 100 Bio UV−vis spectrophotometer or an Applied Photophysics SX-17MV stopped-flow reaction analyzer, both thermostatted at 25 °C. When the [HPNPP] was too large to evaluate at 320 nm, progressively higher wavelengths (up to 340 nm) and shorter path cells (0.2 cm) were employed. In all cases except for the situation portrayed in Figure 2, the pseudo-first-order rate constants (k_{obs}) were obtained by fitting the UV−vis absorbance (abs.) vs time traces to a standard exponential model. All reactions were followed to at least three half-times and found to exhibit good pseudo-first-order rate behavior, unless specified. The second-order rate constants (k_2^{obs}) were determined from the slopes of k_{obs} vs $[\text{active catalyst}]_{total}$ plots.

Stock solutions of **1**, [12]aneN_3, the metal ions as their triflate salts, and NaOMe in methanol were prepared at 0.05 M in the

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[13] (a) Cleland, W. W.; Frey, P. A.; Gerlt, J. A. *J. Biol. Chem.* **1998**, *273*, 25529. (b) Simonson, T.; Carlsson, F.; Case, D. A. *J. Am. Chem. Soc.* **2004**, *126*, 4167 and references therein.

[14] The term "effective dielectric constant" is an interesting one when discussing the active site of an enzyme as D_e is a bulk macroscopic property resulting from an isotropic average of a large number of solvent molecules, whereas the situation within the active site of an enzyme, due to the molecular dimensions, is controlled more by specific interactions. See: Richard, J. P.; Ames, T. L. *Bioorg. Chem.* **2004**, *32*, 354.

[15] (a) Breslow, R.; Berger, D.; Huang, D.-L. *J. Am. Chem. Soc.* **1990**, *112*, 3686. Authors report that 0.5 mM Zn^{2+} at pH 7 in water at 37 °C produces a pseudo-first-order rate constant of 1.71×10^{-2} h^{-1} (4.75×10^{-6} s^{-1}) from which a second-order rate constant of 9.5×10^{-3} M^{-1} s^{-1} can be calculated at that pH. By way of comparison, the second-order rate constant for HO^--promoted cyclization of **1** is $k_2^{HO^-} = 0.065$ M^{-1} s^{-1} at 25 °C.[19b]. (b) Chin, K. O. A.; Morrow, J. R. *Inorg. Chem.* **1994**, *33*, 5036.

[16] Interesting studies of this reaction showing the dramatic effects of metal ion in nonaqueous media have also been reported, however, without detailed analysis or consideration of pH effects: (a) Kondo, S.-I.; Yoshida, K.; Yano, Y. *J. Chem. Res., Synop.* **1999**, 106. Authors reported that Zn(II) and Cu(II) accelerate the cyclization of **1** in acetonitrile by 10^{5-6}-fold relative to the situation without metal ion, but the addition of even a few percent of water slows down the reaction "due to a weakening of the electrophilicity of the metal ions". (b) Stulz, E.; Leuman, C. *Chem. Commun.* **1999**, 239. Authors describe a Zr(IV) complex promoting the cyclization of **1** in benzene−methanol.

[17] Tsang, J. S.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 1559.

[18] (a) For measurement of $^{s}_{s}\mathrm{pH}$, definition of terms, and titration of metal ions in methanol see: Gibson, G.; Neverov, A. A.; Brown, R. S. *Can. J. Chem.* **2003**, *81*, 495. (b) For measurement of $^{s}_{s}\mathrm{pH}$ and titration of metal ions in ethanol see: Gibson, G. T. T.; Mohamed, M. F.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.,* in press.

[19] (a) Kimura, E.; Shiota, T.; Kooike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805. Authors report that the pK_a for the aquo form of [12]aneN_3:$Zn^{2+}(H_2O)$ is 7.3. (b) Bonfá, L.; Gatos, M.; Mancin, F.; Tecilla, P.; Tonellato, U. *Inorg. Chem.* **2003**, *42*, 3943. Authors report a pK_a of 7.44 and an apparent second-order rate constant for the transesterification of **1** by the deprotonated complex in water of 0.018 M^{-1} s^{-1} at 25 °C.

appropriate solvent. These were added to a UV cell, diluted with anhydrous alcohol to the desired concentrations, and placed in the thermostatted cell holder to thermally equilibrate, and the reaction was initiated by the addition of **1** to achieve the desired final concentration. The rate constants for cyclization of **1** in the presence of varying [Zn^{2+}] (4×10^{-7} to 4×10^{-3} M) were determined in the presence of $^1/_2$ equiv of NaOR (per Zn^{2+}) such that half of the Zn^{2+} in the solution was associated with alkoxide to help control the solution $^{s}_{s}$pH, and the [**1**] was varied from 8×10^{-6} to 2.5×10^{-3} M. For the kinetic experiment where [Zn^{2+}] = 2[NaOEt] = 4×10^{-4} M while varying the [**1**] from 4×10^{-6} to 2.6×10^{-4} M, the abs. vs time profiles did not fit the usual pseudo-first-order kinetic model for the entire production of product. Thus, the rates for these reactions were determined by fitting the linear portion within the first 10% of the abs. vs time plots by linear regression and the data are shown in Figure 2. For the kinetics of cyclization of **1** in the presence of added diphenyl phosphate (**7**), the following conditions were used: [**1**] = 8×10^{-6} M, [Zn^{2+}] = 2[NaOEt] = 8×10^{-4} M, [diphenyl phosphate] (added as the acid) from 0 to 2.5×10^{-4} M. For the determination of the base-catalyzed ethanolysis of **1**, the conditions were as follows: [**1**] = 8×10^{-6} M, 2×10^{-3} to 10×10^{-3} M of NaOEt, and 1×10^{-3} to 6×10^{-3} M of tetrabutylammonium hydroxide. For the base-catalyzed ethanolysis of 8×10^{-5} M **6**, we used between 2×10^{-3} and 1×10^{-1} M of NaOEt and tetrabutylammonium hydroxide.

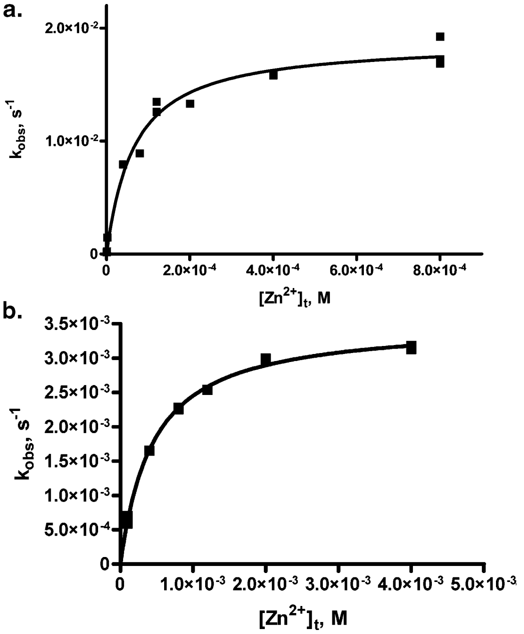

Figure 1. (a) Plot of k_{obs} vs [Zn^{2+}]$_{total}$ for the decomposition of **1** (8×10^{-6} M) in anhydrous ethanol at [$^-$OEt]/[Zn^{2+}]$_t$ = 0.5. Fitting the data to eq 1 gives an apparent dissociation constant K_d of $(6.2 \pm 0.1) \times 10^{-5}$ M and a k_{max} of 1.88×10^{-2} s^{-1}; $r^2 = 0.9811$. (b) A plot of k_{obs} vs [Zn^{2+}]$_t$ for the cyclization of **1** (4×10^{-5} M) in anhydrous methanol at [$^-$OMe]/[Zn^{2+}]$_t$ = 0.5. Fitting the data to eq 1 gives an apparent dissociation constant K_d of $(4.27 \pm 0.03) \times 10^{-4}$ M and a k_{max} of 3.52×10^{-3} s^{-1}; $r^2 = 0.9967$.

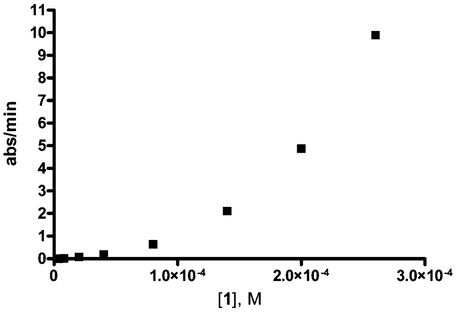

Figure 2. A plot of the rates (abs/min) vs total [**1**] for the cyclization of **1** in the presence of 0.4 mM [Zn^{2+}] at [$^-$OEt]/[Zn^{2+}]$_t$ = 0.5 in anhydrous ethanol at T = 25 °C.

Results

a. Ethoxide-Promoted Ethanolysis Reactions of HPNPP (1). Ethoxide-catalyzed cyclizations of 8×10^{-6} M of **1** were conducted using $(2-10) \times 10^{-3}$ M of NaOEt or $(1-6) \times 10^{-3}$ M of tetrabutylammonium hydroxide (1 M in methanol diluted in ethanol solution to desired concentrations). For the NaOEt-promoted reactions, the plots of k_{obs} vs [NaOEt] (not shown) were significantly upward curving, indicative of a higher than first-order dependence on NaOEt or its constituents due to ion-pairing phenomena that have been observed before by Buncel and co-workers for the NaOEt-catalyzed ethanolysis of phosphinates and phosphates.[20] The upward curvature is very slight in the presence of tetrabutylammonium counterions, so the ethoxide-promoted rate constants were evaluated by determining the gradients of the k_{obs} vs [tetrabutylammonium hydroxide] plot, which gives a second-order rate constant for ethanolysis of **1** at 25 °C of $(7.5 \pm 0.3) \times 10^{-3}$ M^{-1} s^{-1}. By way of comparison, the rate constant for methoxide attack on **1**[17] is 2.56×10^{-3} M^{-1} s^{-1}.

b. Zn^{2+} Catalysis of the Cleavage of 1 in Ethanol and Methanol. Shown in Figure 1 are plots of the pseudo-first-order rate constant (k_{obs}) for ethanolysis and methanolysis of HPNPP (**1**) as a function of [Zn^{2+}]$_{total}$ when the [$^-$OR]/[Zn^{2+}] ratio is 0.5. This ratio was chosen to buffer the system at the half-neutralization $^{s}_{s}$pH of ~7 in ethanol[18b] and ~9.5 in methanol at [Zn^{2+}]$_t$ = ~1−2 mM,[18a] and in each case the active Zn^{2+} species contains alkoxide. The data appear to exhibit saturation behavior and can be fit by eq 1,[21] which is a universal expression for both strong and weak binding scenarios where K_d refers to the dissociation constant for Zn^{2+}:**1** $\rightleftharpoons$ Zn^{2+} + **1**

$$k_{obs} = k_{cat}(1 + K_d[\mathbf{1}] + [Zn^{2+}]K_dX)/([\mathbf{1}](2K_d)) \qquad (1)$$

[**1**] and [Zn^{2+}] are total concentrations, and X is given in eq 2.

$$X = \{(1 + 2K_d[\mathbf{1}] + 2[Zn^{2+}]K_d + K_d^{\,2}[\mathbf{1}]^2 - 2K_d^{\,2}[Zn^{2+}][\mathbf{1}] + [Zn^{2+}]^2K_d^{\,2}\}^{0.5} \qquad (2)$$

However, the situation is more complicated than can be assessed by the one site binding model as we need to consider also the various equilibria of Zn^{2+} association with alkoxide

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[20] (a) Onyido, I.; Albright, K.; Buncel, E. *Org. Biomol. Chem.* **2005**, *3*, 1468. (b) Buncel, E.; Albright, K. G.; Onyido, I. *Org. Biomol. Chem.* **2004**, *2*, 610. (c) Nagelkerke, R.; Thatcher, G. R. J.; Buncel, E. *Org. Biomol. Chem.* **2003**, *1*, 163.

[21] Equation 1 was obtained from the equations for equilibrium binding and for conservation of mass by using the commercially available *MAPLE* software, *Maple V*, Release 5; Waterloo Maple, Inc.: Waterloo, ON, Canada.

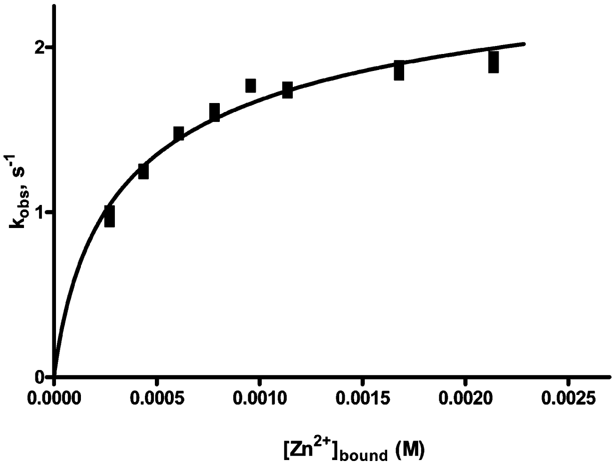
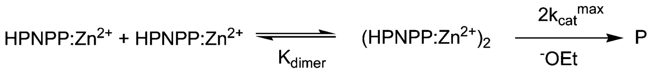


Figure 3. A plot of k_{obs} vs increasing $[Zn^{2+}]_{bound}$ for the cyclization of **1** in anhydrous ethanol with $[HPNPP]/[Zn^{2+}]_{total}/NaOEt = 1/1/0.5$. The $[Zn^{2+}]_{bound}$ data used are corrected for the amount of unbound Zn^{2+} using the stability constant K_d of $(6.2 \pm 0.1) \times 10^{-5}$ M given in Figure 1. Fitting the k_{obs} vs concentration data to a dimerization model (eq 3) gives a dissociation constant K_{dimer} for the presumed $(HPNPP{:}Zn^{2+})_2$ complex of $(6.2 \pm 0.5) \times 10^{-4}$ M and a maximum k_{cat}^{max} of 2.92 ± 0.06 s^{-1}; $r^2 = 0.9586$. The measured ${}^{s}_{s}$pH at 2 mM is 7.1.

Scheme 1



$$HPNPP{:}Zn^{2+} + HPNPP{:}Zn^{2+} \underset{K_{dimer}}{\rightleftharpoons} (HPNPP{:}Zn^{2+})_2 \xrightarrow[{}^{-}OEt]{2k_{cat}^{max}} P$$

and its oligomerization to produce dimers and higher-order aggregates (vide infra). Nevertheless, what can be established is that at $[Zn^{2+}]_{total} > 0.4$ and 5 mM in ethanol and methanol, respectively, essentially all the HPNPP is complexed to Zn^{2+}.

The reaction in ethanol, but not in methanol, has a higher than first-order dependence on [HPNPP]. Shown in Figure 2 is a plot of the rates of the cyclization of varying [HPNPP] in ethanol in the presence of 0.4 mM $[Zn^{2+}]_t$ at $[{}^{-}OEt]/[Zn^{2+}]_t = 0.5$ determined within the initial 10% of the abs. vs time plots. According to Figure 1a, at this concentration the HPNPP in ethanol is largely bound as a kinetically competent complex, but the Figure 2 plot shows an upward curvature suggestive of the involvement of two molecules of HPNPP. The log/log representation of Figure 2 (not shown) is linear with a gradient of 1.86 ± 0.05 ($r^2 = 0.9961$).

A second set of experiments was conducted under conditions where the k_{obs} for the cyclization of HPNPP was monitored as a function of increasing $[Zn^{2+}]/[HPNPP]/[NaOEt]$ values in a 1/1/0.5 ratio. Under these conditions, the HPNPP is essentially fully bound by the Zn^{2+}, and if the 1:1 complex were solely responsible for the catalysis, the observed rate constant for the reaction should be independent of concentration. However, the plot of k_{obs} vs $[Zn^{2+}]_{bound}$ (referring to the amount of Zn^{2+} which is bound to HPNPP as defined in the Figure 3 caption) between 0.4 and 2.5 mM shows evidence of a second saturation process consistent with the equilibrium binding of two $HPNPP{:}Zn^{2+}$ complexes to yield a dimer $(HPNPP{:}Zn^{2+})_2$ within which the cyclization of HPNPP occurs as shown in Scheme 1. The fit of the data to this model using the derived eq 3a gives values for the dimerization constant (K_{dimer}) and maximum catalytic rate constant (k_{cat}^{max}) of $(6.2 \pm 0.5) \times 10^{-4}$ M and 2.92 ± 0.06 s^{-1}, measured at a ${}^{s}_{s}$pH of 7.1 at 2 mM concentration of complex.

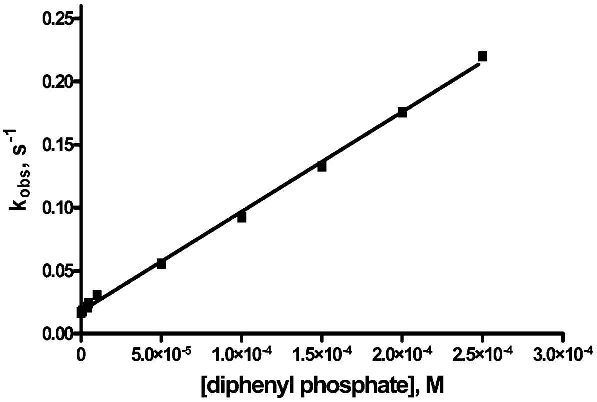


Figure 4. A plot of k_{obs} vs total [diphenyl phosphate (**7**)] for the cyclization of **1** (8×10^{-6} M) in the presence of $[Zn^{2+}] = 0.8$ mM in anhydrous ethanol at $[{}^{-}OEt]/[Zn^{2+}]_t = 0.5$. The best fit line is described as $k_{obs} = (1.77 \pm 0.12) \times 10^{-2}$ s^{-1} + (791 ± 11) [**7**]; $r^2 = 0.9983$. Diphenyl phosphate (**7**) was added as the acid, and the ${}^{s}_{s}$pH values measured after the reactions were 7.0 ± 0.2.

$$k_{obs} = 2k_{cat}^{max}[(HPNPP{:}Zn^{2+})_2]/[HPNPP] \qquad (3)$$

where

$$[(HPNPP{:}Zn^{2+})_2] = (4X + K_{dimer} - K_{dimer}(8X/K_{dimer} + 1)^{0.5})/8 \qquad (3a)$$

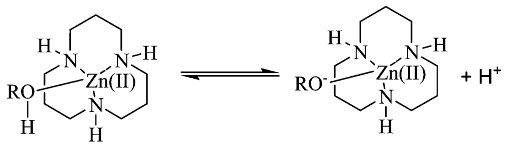
and X correspond to the x-axis data in Figure 3 which is corrected for the amount of unbound Zn^{2+}.

The process shown in Scheme 1 is predicated on the assumption that the cyclization reaction requires ethoxide, either coordinated to the complex or external to it, to act as a base to deprotonate the hydroxypropyl groups within the $(HPNPP{:}Zn^{2+})_2$. However, it is not clear from the data what the role of the second phosphate is other than to act as a template that stabilizes the dinuclear Zn^{2+} core in much the same way as that found in our earlier study of the La^{3+}-catalyzed cleavage of HPNPP.[17] If so, it is reasonable that a surrogate phosphate diester, for example, diphenyl phosphate (**7**), should catalyze the reaction of **1**:Zn^{2+} through its binding to second Zn^{2+} and formation of a reactive dimer (**7**:Zn^{2+})-(**1**:Zn^{2+}). Indeed, as shown in Figure 4, when the k_{obs} for cyclization of **1** in a solution containing 8×10^{-6} M **1** and 8×10^{-4} M Zn^{2+}:0.5(NaOEt) is monitored as a function of varying **7** ($(0-2.5) \times 10^{-4}$ M), the gradient of the linear plot is 791 ± 11 M^{-1} s^{-1}, indicating a marked catalysis of the reaction.

c. Binding of Zn^{2+} to Diphenyl Phosphate and bis(*p*-Nitrophenyl) Phosphate. The cyclization reaction of **1** in the presence of Zn^{2+} appears to require preliminary binding followed by a second equilibrium binding of two (**1**:Zn^{2+}) complexes to form a reactive dimer. Although **1** reacts too fast in the presence of Zn^{2+} to determine the 1:1 equilibrium binding constant, some idea of this might be determined from Zn^{2+} binding to the far less reactive substrate diphenyl phosphate, the binding constant of which was determined in two ways. First, the formation constant between Zn^{2+} and **7** in ethanol was calculated by fitting triplicate potentiometric titration data of solutions containing 1 mM of $Zn(OTf)_2$ or **7** along with 1 mM $HClO_4$ and solutions comprising 1 mM in each of $HClO_4$, $Zn(OTf)_2$, and **7** using the computer program Hyperquad 2000 (version 2.1 NT)[22] with the autoprotolysis constant of pure ethanol taken to be $10^{-19.1}$ at 25 °C.[18b] The $^{s}_{s}\mathrm{p}K_a$ of **7** in ethanol was determined to be 4.22 ± 0.20, which was set as a constant, and the titration data for Zn^{2+} with **7** were fitted to a 1:1 binding model via eq 1 to yield a K_d of $(5.9 \pm 0.6) \times 10^{-6}$ M.

In the second set of experiments, the binding of 2×10^{-5} M **8** (treated with 1 equiv of NaOEt to make the anion) with varying $[Zn^{2+}]$ and separately with varying $[Zn^{2+}$:0.5-(NaOEt)] in ethanol was determined spectrophotometrically at 260 nm. Analysis of the $\mathrm{abs.}_{260nm}$ vs $[Zn^{2+}]$ or $[Zn^{2+}$:0.5-(NaOEt)] data using the universal eq 1 for an assumed 1:1 complex gave dissociation constants for the **8**:Zn^{2+} complexes under the two sets of conditions as $K_d = (3.98 \pm 0.05) \times 10^{-6}$ M and $(1.12 \pm 0.04) \times 10^{-5}$ M, respectively. Given the differences in the conditions used to determine the binding constants of **7** and **8** as well as the fact that the $^{s}_{s}\mathrm{p}K_a$ of **7** has an error of at least 0.2 units, we cannot say the binding constants are different, but we can say that each binds to the Zn^{2+} strongly, which leads to the conclusion that the 1:1 HPNPP:Zn^{2+} complex must have a similarly small K_d value confirming what was determined from analysis of the data in Figure 1a.

d. Cyclization of 1 Promoted by Zn(II):12 and N_3 in Ethanol and Methanol. Whereas the catalysis of the cyclization of **1** by Zn^{2+} in methanol and ethanol is complicated by the presence of dimeric and oligomeric Zn^{2+}-alkoxide species, complexation to the macrocyclic ligand [12]aneN_3 reduces the number of species in the $^{s}_{s}\mathrm{pH}$ regions of interest to the Zn^{2+}-complex (Zn(II):[12]aneN_3) and, by dissociation of a coordinated ROH, its alkoxy form **9** as in eq 4.



(ROH:Zn(II):[12]aneN_3) **9** (4)

The $^{s}_{s}\mathrm{p}K_a$ values for the process in eq 4 are 7.5 in ethanol[23] and 9.2[24] in methanol.

Presented in Figure 5 is a plot of the k_{obs} for the cyclization reaction of **1** in methanol vs $[(Zn(II):[12]aneN_3)]_{total}$ under conditions where the $[^{-}OCH_3]/[Zn^{2+}]_t = 0.5$ which sets the $^{s}_{s}\mathrm{pH}$ at 9.2−9.3.[9] The plot shows upward curvature consistent with a process bimolecular in $[Zn(II):([12]aneN_3)]_t$. Nonlinear least-squares (NLLSQ) fitting of the data to the expression $k_{obs} = k_1\ [Zn(II):[12]aneN_3] + k_3^{obs}\ [Zn(II):[12]aneN_3]^2$ gives $k_1 = 18.9\ M^{-1}\ s^{-1}$ and $k_3^{obs} = (1.8 \pm 0.4) \times 10^3\ M^{-2}\ s^{-1}$, where the best fit line through the data is shown in Figure 5. In no case do we observe evidence of saturation binding in methanol up to a $[Zn(II):([12]aneN_3)]_t$ of 5×10^{-3} M, so the K_{dis} for dissociation of any HPNPP:Zn(II):[12]aneN_3 complex must be at least 5× higher.

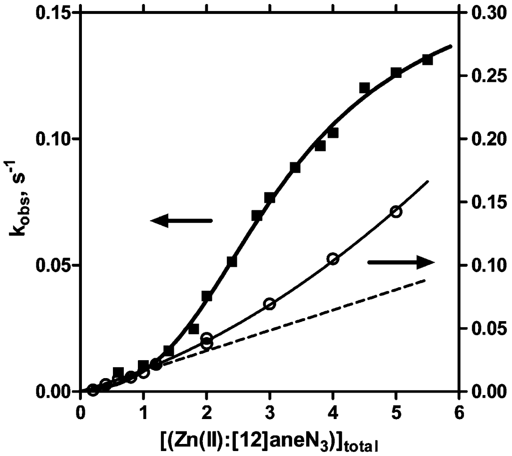


Figure 5. Plot of k_{obs} vs total $[(Zn(II):[12]aneN_3)]_{total}$ for the cyclization of **1** (8×10^{-5} M) in ethanol (■, left *y*-axis) and methanol (○, right *y*-axis) under conditions where the $[^{-}OR]/[Zn(II)]_{total}$ is 0.5. Dotted line corresponds to process first order in $[[12]aneN_3]$, whereas curved line is the fit in methanol to the expression $k_{obs} = k_1\ [Zn(II):[12]aneN_3] + k_3^{obs}[Zn(II):[12]aneN_3]^2$. Heavy solid line through the solid squares is the fit of the data to eq 6a.

The situation in ethanol is similar, but the upward curvature is manifested at lower [Zn(II)] concentration as illustrated in Figure 5, which shows the plot of k_{obs} for the cyclization of **1** vs $[(Zn(II):[12]aneN_3)]_{total}$ under conditions where the $[^{-}OEt]/[Zn^{2+}]_t = 0.5$, setting the measured $^{s}_{s}\mathrm{pH}$ at 8.1 ± 0.2. This plot shows a downward curvature suggestive of a saturation phenomenon which is not seen in methanol or water at these concentrations. To test that the kinetic behavior exhibited in Figure 5 does not result from a medium-induced agglomeration of Zn^{2+} species or inhibition by increasing [OTf^{-} counterion] in ethanol, the k_{obs} for the [Zn(II):[12]aneN_3]-catalyzed ethanolysis of the weak binding substrate **6** was studied at a $[^{-}OEt]/[Zn^{2+}]_t = 0.5$ at $[Zn^{2+}]_t$ between 0.1 and 6 mM, with a measured $^{s}_{s}\mathrm{pH} = 8.3 \pm 0.1$. The plot (not shown) is linear with a gradient of $0.81 \pm 0.01\ M^{-1}\ s^{-1}$. Additional studies indicated that the active species is EtO^{-}:Zn(II):[12]aneN_3,[25] which is consistent with the situation in methanol[24] where the second-order rate constant for CH_3O^{-}:Zn(II):[12]aneN_3-promoted methanolysis of paraoxon is $0.84\ M^{-1}\ s^{-1}$.

Overall the behavior exhibited for strongly binding **1** with the Zn^{2+} complex is consistent with a minimal process given in eq 5 which is predicated on the mechanism being first order in $[\mathrm{complex}]_{total}$ which accounts for k_1 and a more complicated event dependent on both [Zn(II):[12]aneN_3] and [EtO^{-}:Zn(II):[12]aneN_3] reacting with **1** as it is in methanol. For technical reasons relating to a too high variance of $^{s}_{s}\mathrm{pH}$

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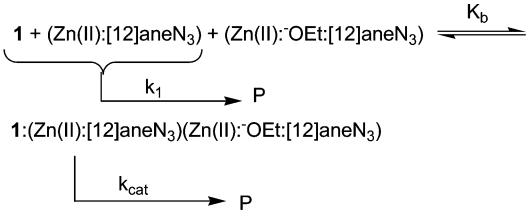
(22) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739.

(23) The $^{s}_{s}\mathrm{p}K_a$ is determined from Hyperquad fitting of the potentiometric titration data as described in ref 18b. However, the working $^{s}_{s}\mathrm{pH}$ for a solution formulated from 1 mM of [12]aneN_3, 0.5 mM NaOEt, and 1 mM $Zn(OTf)_2$, added in the indicated order, is 8.2.

(24) Desloges, W.; Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2004**, *43*, 6752.

(25) Since the active species for the ethanolysis of *p*-nitrophenyl acetate is EtO^{-}:Zn(II):[12]aneN_3, the true second-order rate constant for the reaction would be twice the gradient of the plot, or $1.62\ M^{-1}\ s^{-1}$.

at a $[^{-}OEt]/[Zn^{2+}]_t = 1.0$, we have not specifically tested whether the bimolecular behavior in ethanol depends on the presence of both [Zn(II):[12]aneN_3] and [EtO^-:Zn(II):[12]aneN_3], but on the basis of the results in methanol, there is no reason to suggest it does not. The fitting of the Figure 5 data to the expression derived for it in eq 6 where [A] and [B] are [Zn(II):[12]aneN_3] and [EtO^-:Zn(II):[12]aneN_3] (equal concentrations) yields a k_1 of 3.69 $M^{-1}\ s^{-1}$, a K_b of $2.85 \times 10^{-8}\ M^{-2}$, and a k_{cat} of 0.13 s^{-1}. However, the predicted line does not fit the data well at low concentrations of complex due to its apparent dissociation. The actual line shown through the data in Figure 5 is corrected for this dissociation through the application of eq 6a.



(5)

$$k_{obs} = k_{cat}K_b[A][B]/(1 + K_b[A][B]) + k_1[A] \qquad (6)$$

$$k_{obs} = k_{cat}K_b\,[A]^3/(1 + K_b[A]^3) + k_1[A] \qquad (6a)$$

Discussion

a. General Considerations. The accepted general process for metal-ion-catalyzed processes of the sort we consider here involves pre-equilibrium binding with the substrate, followed by a reaction of the complex as shown in eq 7. Whether the metal ion is complexed by ligands or bears an associated lyate, or whether the substrate is neutral or anionic, these appear to be just the sort of processes that are expected to experience large rate accelerations in media of reduced dielectric constant. According to the Debye−Hückel model for association of spherical ions in a medium of dielectric constant D_r, the electrostatic potential energy of attraction for oppositely charged ions is

$$\text{Lig:M}^{X+} + \text{Subst.} \overset{K_b}{\rightleftharpoons} \text{Lig:M}^{X+}\text{:Subst.} \xrightarrow{k_{cat}} \text{Lig:M}^{X+} + \text{P} \qquad (7)$$

$$\text{P.E.} = (z_+e)(z_-e)/(4\pi D_0 D_r r) \qquad (8)$$

where r is the distance between the centers of the ions, z_+e and z_-e are their charges in coulombs (e being the proton charge), D_0 is the permittivity of a vacuum, and r is the separation of the ions.[26] A change from water ($D_r = 78$) to methanol or ethanol (D_r = 31.5, 24.3, respectively[27]) increases the potential energy of the attraction for oppositely charged ions by respective factors of 2.5 and 3.2. Ignoring specific changes in solvent effects and changes in the entropy of binding, for a hypothetical process of $M^{x+} + A^{y-} \rightleftharpoons M^{x+}{:}A^{y-}$ where the electrostatic binding energy is, for example, 3 kcal/mol in water, all of which is expressed in the free energy of ion binding, the calculated binding constant according to $\log K_b = (-\Delta G_{H_2O} + \Delta G_{ROH})/2.303RT$ increases 1.8×10^3-fold and 8.9×10^6-fold in passing from water to methanol and from water to ethanol, respectively.

Our earlier studies indicated that metal ion catalysis of neutral carboxylate esters[28] and neutral phosphate, phosphorothioate, phosphonate, and phosphonothioate esters[29] is greatly accelerated in methanol solvent relative to water, the main accelerating effects of the solvent change being proposed as increased preassociation M^{X+}/C═O or M^{X+}/P═O binding and a changed activity of the metal-bound methoxide. Larger binding constants could be a prelude to enhanced rates of the subsequent chemical reactions since the ion-paired intermediate complexes now exist long enough for several intracomplex collisions from which a productive chemical reaction could occur. This will particularly be so for a metal-ion-catalyzed reaction where there is charge neutralization of the substrate (as in the reaction of a negatively charged phosphate diester) in the rate-limiting transition state for the reaction.

b. Zn^{2+} in Methanol and Ethanol. The metal alkoxides of Zn^{2+} are generally considered to be oligomeric or polymeric in solution.[30] Our previous work showed that Zn^{2+}, when present in methanol solution at ∼0.1−2 mM concentrations along with 0.1−0.4 equiv of $^{-}OCH_3$ per Zn^{2+}, exists as a monomeric species ($Zn^{2+}(^{-}OCH_3)$) which is in equilibrium with dimers $(Zn^{2+}(^{-}OCH_3))_2$ and $(Zn^{2+}(^{-}OCH_3)_2)_2$.[24] Thus, a plot of k_{obs} for the methanolysis of the neutral and weakly binding **6** vs $[Zn^{2+}]_{total}$ at a $[^{-}OCH_3]/[Zn^{2+}]_{total} = 0.3$ is bowed downward, fitting a square root dependence on $[Zn^{2+}]_{total}$ as expected for a catalytically active monomer in equilibrium with inactive dimers. The Zn^{2+} is not active without some methoxide, and activity maximizes at $[^{-}OCH_3]/[Zn^{2+}]_{total} = 0.1-0.4$, but above a $[^{-}OCH_3]/[Zn^{2+}]_{total} = 1$ the catalytic efficacy drops significantly, probably due to predominant formation of inactive dimers and oligomers of stoichiometry $(Zn^{2+}(^{-}OCH_3)_2)_{2,3,...n}$.

Oligomerization also occurs with Zn^{2+} in ethanol, and the potentiometric titration data[18b] at $[Zn^{2+}]_{total}$ = ∼1 mM are

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(26) Levine, I. N. *Physical Chemistry*, 4th ed.; McGraw-Hill, Inc.: New York, 1978; pp 276−281.

(27) Harned, H. S.; Owen, B. B. *The Physical Chemistry of Electrolytic Solutions*; ACS Monograph Series 137, 3rd ed.; Reinhold Publishing: New York, 1957; p 161.

(28) (a) Brown, R. S.; Neverov, A. A. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1039. (b) Brown, R. S.; Neverov, A. A.; Tsang, J. S. W.; Gibson, G. T. T.; Montoya-Pelaez, P. J. *Can. J. Chem.* **2004**, *82*, 1791. (c) Neverov, A. A.; McDonald, T.; Gibson, G.; Brown, R. S. *Can. J. Chem.* **2001**, *79*, 1704. (d) Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2001**, *40*, 3588. (e) Neverov, A. A.; Montoya-Pelaez, P. J.; Brown, R. S. *J. Am. Chem. Soc.* **2001**, *123*, 210. (f) Neverov, A. A.; Sunderland, N. E.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 65.

(29) (a) Tsang, J. S.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 7602. (b) Tsang, J. S. W.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2004**, *2*, 3457. (c) Liu, T.; Neverov, A. A.; Tsang, J. S. W.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 1525. (d) Lu, Z.-L.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 3379. (e) Lewis, R. E.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 4082. (f) Maxwell, C.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 4329. (g) Melnychuk, S. A.; Neverov, A. A.; Brown, R. S. *Angew. Chem., Int. Ed.* **2006**, *45*, 1767.

(30) (a) Tesmer, M.; Müller, B.; Vahrenkamp, H. *J. Chem. Soc., Chem. Commun*. **1997**, 721. (b) Mehotra, R. C.; Singh, A.; Tripathi, U. M. *Chem. Rev.* **1991**, *91*, 1287. (c) Caulton, K. G.; Hubert-Pfalzgraf, L. G. *Chem. Rev.* **1990**, *90*, 969.

satisfactorily fit by a minimal model that comprises $Zn^{2+}{}_3(^-OCH_3)_2$, $Zn^{2+}{}_3(^-OCH_3)_5$, and $Zn^{2+}{}_4(^-OEt)_8$. A recent kinetic study in our lab of the Zn^{2+}-catalyzed ethanolysis of paraoxon (diethyl *p*-nitrophenyl phosphate) in the presence of varying Zn^{2+} with a constant $[^-OEt]/[Zn^{2+}]$ ratio of 0.25 gives a log/log plot of k_{obs} vs $[Zn^{2+}]$ having a gradient of ~0.3, consistent with the presence of inactive trimeric Zn^{2+} species in equilibrium with an active monomer $Zn^{2+}(^-OEt)$.[31]

c. Reaction of 1 with Zn^{2+} in the Absence of Ligands. (i) In water, Zn^{2+} catalysis of the cyclization of **1** is poor relative to the background reaction and proceeds with an apparent second-order rate constant of 9.5×10^{-3} M^{-1} s^{-1} at a pH of 7, with no evidence for the involvement of higher-order terms in metal ion or substrate and no evidence for substrate:metal ion binding.[15] In water, the metal ion and the substrate are sufficiently well solvated that binding of the sort we see in alcohol is not observed. There is evidence presented here that phosphate diesters including **1** and **7** are completely bound to Zn^{2+} in ethanol above a $[Zn^{2+}]_t$ of $\sim 1 \times 10^{-4}$ M. Hyperquad analysis of the potentiometric titration data from a solution containing 1 mM each of **7**, Zn^{2+}, and $HClO_4$ provides a dissociation constant (K_d) for the 1:1 (Zn^{2+}:**7**) complex of $(5.9 \pm 0.6) \times 10^{-6}$ M. We take this value as being an estimate of that of the 1:1 (Zn^{2+}:**1**) complex for which we have a tentative kinetic binding constant of $(6.2 \pm 0.1) \times 10^{-5}$ M from the data in Figure 1a, although we cannot determine a thermodynamic binding constant since the metal-catalyzed reaction is too fast. The apparent dissociation constant value determined for a complex formed between bis(*p*-nitrophenyl) phosphate anion (**8**) and $[Zn^{2+}]$ or Zn^{2+} in the presence of 0.5 equiv of NaOEt in ethanol, termed here as $[Zn^{2+}$:0.5(NaOEt)], was spectrophotometrically determined at 260 nm as $K_d = (3.98 \pm 0.05) \times 10^{-6}$ and $(1.12 \pm 0.04) \times 10^{-5}$ M, respectively. These values are sufficiently close to that determined from the potentiometric titration of (Zn^{2+}:**7**) that one can say that under the conditions chosen for determining the kinetics of cyclization, namely, in the presence of excess catalyst, the **1** will be substantially or completely bound as a 1:1 complex at $[Zn^{2+}]_t > 5 \times 10^{-5}$ M.

(ii) Figure 1 depict plots of k_{obs} vs $[Zn^{2+}$:0.5(NaOR)] for the cyclization of **1** in ethanol and methanol where the respective ${}^{s}_{s}pH$ values are ~7[18b] and 9.5[18a] at $[Zn^{2+}]_t$ = ~1−2 mM. Although one must be mindful that Zn^{2+} ions in alcohols are aggregated and thus not fully available for binding with weak substrates, for stronger binding substrates such as the ones dealt with here, the k_{obs} vs $[Zn^{2+}]_{total}$ data at $[^-OEt]/[Zn^{2+}] = 0.5$ does adhere satisfactorily to a standard one-site strong binding model eq 1. Both plots are analyzed in this way to give conditional k_{max} (K_d) constants in methanol of 3.5×10^{-3} s^{-1} (4.2×10^{-4} M) and in ethanol of 1.9×10^{-2} s^{-1} (6.2×10^{-5} M), indicating that not only is the binding stronger in ethanol but the rate constant when fully bound is larger than is the case in methanol even though the ${}^{s}_{s}pH$ in ethanol is over 2 units lower. However, the ethanol process is more complex than is the case in methanol because there is evidence for a higher than first-order dependence on **1** as is demonstrated by the data presented in Figure 2. For example, if we determine the k_{obs} for the reaction starting with an initial [**1**] = $[Zn^{2+}$:0.5(NaOEt)] = 0.4 mM and then increase the concentration of each keeping the [**1**]/$[Zn^{2+}$:0.5(NaOEt)] ratio at unity, there is additional saturation shown in Figure 3 indicative of a process where two (Zn^{2+}:**1)** complexes come together to form a kinetically active dimeric species (Zn^{2+}:**1**$)_2$. This behavior is not seen in analogous experiments with **1** and Zn^{2+}:0.5(NaOMe) in methanol, nor is it reported in water,[15] suggesting that the kinetic experiments in those solvents do not involve spontaneously formed dimers at least at the concentrations employed.

The behavior in ethanol with Zn^{2+} is reminiscent of that seen for La^{3+}-bound HPNPP cyclization in methanol[17] where two tightly bound (La^{3+}:**1)** complexes associate with each other to form a (La^{3+}:**1**$)_2$ species. The dimer is then subject to a methoxide-dependent cyclization reaction with a second-order rate constant for cleavage of (La^{3+}:**1**$)_2$ of $k_{cat}{}^{max,OMe} = 1.18 \times 10^8$ M^{-1} s^{-1}. The similarity in the La^{3+}-promoted cleavage of **1** in methanol to what is observed here with Zn^{2+} in ethanol suggests that the reactive dimers are probably doubly activated as **(1**:$Zn^{2+})_2$ as shown in Scheme 2 where the actual catalytic role of the second phosphate in the dimer is to act as a template in stabilizing the dinuclear Zn^{2+} core. If the template role is operative, any phosphate monoanion should be capable of performing this role which is verified by the data in Figure 4 where low concentrations of diphenyl phosphate (from 1×10^{-6} to 2.5×10^{-4} M) exert a profound accelerating effect on the cyclization reaction of (Zn^{2+}:**1)**, the second-order rate constant being 790 M^{-1} s^{-1}, which is far too fast for any general base role.

There are additional considerations that cast some important light on the chemical process proposed in Scheme 2. First, each dimer releases 2 equiv of *p*-nitrophenol in sequential steps, but the kinetics for the appearance of *p*-nitrophenol are strictly first order, so the second ethanolysis must be at least as fast or faster than the first, i.e., $k_{cat}{}^{OEt} \geq k_{cat2}{}^{OEt}$. We envision that the phosphate products of the HPNPP cyclization and opening with ethoxide can also fulfill a template role so that, in principle, the product of the reaction actually serves to catalyze the loss of HPNPP by maintaining the dimeric form of the catalyst. Second, the observed pseudo-first-order rate constant ($k_{cat}{}^{max}$) is 2.9 s^{-1} at ${}^{s}_{s}pH$ 7.1 at 2 mM $[Zn^{2+}$:0.5(NaOEt)]. Strictly speaking, the data do not tell whether the required ^-OEt is an external or internal one, but consideration of the rates excludes an external ^-OEt acting as a base since the observed process is at least 100× faster than that permitted by a diffusion-limited abstraction of the 2-hydroxypropyl hydrogen by free [ethoxide] at the concentrations set at ${}^{s}_{s}pH$ 7.1.[32] The lack of general base assistance to the cyclization of **1** was noted before in processes not nearly as fast as that presented here on the bases of (1) an absence of general catalysis of

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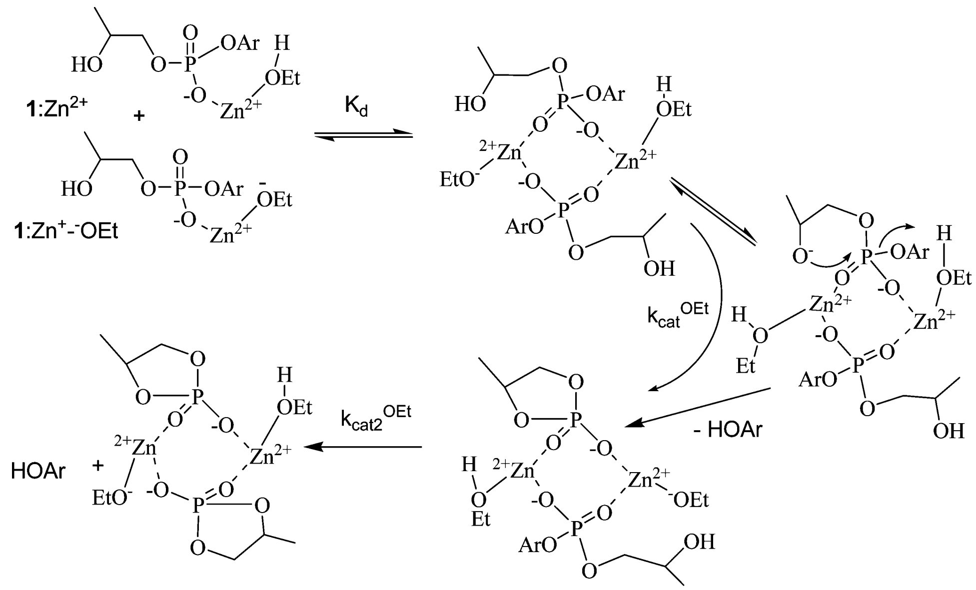
(31) Dong, Y.; Neverov, A. A.; Brown, R. S., unpublished results.

(32) A diffusion-limited proton abstraction would occur at $k_{dif} = 10^{10}$ M^{-1} s^{-1}, so at ${}^{s}_{s}pH$ 7.1 where $[^-OEt] = 10^{-12}$, the maximum pseudo-first-order rate constant would be $k_{cat}{}^{max} = 10^{-2}$ s^{-1}.

Scheme 2 ^{a}



^{a} (OAr = p-nitrophenyl)

cyclization of **1** in the presence of metal ions by the strong piperidine base,[3] (2) the lack of a primary deuterium kinetic isotope effect on the cyclization of **1** when catalyzed by a dinuclear Zn^{2+} complex,[5c] and (3) the absence of buffer catalysis on the cyclization of **1** promoted by a mononuclear Zn^{2+} complex where a specific base-catalyzed process was proposed.[33] Thus, the proposed mechanism in Scheme 2 involves two relatively nonreactive HPNPP:Zn^{2+} complexes (one suggested to have a coordinated ethoxide or the kinetic equivalent of an O-deprotonated hydroxypropyl group and another to have coordinated ethanols) associating to form a reactive (HPNPP:Zn^{2+})$_2$ dimer with a Zn^{2+}-coordinated ethoxide (or its kinetic equivalent) which acts as an internal base to deprotonate the HPNPP. This process would be facilitated by the dimeric nature of the complex where the large positive charge on the Zn^{2+} core stabilizes the anionic deprotonated propanolate[5c] which subsequently cyclizes with the expulsion of p-nitrophenolate. The latter in turn acquires a proton to re-create the $Zn^{2+}-^{-}OEt$ in the nascent complex. The second equivalent of p-nitrophenol is released from the complex in a similar two-step pathway summarized as $k_{cat2}{}^{OEt}$.

It is evident from all the above that the Zn^{2+} catalysis of the reaction of **1** exhibited in ethanol is quite spectacular, and there are several ways to quantitate this. If one simply evaluates the data of Figure 1a to determine an apparent second-order rate constant ($k_2{}^{app}$) from the k_{cat}/K_d values, the value is ~300 M^{-1} s^{-1}. However, from the subsequent presentation it is clear that the great bulk of the reaction occurs from a catalytically active (HPNPP:Zn^{2+})$_2$ dimer which, for the conditions of the data in Figure 1a, is present only to only a minor extent. As such, the $k_2{}^{app}$ number should increase with the concentration of substrate. Correspondingly, if one looks at the data of Figure 3 where the bulk of the substrate is bound to Zn^{2+} as the productive (HPNPP:Zn^{2+})$_2$ dimer, an alternative apparent second-order rate constant of 4.2×10^3 M^{-1} s^{-1} can be calculated from $k_{cat}{}^{max}/K_{dimer}$. The latter number could be compared directly with the second-order rate constant for the ethoxide reaction of 7.5×10^{-3} M^{-1} s^{-1}, showing that the (HPNPP:Zn^{2+})$_2$ dimer is at least 5.6×10^5 times more reactive than ethoxide.

An alternative comparison looks at the accelerations provided for the reaction relative to a background process under the experimental conditions. The $k_{obs}{}^{max}$ from the Figure 3 data (2.9 s^{-1}) computed for cyclization within the dimeric complex can be compared with the rate constant computed for the ethoxide reaction on **1** at $^{s}_{s}$pH 7.1 which is $k_{obs}{}^{OEt} = 7.5 \times 10^{-3}\ M^{-1}\ s^{-1} \times 10^{-12}\ M = 7.5 \times 10^{-15}\ s^{-1}$, meaning the catalytic reaction is accelerated by $\sim 4 \times 10^{14}$-fold! As far as we know, this is the greatest acceleration reported for the cyclization of **1** brought about by any simple catalytic system and attests to the remarkable medium effect provided by ethanol for a metal-catalyzed reaction of a phosphate diester RNA model. In true reductionist[12] terms, this remarkable acceleration is realized without any supporting ligands and depends only on the a spontaneous self-assembly of two metal ions and two substrates brought about by a composite and perhaps complex effect of the reduced polarity/dielectric constant medium.

d. Zn(II)[12]aneN$_3$ in Methanol and Ethanol. The [12]aneN$_3$ ligand binds Zn^{2+} strongly enough in methanol and ethanol so that at the concentrations employed here (0.1−6 mM) the most important species are ROH:Zn(II):[12]aneN$_3$ and its basic form, **9**. This ligand also binds Zn^{2+} strongly in water, although there is no evidence that this complex binds phosphate diesters strongly in water. In fact, Kimura[34] has noted that Zn(II) complexes in general bind phosphate diesters in water poorly, the K_b values being <0.5 M^{-1}. A recent study[35] indicates the reaction of HO^{-}:Zn(II):[12]aneN$_3$ with **1** in water is second-order overall ($k_2{}^{obs}$ =

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(33) Feng, G.; Marque-Rivas, J. C.; Torres, Martín de Rosales, R.; Williams, N. H. *J. Am Chem. Soc.* **2005**, *127*, 13470.

(34) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, *113*, 8935.

0.018 M^{-1} s^{-1}) without evidence of saturation substrate binding between 0.1 and 1 mM in catalyst and without evidence for higher-order terms in complex. However, in methanol other aspects of the reaction with **1** emerge as is evidenced by the upward curving plot of k_{obs} vs [(Zn(II):[12]aneN_3)]$_{total}$ shown in Figure 5 under conditions where the [$^{-}OCH_3$]/[Zn^{2+}]$_t$ = 0.5, at $^{s}_{s}$pH = 9.5. The methanol data are analyzed in terms of an overall process comprising a second-order reaction dependent on [CH_3O^-:Zn(II):[12]aneN_3] and a third-order reaction which is dependent on both [CH_3OH:Zn(II):[12]aneN_3] and the concentration of the basic form [CH_3O^-:Zn(II):[12]aneN_3].[9] Although there is no evidence of a saturation binding of **1** in methanol by either component of the catalyst, there is strong evidence of a medium-induced rate acceleration of at least 2100-fold over that in water since the k_2^{obs} value for the CH_3O^-:Zn(II):[12]aneN_3-promoted cyclization of **1** is 38 M^{-1} s^{-1} (2 × 18.9 M^{-1} s^{-1} accounting for the fact that only half the total [Zn^{2+}] is in the form of the active CH_3O^-:Zn(II):[12]aneN_3).

A change to ethanol brings on yet another facet to the k_{obs} vs [EtO^-:Zn(II):[12]aneN_3] plot as shown in Figure 5, shifting it left, to lower [complex]$_t$ values, clearly showing the third-order process but now with evidence for a saturation binding of **1** as in eq 5. This behavior must be a consequence of stronger binding of the complex to anionic phosphate **1** in ethanol than in methanol since only a linear dependence on [EtO^-:Zn(II):[12]aneN_3] is observed for the ethanolysis reaction of the weakly binding neutral carboxylate ester **6**. When fully bound as **1**:[EtOH:Zn(II):[12]aneN_3]:[EtO^-:Zn(II):[12]aneN_3] or its deprotonated kinetic equivalent $\mathbf{1}^-$:[EtOH:Zn(II):[12]aneN_3]:[EtOH:Zn(II):[12]aneN_3] (no structure implied from the data), the catalytically active ternary complex decomposes with a computed k_{cat} of 0.13 s^{-1} at $^{s}_{s}$pH = 8.3. The acceleration of the cyclization reaction of complexed **1** in ethanol relative to the background ethoxide-promoted reaction (7.5 × 10^{-3} M^{-1} s^{-1}) at this $^{s}_{s}$pH is also spectacular at 1.7 × 10^{12}!

Conclusions

In modeling the chemistry of Zn^{2+}-containing enzymes that promote phosphoryl transfer reactions, numerous studies have employed more or less complicated synthetic complexes to bind one or two metal ion(s) and then investigated the catalysis of the cyclization of simple surrogates for RNA such as the phosphate diester **1**. The great bulk of these studies looked at the phosphoryl transfer reactions in water, or where solubility of the substrate or complex dictates, mixed solvents which are largely aqueous. Despite a great deal of effort which has led to an increased understanding of how enzymatic catalysis might occur, it is generally held that "none of several models so far described approaches the enormous catalytic efficiency of natural enzymes"[35] although it is our contention that our previously published La^{3+} work[17] and more recent study[9] on the cyclization of **1** promoted by di-Zn^{2+} complex **5** in methanol show that a relatively simple system comprising dinuclear metal complexes and a medium effect does give rate accelerations approaching those of enzymes.

In the above study we have shown that a very simple approach of changing the medium from water to methanol and then to ethanol for the cyclization reaction of **1** promoted by Zn^{2+} ion provides the largest accelerations of the cyclization of **1** reported to date without the need for a sophisticated dinucleating ligand. Specifically, a very simple system comprising Zn^{2+}, **1**, and a medium effect imbued by ethanol encourages self-aggregation of four independent particles to generate a (Zn^{2+}:**1**)$_2$ complex within which the cyclization of **1** occurs with a rate constant of 2.9 s^{-1} at $^{s}_{s}$pH 7.1. This is 4 × 10^{14}-fold faster than the background reaction, providing an acceleration within the realm of those achieved by enzymes. There are several features of these Zn^{2+}-catalyzed reactions that become evident on changing the medium from water to methanol and then to ethanol:

1. Due to its heavy solvation of both the anionic substrate and the metal ions and their complexes, water discourages substrate:metal ion binding which is required for catalysis. Dampening this heavy solvation by moving to less polar media with lower dielectric constants confers greater binding and also should favor a kinetic process where the (+) and (−) charges of the substrate and metal ion are somewhat neutralized in the transition state for the phosphoryl transfer process. Whereas it is tempting to suggest that these catalysts derive much of their overall acceleration in alcohol through an apparent retardation of the background lyoxide reaction due to the wider $^{s}_{s}$pH range engendered by the high autoprotolysis constants of the alcohols relative to water, in our opinion, this is not the whole situation. If the stability of the metal:lyoxide complexes (defined as K_{eq} for dissociation of M^{2+}:$^{-}OR \rightleftharpoons M^{2+} + {}^{-}OR$) remains the same in ethanol as it is in water, the $^{s}_{s}$pH at which the catalysts are active would increase as a function of the autoprotolysis constant of the medium. This means that there would be no net increase in the relative rates unless the catalysts were in fact more active in the lower dielectric constant medium. However, the observation is that the alcohol solvents provide a much higher electrostatic stabilization of the $Zn^{2+}-{}^{-}OR$ complexes than does water, so that they are able to operate at lower $^{s}_{s}$pH values. It should be appreciated that the electrostatic stabilization is an important key to providing the high catalysis in alcohol relative to water. Contrary to the situation in alcohols, the bulk of the presently reported metal-containing catalysts[36] in water have catalytic rate constants that are no better than hydroxide itself. In such cases, it must be stressed that all the apparent excess catalytic activity over the background solution stems from the ability to operate at lower pH values where the rate of the background reaction is suppressed.

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(35) Bonfá, L.; Gatos, M.; Mancin, F.; Tecilla, P.; Tonellato, U. *Inorg. Chem.* **2003**, *42*, 3943.

(36) During the revision of this manuscript we became aware of a recent publication (Feng, G.; Natale, D.; Prabaharan, R.; Mareque-Rivas, J. C.; Williams, N. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 7056) where a dinuclear Zn^{2+}-containing catalyst exhibits a k_{cat}/K_M of 53 M^{-1} s^{-1} and a Michaelis−Mentin type reaction of HPNPP providing about 10^6-fold acceleration over the background reaction at pH 7.4.

2. In the case of Zn^{2+} in the absence of any ligands, the change from water to methanol brings on a saturation binding of **1** and a maximal rate constant for decomposition of the (Zn^{2+}:**1**) complex of 3.52×10^{-3} s^{-1} at ${}^{s}_{s}$pH 9.5. Relative to the background methoxide reaction at that ${}^{s}_{s}$pH (2.56×10^{-3} M^{-1} s^{-1})[17], this amounts to an acceleration in the decomposition of **1** of 2.5×10^{7}-fold. In passing to ethanol, a (Zn^{2+}:**1**)$_2$ complex is formed, and its maximal rate constant for decomposition is 2.92 ± 0.06 s^{-1} at ${}^{s}_{s}$pH 7.1, which is 4×10^{14}-fold greater than the background ethoxide reaction at that ${}^{s}_{s}$pH. This indicates that the two metal ion mechanism involving a putative doubly bridged motif for the phosphate:Zn^{2+} binding offers a profound catalytic advantage over the one metal ion mechanism, a fact that has been noted before.[2b]

3. When complexed to the simple cyclic ligand, [12]aneN_3, Zn^{2+} in water still shows no evidence of saturation binding to **1**, and the second-order rate constant for the HO^-:Zn(II):[12]andN_3 reaction is 0.018 M^{-1} s^{-1}, which is about 3× slower than the hydroxide reaction, $k_2^{HO^-} = 0.065$ M^{-1} s^{-1} at 25 °C.[19b] Apparently, the combination of an electrophilic metal ion and an internal hydroxide nucleophile in water is not better than free hydroxide itself, a general phenomenon which has been noted before,[9] but one which must be overcome if any catalytic system is to be effective in water. However, in methanol the CH_3O^-:Zn(II):[12]aneN_3 species is at least 2100-fold better than methoxide in promoting cyclization of **1**. Methanol also brings about a reaction that is second order in complex, and this is also evident in ethanol along with a saturation binding to form a ternary complex **1**:[EtOH:Zn(II):[12]aneN_3]:[EtO^-:Zn(II):[12]aneN_3] that spontaneously decomposes at ${}^{s}_{s}$pH 8.3 with a $k_{cat} = 0.13$ s^{-1}. Relative to the background ethoxide reaction, this constitutes a 1.7×10^{12}-fold acceleration of the decomposition of **1**.

In sum, we believe that the unprecedented acceleration of the HPNPP cyclization afforded by Zn^{2+} in alcohols described above is attributable to a catalytically beneficial medium effect which operates in at least two important ways. First, the lower dielectric constant/polarity seems to recruit clear bifunctional catalysts whose activities, as judged by the second-order rate constants, exceed that of the lyate by a magnitude of 10^3-10^6. Moreover, because of the stabilization of the Zn^{2+}:$^-$OR forms, these catalysts operate in a ${}^{s}_{s}$pH regime which is a manifestation of the greater stabilization of coordinated $^-$OR but higher kinetic reactivity in the lower dielectric constant/polarity medium.

It is a general belief that the active sites of enzymes have effective dielectric constants that are lower than that of water, and it is a venerable hypothesis that this reduction in some way is responsible in part, or in large part, for the exalted catalysis seen in enzymatic systems. The present results tend to support this contention at least in the cases of metal ion catalysis of an RNA model system. It will be of interest to see whether other systems, including those already studied in aqueous media, when investigated in low-polarity media such as the alcohols we have employed here and previously, also exhibit catalysis which is far larger than observed in water.

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