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Structure and Nuclease Activity of Simple Dinuclear Metal Complexes: Quantitative Dissection of the Role of Metal Ions

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Introduction

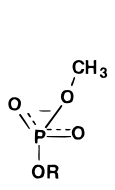
More and more frequently, catalytic systems based around two metal ions are reported in chemistry (e.g., Sharpless epoxidation, Corey's chemzyme) and in biology (e.g., methane monooxygenase,[1] aminopeptidase, urease, phosphoesterases).[2] Over the years, we have been particularly interested in simple dinuclear metal complexes that hydrolyze phosphate diesters. In nature, many enzymes that catalyze phosphate ester hydrolysis are activated by two or more metal ions.[2] These include phosphate monoesterases, diesterases, and triesterases. Enzymes that catalyze the replication of DNA and RNA and ribozymes that catalyze the intermolecular transesterification of RNA are also activated by more than one metal ion. Currently there is considerable interest in understanding the role of the metal ions in these metalloenzymes, and in developing ever more reactive chemical systems that efficiently hydrolyze the phosphate diester bonds of DNA and RNA sequence specifically.[3] Excellent reviews on dinuclear metalloenzymes that catalyze the transfer of phosphoryl groups have appeared recently.[2] In an earlier Account one of us (J.C.) reviewed mononuclear metal complexes that hydrolyze activated or unactivated esters, amides, nitriles, and phosphate esters by a unified mechanism.[4] The principal aim of this Account is to dissect and quantify the modes of activation that metal ions can provide in simple dinuclear metal complexes that hydrolyze phosphate diesters, with particular emphasis on the structures and cooperativity involved.

Stability of Phosphate Diesters

To design metal complexes that hydrolyze phosphate diesters, it is of primary importance to know the reactivity and the hydrolysis mechanism of the metal-free phosphates.

Half-Life of RNA. Over the years, solvent-catalyzed (H_2O, H^+, OH^-) hydrolysis of RNA has been studied in some detail.[5] It is well established that the mechanism for the solvent-catalyzed hydrolysis of RNA involves intramolecular transesterification (by the 2′-OH group) followed by hydrolysis of the cyclic phosphate intermediate. A pH−rate profile for the transesterification of UpU has been reported by Lönnberg's group,[6] showing that at pH 7 hydroxide-catalyzed transesterification dominates over the water- or hydronium-catalyzed transesterifications. Extrapolating the second-order rate constant for hydroxide-catalyzed transesterification to 25 °C gives a value of 2×10^{-3} M^{-1} s^{-1}.[7] So, at pH 7 and 25 °C, the pseudo-first-order rate constant for the cleavage of UpU is expected to be 2×10^{-10} s^{-1} (2×10^{-3} M^{-1} s^{-1} $\times$ 10^{-7} M), which represents a half-life of about 110 years.

Half-Life of DNA. The second-order rate constant for hydroxide-catalyzed hydrolysis of the phosphate diester bond of DNA has never been directly measured. The enormous difference in the reactivity of the phosphate diester bonds of RNA and DNA must be emphasized since it can be tens of millions times underestimated even by the well-respected grandfather of phosphate chemistry.[8] Model studies show that the 2′-OH group in RNA provides about 10^9-fold rate acceleration for cleaving phosphate diesters;[9] i.e., for R = *p*-nitrophenyl, the relative rate constants for hydroxide-catalyzed cleavage of **1**, **2**, and **3**



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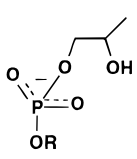

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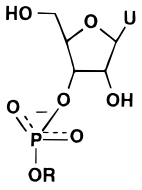

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are $1{:}10^5{:}3 \times 10^9$ at 25 °C. This rate acceleration is similar with good and poor leaving groups, as shown in the Brönsted plots for the hydroxide-catalyzed cleavage of **1**,

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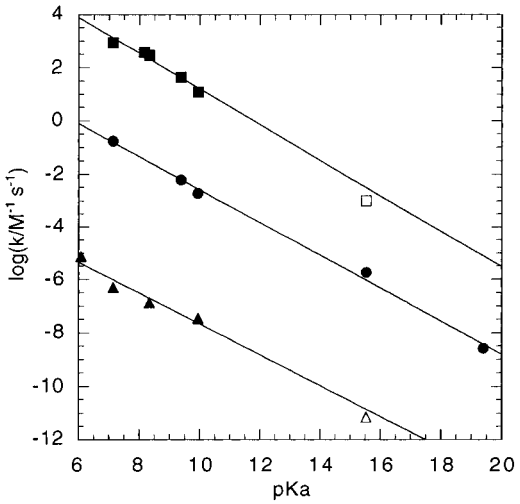


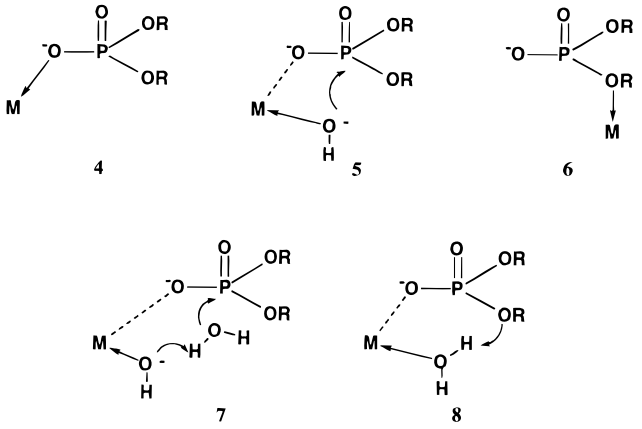
FIGURE 1. Brönsted plot for hydroxide-catalyzed cleavage of **3** (squares), **2** (circles), and **1** (triangles). The rate constants were extrapolated to or determined at 25 °C, and only substituted aryl groups without 2-substituents were used. The open square and the open triangle represent the data for UpU and dimethyl phosphate, respectively (not used in the fits). The best linear fit gave β values of −0.58 (**1**), −0.62 (**2**), and −0.67 (**3**) (intercepts: −1.89 (**1**), 3.62 (**2**), and 7.91 (**3**)).

2, and **3** (Figure 1).[10] The second-order rate constant for hydroxide-catalyzed hydrolysis of dimethyl phosphate (6.8×10^{-12} M^{-1} s^{-1}),[11] extrapolated from high temperatures, is in remarkably good agreement with that obtained from the Brönsted plot (open triangle in Figure 1). Likewise, the reactivity of UpU (discussed above, open square in Figure 1) is in good agreement with that extrapolated from the Brönsted plot.

As **3** is expected to react a few billion times more rapidly than **1** with methoxide as the leaving group, the second-order rate constant for hydroxide-catalyzed hydrolysis of TpT should also be billions of times smaller than that for UpU (2×10^{-3} M^{-1} s^{-1}). The reactivity of the phosphate diester bond in TpT ($k \approx (2 \times 10^{-3})/(3 \times 10^{9}) \approx 10^{-12}$ M^{-1} s^{-1} at 25 °C) should be comparable to that of dimethyl phosphate[10] (6.8×10^{-12} M^{-1} s^{-1}). The half-life of a typical phosphate diester bond in DNA in neutral water at 25 °C is expected to be on the order of tens to hundreds of billions of years[12] assuming that the hydroxide rate dominates the water rate at pH 7 as in RNA cleavage.[6] This half-life is even longer than what is thought to be the age of life on earth (~4 billions years). *To hydrolyze this bond within a few minutes, a catalyst would have to provide over 10^{17}-fold rate acceleration*. As formidable as it may seem to develop such reactive catalysts, it is nevertheless a soluble problem since nature has already found ways to hydrolyze DNA within 1 s using metalloenzymes, and below we consider how metal ions can be used to enhance this reaction.

Dinuclear Transition Metal Complexes

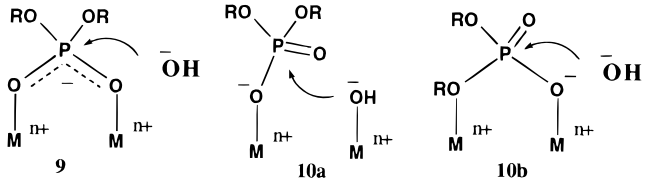
Over the past decade, more than a dozen highly revealing crystal structures of dinuclear and trinuclear metalloenzymes that catalyze phosphoryl transfer reactions have been reported.[2] Furthermore, many elegant dinuclear and trinuclear metal complexes have been developed as structural or functional models of the enzymes.[13] One of the challenging problems of these enzymic and model systems is to elucidate the overall catalytic mechanism and to identify the roles of the metal ions. There are three direct (inner sphere) modes of activation that a metal ion can provide for accelerating the rate of phosphate ester hydrolysis. They are Lewis acid activation (**4**, coordination of phosphoryl oxygen(s) to the metal), nucleophile activation (**5**, coordination of a nucleophile such as a hydroxide to the metal), and leaving group activation[14] (**6**, coordination of the leaving group oxygen to the metal). Additionally there are three indirect (outer sphere) modes of activation that metal ions can provide. Metal-coordinated hydroxides could act as an intramolecular general base catalyst (**7**), and metal-coordinated water molecules as an intramolecular general acid catalyst (**8**). Electrostatic interaction between the metal and uncoordinated phosphate ester may also provide some rate accelerations for the hydrolysis reaction.[15]



In this Account we are mainly concerned with the three direct modes of activation. Since each metal center in di- and trinuclear metal complexes can have multiple roles, it is fundamentally important to be able to dissect quantitatively the rate accelerations observed in terms of the individual modes of activation. Such studies may reveal if the individual modes of activation are more or less than additive.

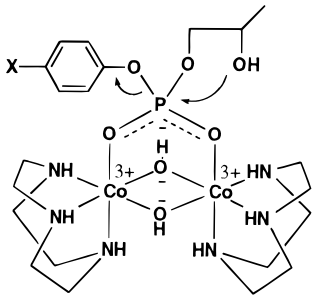
Lewis Acid Activation. It has been proposed and/or shown that phosphates bridge dinuclear metal centers in the active sites of phosphoesterases (e.g., EcoRV endonuclease, mamalian protein phosphatase-1, fructose-1,6-biphosphatase).[2] In principle, dinuclear metal complexes should be able to provide double Lewis acid activation for hydrolyzing phosphates by initially bridging the two metal centers with the two phosphoryl oxygens (**9**). However, it is generally difficult to quantify double Lewis acid activation because the hydrolysis reaction may proceed by a different mechanism involving single Lewis acid activation/metal hydroxide activation (**10a**) or single Lewis acid activation/leaving group activation (**10b**). Mechanisms **9**, **10a**, and **10b** are kinetically indistinguish-

able for substitutionally labile metal complexes as the three species could be in rapid equilibrium prior to hydrolysis.



9 **10a** **10b**

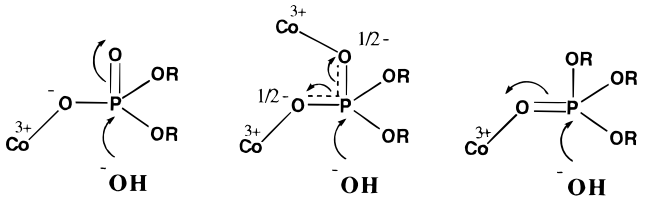
To quantify double Lewis acid activation for cleaving phosphate diesters, we studied the reaction of **11a** which has two substitutionally innert Co(III) centers. The second-order rate constant for hydroxide-catalyzed transesterification of **11a** is 430 ± 20 M^{-1} s^{-1} at 25 °C.[16] By



11a: X = p-NO_2
11b: X = H

comparison, the second-order rate constant for hydroxide-catalyzed transesterification of the free diester in solution is 9.8 × 10^{-4} M^{-1} s^{-1} at 25 °C. Remarkably, double Lewis acid activation in the model system provides about 4 × 10^5-fold rate acceleration for cleaving the diester, fairly independent of the leaving group basicity.[17]

Sargeson's research team showed that single Lewis activation by Co(III) can provide a rate acceleration of about 400-fold for hydrolysis of a phosphate triester.[18] Furthermore, they showed that single Lewis acid activation by Co(III) provides a rate acceleration of about 50-fold for hydrolysis of a coordinated phosphate diester.[19] The rate acceleration due to double Lewis acid activation in **11** is comparable in value to the square of the rate acceleration due to single Lewis acid activation for phosphate triester hydrolysis (400^2) but more than the square of the rate acceleration due to single Lewis acid activation (50^2) for phosphate diester hydrolysis. Hence, there appears to be considerable cooperativity between the two metal centers in double Lewis acid activation for phosphate diester transesterification.[20] The rate acceleration may in part be due to the strain in the O−P−O bond angle as the X-ray structure reveals that the value of the bond angle (117.4°)[21] is considerably larger than the tetrahedral value (109°) and close to what it should be in the trigonal bipyramidal transition state (120°). Additionally, the developing negative charge in the single Lewis acid activated phosphate diester cleavage reaction is not stabilized by direct coordination to the metal ion (**12**) as it is in the double Lewis acid activated diester cleavage (**13**) or in the single Lewis acid activated phosphate triester hydrolysis (**14**).

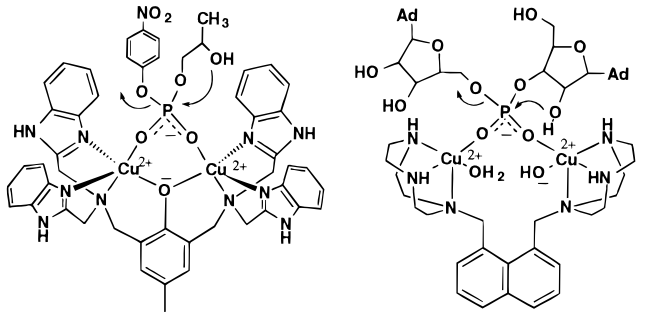


12 **13** **14**

In principle, it should be possible to obtain double Lewis acid activation for hydrolyzing phosphate diesters with mononuclear metal complexes by chelating the phosphate to the metal. Although acetate,[22] inorganic phosphate,[23] and phosphate monoesters[24] have been shown to chelate to Co(III), it has proven difficult to chelate phosphate diesters and weakly basic carboxylates to the metal. However, we presented some kinetic evidence for chelation of a phosphate diester to a Cu(II) center.[25]

As well as the remarkable effects on reactivity, there is significant cooperativity between the two metal centers in the dinuclear Co(III) complex (**11**) for coordinating phosphate diesters. The equilibrium constant for monodentate coordination of dimethyl phosphate to a mononuclear Co(III) complex is only about 4 M^{-1},[26] but is greater than 330 M^{-1} for bridging dimethyl phosphate to the dinuclear Co(III) center in **11**.[21]

Although Co(III) complexes are very useful for quantifying the effects of different types of activation, it is generally difficult to obtain turnover because they are substitutionally inert, so we have extended our studies with dinuclear Cu(II) complexes (**15** and **16**), which are



15 **16**

substitutionally labile. X-ray crystallographic studies reveal that a phosphate can bridge the two metal centers in **15** and **16** as it does in **11**.[27] The intermetal distances in **11′**, **15′**, and **16′** are 2.9,[21] 3.6,[28] and 5.0 Å,[29] respectively (Figure 2). Intermetal distances in dinuclear metalloenzymes that process phosphate esters also range between about 3 and 5 Å.[2] The dinuclear metal centers in **15** and **16** provide 5−6 orders of magnitude rate acceleration for the transesterification of 2-hydroxypropyl *p*-nitrophenyl phosphate (**2**) and RNA. On the basis of the results of the studies on the cobalt complex (**11**) and the crystal structures and kinetic studies of the copper complexes (**15′** and **16′**, Figure 2), we propose that the above rate accelerations for the transesterification reactions are at

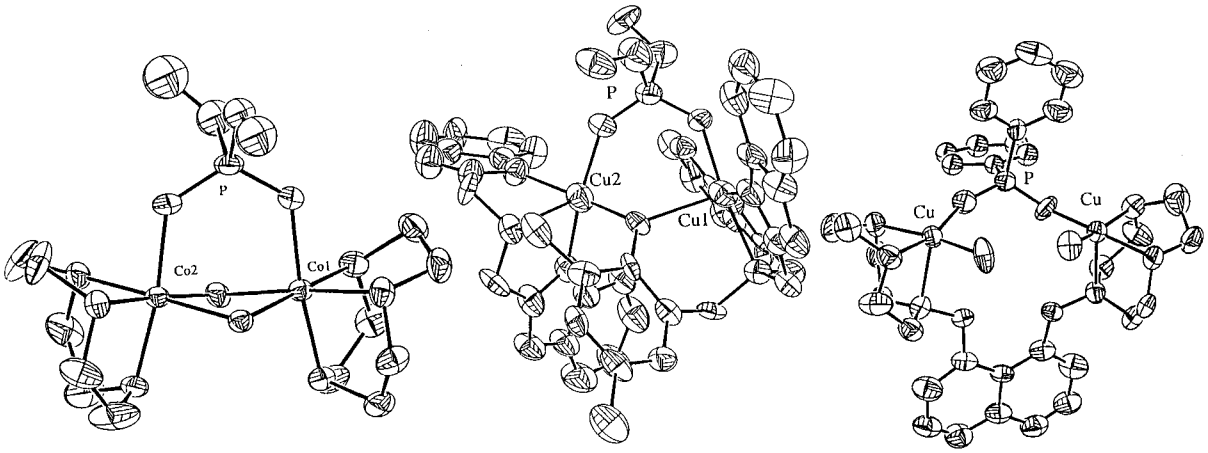
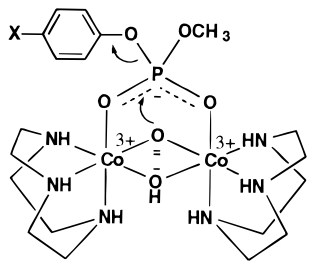


FIGURE 2. ORTEP diagrams of **11′**, **15′**, and **16′** from left to right. The phosphates in **11**, **15**, and **16** have been replaced with dimethyl phosphate, dibenzyl phosphate, and diphenyl phosphinate in **11′**, **15′**, and **16′**, respectively. For compound **16′**, there are two bridging phosphinates in the original structure. The one in the front has been omitted here for clarity of view.

least in part due to double Lewis acid activation. In addition, the metal hydroxide in **16** may act as an intramolecular general base catalyst for cleaving RNA (as in **7**).[30]

Double Lewis acid activation is expected to provide comparable rate accelerations for cleaving RNA and DNA. However, this mode of activation by itself will have a more significant effect on efficient cleavage of RNA. DNA would require 9 additional orders of magnitude rate acceleration to be as reactive as RNA (Figure 1).

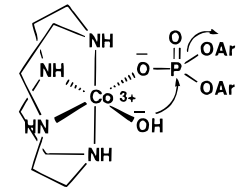
Nucleophile Activation. It is well-known that metal hydroxides and metal alkoxides can function as effective nucleophiles for cleaving phophates or amides. Recently, it has been suggested that hydroxide coordinated to two metal ions in aminopeptidases and phosphatases may function as nucleophiles.[2] However, it is difficult to obtain evidence for such a mechanism in enzyme-catalyzed reactions. The slow ligand exchange rate of Co(III) complexes makes them ideal not only for quantifying Lewis acid activation, but also for studying reaction mechanisms in detail. Thus, we could use ^{18}O isotope labeling studies to show that the bridging oxide in **17** acts as a very



17a: X = m-F, p-NO_2
17b: X = p-NO_2
17c: X = m-NO_2
17d: X = H

effective nucleophilic catalyst in hydrolyzing the bridging phosphate diester.[17,21] Hydroxide when coordinated to one metal ion is expected to be much more nucleophilic than when coordinated to two metal ions. On the other hand, the second metal ion can help generate the nucleophilic oxide by lowering the pK_a of the bridging hydroxide.[31] Overall, double Lewis acid activation (including possible strain induced as mentioned previously) in combination with the "bridging oxide" activation gives about 11 orders of magnitude rate acceleration for hydrolyzing the phosphate diester in **17b**.[21] Interestingly, the active site structure of kidney bean purple acid phosphatase[2] is remarkably similar to the dinuclear center in **17**.

Detailed mechanistic analysis on the reactions of **11** and **17** show that the above-mentioned oxide activation provides large rate accelerations for hydrolyzing phosphate diesters with good leaving groups but not for hydrolyzing those with poor leaving groups.[17] In contrast, metal hydroxides provide comparable rate acceleration for cleaving phosphate diesters with good (**18**)[26] or poor (**19**) leaving groups.[32] In another study,[33] we have shown that a metal alkoxide (**21**) is more reactive than the corresponding metal hydroxide (**20**) for cleaving phosphate diesters with very good leaving groups but the order of reactivity is reversed for cleaving phosphate diesters with poorer leaving groups.



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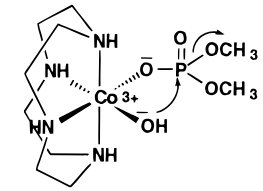

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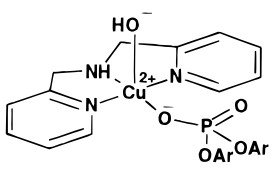

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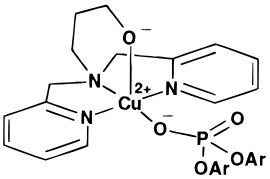

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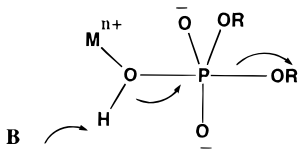
The studies of these three nucleophiles (metal bridging

oxide, metal hydroxide, and metal alkoxide) indicate that, for cleaving phosphates with poor leaving groups, a deprotonatable nucleophile (such as a metal hydroxide but not metal alkoxide or metal bridging oxide) is required. We suggest that the expulsion of poor leaving groups (as in RNA and DNA hydrolysis without leaving group activation) is accompanied by deprotonation of the metal hydroxide (**22**). This deprotonation may be coupled to protonation of the leaving group oxygen.



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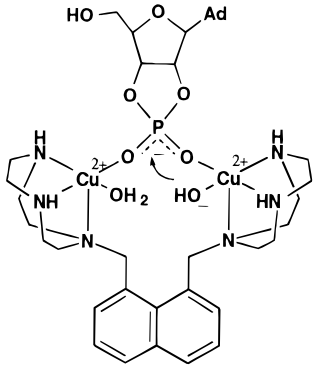
The dinuclear Cu(II) complex that provides 5 orders of magnitude rate acceleration for cleaving ApA gives 8 orders of magnitude rate acceleration for hydrolyzing 2′,3′-cAMP.[29] We proposed that this additional rate acceleration may be due to the intramolecular metal hydroxide nucleophile in addition to the double Lewis acid activation (**23**). Single Lewis acid activation in combination with



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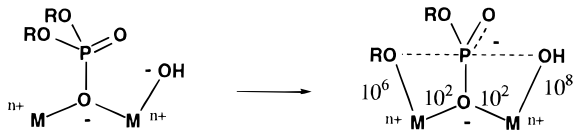
metal hydroxide activation can provide up to 10 orders of magnitude rate acceleration for hydrolyzing phosphates with good[26] or poor leaving groups.[32] The relatively modest additional rate acceleration brought about by the metal hydroxide (i.e., 3 orders of magnitude) in **23** may be due to the poor positioning of the nucleophile imposed by binding the phosphate to the two metal centers. It may also appear modest because the metal hydroxide could be providing general base catalysis for the intramolecular transesterification of ApA (**16**).[30]

It should be noted that nucleophile activation is very important for DNA hydrolysis, but not for RNA hydrolysis. It would be difficult to design a catalyst which has a nucleophile that can compete effectively with the 2′-OH group of RNA as this group provides over 9 orders of magnitude rate acceleration for displacing the leaving group (Figure 1). However, the metal-activated nucleophile could act as an intramolecular general base and assist in the nucleophilic attack of the 2′-OH group (as in **7**).[30]

Leaving Group Activation. Although it is difficult to coordinate a leaving group oxygen of a phosphate ester to a metal ion, it could greatly accelerate the rate of the hydrolysis reaction. The Brönsted plot in Figure 1 shows that the rate of hydrolysis of phosphate diesters is fairly sensitive to the pK_a of the leaving alcohol ($\beta_{lg} \approx -0.6$), and as the pK_a value of water or an alcohol may drop by 10 units upon coordination to a metal complex (e.g., Co(III), Cu(II), Ln(III)), about 6 orders of magnitude rate acceleration (0.6×10) can be expected from leaving group activation, discounting steric or electrostatic effects. In alkaline phosphatase catalyzed hydrolysis of phosphate monoesters, it has been suggested that the leaving group oxygen coordinates to the Mg(II) ion at the active site.[2] In simple systems, it would be difficult to directly detect metal ions bound to the leaving group ester-like oxygen, which has an effective charge of +0.74 in phosphate diesters.[34]

Additivity of Types of Activation. If the logarithm of rate accelerations from Lewis acid activation (**4**, 10^2-fold), nucleophile activation (**5**, 10^8-fold), and leaving group activation (**6**, 10^6-fold) are additive, combining all three could give up to 16 orders of magnitude rate acceleration for phosphate diester hydrolysis. This would decrease the half-life of DNA phosphate diester bonds from tens of billions of years to about 10 min at pH 7 and 25 °C. In some cases these types of activation are expected to be more than additive while in other cases they may be less than additive. We have already seen that the two Lewis acid activations in **11** may be more than additive. In contrast, mononuclear *cis*-diaqua-Co(III) complexes provide comparable rate accelerations for hydrolyzing bis(*p*-nitrophenyl) phosphate[26] and dimethyl phosphate,[32] so in this case the logarithm of the rate acceleration from leaving group activation should be additive to that from Lewis acid and metal hydroxide activation. However, not all types of nucleophile activation are expected to be additive with leaving group activation. As already mentioned, metal alkoxides (**21**) and metal-bridging oxides (**17**) provide large rate acceleration for cleaving phosphates with good leaving groups but not for cleaving those with poorer leaving groups. Coordination of a metal to the poor leaving group oxygen should lower the basicity of the leaving group and make two types of nucleophiles (**17**, **21**) highly reactive again. Hence, for these two systems, a synergistic effect between nucleophile activation and leaving group activation is anticipated.

Quantitative dissection of the role of metal ions in simple compounds may give valuable insights into how much rate acceleration can be obtained from metal ions in phosphodiesterases. On the basis of their crystallographic data, Steitz and Beese proposed an interesting mechanism for 3′,5′-exonuclease-catalyzed hydrolysis of DNA.[2] Essentially the same mechanism has been proposed for several polymerase-catalyzed syntheses of DNA.[35] Their mechanism includes two Lewis acid activations, one nucleophile activation, and one leaving group activation (**24**). The above analysis indicates that such four-point



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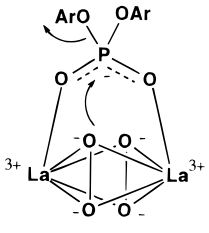
contact between the metal ions and the transition state could give up to 18 orders of magnitude rate acceleration for the reaction if the individual rate accelerations are multiplicative (leaving group activation (10^6) × two Lewis acid activations ($10^2 \times 10^2$) × nucleophile activation (10^8)). In would be interesting to develop a simple model system based on this proposed enzyme mechanism.[36]

Dinuclear Lanthanides

It has been known for many decades that lanthanides as monomers, dimers, or gels are highly reactive for hydrolyzing phosphates.[37] The mechanisms of dinuclear or higher order lanthanides for hydrolyzing phosphates are more difficult to solve than those of dinuclear transition metal complexes. Part of the problem is that ligand-free dinuclear or higher order lanthanides tend to be the most reactive but such species are difficult to solubilize let alone crystallize and characterize. Our results described above for transition metal complexes can be combined with our studies on lanthanide-promoted hydrolysis of phosphate diesters to lead to a general proposal for how these highly reactive species may be working by a unified mechanism.

Bridging Peroxides and Activated Phosphate Diester Hydrolysis. There is enormous cooperativity between hydrogen peroxide and Ln(III) ions for hydrolyzing activated phosphate diesters.[38] On the basis of kinetic and thermodynamic experiments, we proposed that the simplest active form of the Ln(III) complex for hydrolyzing bis(*p*-nitrophenyl) phosphate is a dinuclear species with two bridging peroxides (**25**). Interestingly, a crystal structure of a dinuclear Ce(IV) complex with two bridging peroxides has been reported (**26**),[39] with strong similariy to the proposed diamond core structure of the La(III)/H_2O_2 complex (**25**).



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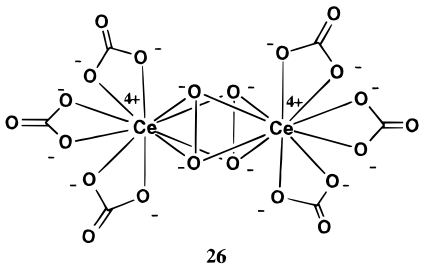


26

^{18}O isotope labeling studies show that one of the bridging peroxides in **25** acts as a nucleophilic catalyst in hydrolyzing the phosphate diester. We proposed that the mechanism of the hydrolysis reaction may involve nucleophilic attack of the bridging peroxide on the bridging phosphate diester (**25**)[38a] much like in the dinuclear Co(III) complex where the bridging oxide attacks the bridging phosphate diester (**17**).[21] As with the bridging oxide (**17**) and the metal alkoxide (**21**), the bridging peroxide provides much greater rate acceleration for hydrolyzing phosphates with good leaving groups than for hydrolyzing those with poor leaving groups. Figure 3 shows the crystal structure of a portion of **27** demonstrating that phosphate diesters can bridge two Ln(III) centers (**27**) as they bridge two alkaline earth or transition metal centers.[40] The intermetal distance in **27** (7 Å) is significantly greater than those in **11′**, **15′**, and **16′**.

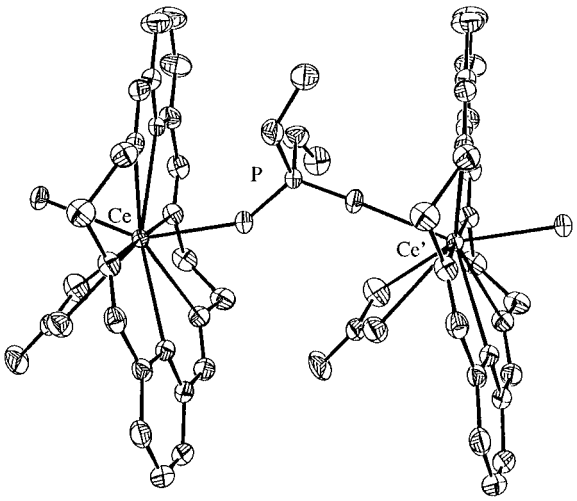
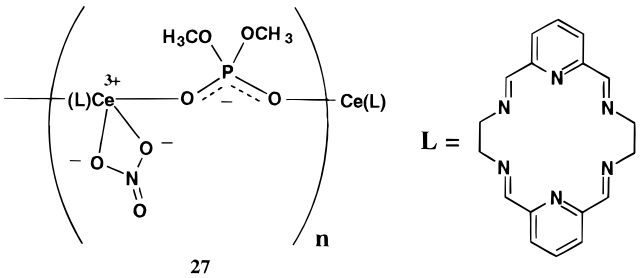
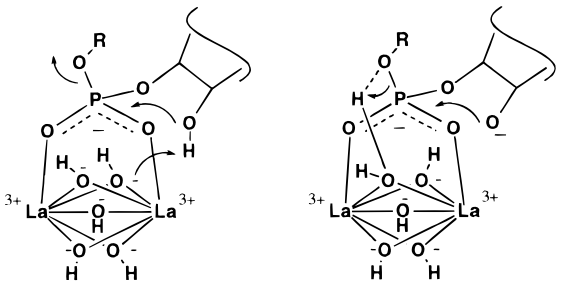


FIGURE 3. ORTEP diagram of a portion of **27**.



27

Bridging Hydroxides and RNA Hydrolysis. There is also enormous cooperativity between hydroxide and Ln(III) ions for hydrolyzing RNA.[41] The hydrolysis rate is second-order in Ln(III) concentration, indicating that a dinuclear Ln(III) complex may be the active species. Furthermore, the hydrolysis rate increases sharply with an increase in pH (fifth-order in hydroxide) but levels off at around pH 9. Here, the half-life of the phosphate diester bond is only about 13 s at 25 °C. Outside the realm of enzymes, this represents the most reactive system known for hydrolyzing RNA. It appears that the two bridging peroxides in **25** can be replaced with five bridging hydroxides to form a highly reactive species for hydrolyzing RNA (**28**).[42] The products of the hydrolysis reaction are adenosine, 2′-AMP, and 3′-AMP, indicating that the 2′-OH group is the initial nucleophile involved, forming 2′,3′-cAMP as an intermediate. Thus, we propose that **28** or its kinetic equivalent (**29**) is the active species for the hydrolysis reaction.



28 **29**

Cerium(IV) and DNA Hydrolysis. Cerium, the only lanthanide that is easily oxidizable to the tetravalent state, is the only lanthanide that hydrolyzes DNA at a reasonable rate.[43] Detailed mechanistic studies have not been possible due to solubility problems with Ce(IV) above pH 4. However, on the basis of studies on other lanthanides and the detailed mechanistic evidence available from the cobalt complexes, it is tempting to suggest that it is dinuclear Ce(IV) with bridging peroxides, hydroxides, or oxides that is the reactive motif required for hydrolyzing DNA.

Unified Mechanism. The mechanisms for hydrolyzing RNA or RNA models with substitutionally inert dinuclear Co(III), labile dinuclear Cu(II), and labile Ln(III) complexes appear to be closely related (**11**, **15**, **16**, and **28** or **29**). Similarities in mechanisms are also apparent for hydrolyzing DNA models with dinuclear metal complexes (**17**, **23,** and **25**). While double Lewis acid activation alone is enough to rapidly hyrolyze RNA which already has a highly efficient internal nucleophile (2′-OH group), DNA hydrolysis requires nucleophile activation in addition to double Lewis acid activation.

Summary

Enormous rate accelerations are necessary to hydrolyze RNA (10^8-fold) or DNA (10^{17}-fold) within minutes. There are three direct modes of activation that metal ions can provide for hydrolyzing phosphate diesters. The rate accelerations due to Lewis acid activation ($<10^2$-fold), intramolecular nucleophile activation (10^8-fold), and leaving group activation (10^6-fold) may in some cases combine simply to give an overall rate acceleration in excess of 10^{16}-fold; in other cases, greater cooperativity between the modes of catalysis is possible.

Double Lewis acid activation obtained by coordinating both phosphoryl oxygens of a phosphate diester to a variety of dinuclear metal complexes (Figures 2 and 3) can give far greater rate accelerations for the hydrolysis reaction (4×10^5-fold for a dinuclear Co(III) complex) than single Lewis acid activation ($<10^2$). This mode of activation is possible with an intermetal distance from 2.9 to 7.0 Å and is particularly useful for cleaving RNA efficiently. For DNA hydrolysis, double Lewis acid activation by itself is not enough for efficient cleavage as it is about 10^9 times more stable than RNA.

The nucleophile activation (metal hydroxide, metal alkoxide, metal-bridging oxide, metal-bridging peroxide) is important for DNA hydrolysis but not for RNA cleavage as its 2′-OH group already acts as a highly efficient internal nucleophile (Figure 1). However, the metal-activated nucleophiles may in some cases act as general base catalysts in cleaving RNA.[30] While all of the above-mentioned nucleophiles can cleave phosphates with good leaving groups, only metal hydroxides appear to cleave those with poor leaving groups. For the other nucleophiles to be effective (metal alkoxide, metal-bridging oxide, metal-bridging peroxide), leaving group activation (coordination of the poor leaving group oxygen to the metal) is required.

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