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Janet R. Morrow[a]
[a] Department of Chemistry, University at Buffalo, State University of New York, Buffalo, New York, USA

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SPEED LIMITS FOR ARTIFICIAL RIBONUCLEASES

JANET R. MORROW

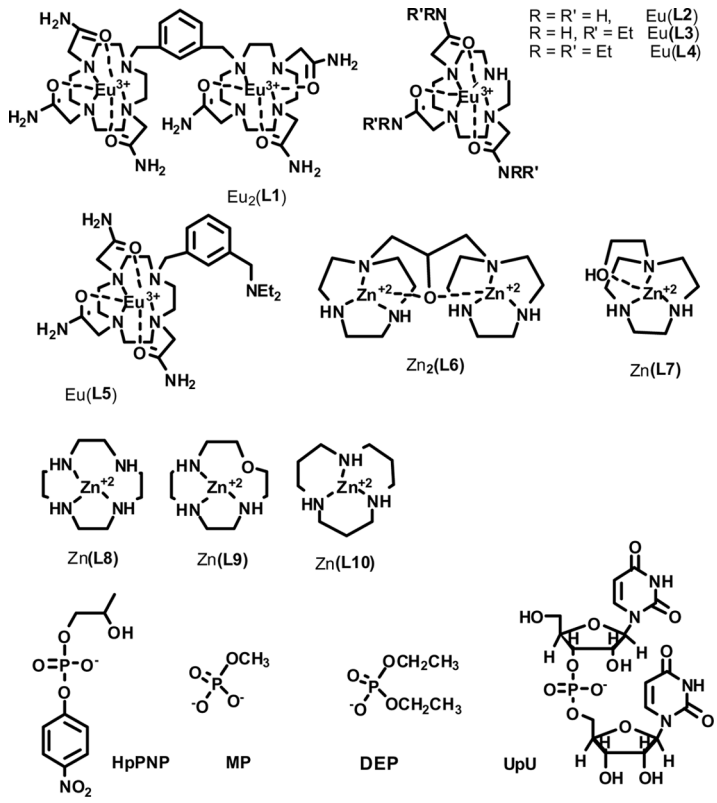
Department of Chemistry, University at Buffalo, State University of New York, Buffalo, New York, USA

There are four major catalytic roles for natural and artificial nucleases that catalyze the hydrolytic cleavage of RNA. For metal ion complexes that act as artificial nucleases, the most important role is the stabilization of the phosphorane-like transition state. In keeping with this role the best catalysts, including Ln(III) complexes and dinuclear Zn(II) complexes, interact strongly with dianionic phosphate esters as crude transition state models. Two other catalytic modes, alignment of the hydroxyl nucleophile for in-line attack and activation of the 2′-hydroxyl, are not important for metal ion catalyzed cleavage of simple RNA analogs. This may impose a speed limit for metal ion catalysis, although additional catalytic roles may be operative in the cleavage of structured RNA.

INTRODUCTION

The development of metal ion catalysts for the hydrolytic cleavage of RNA as artificial nucleases has been a popular area of study for a number of years.[1–4] As an example, several of the metal ion complex catalysts we have developed for cleavage of RNA are shown in Scheme 1. The high level of efficiency of these complexes is unusual for small molecule catalysts that function in water and has attracted efforts to tease out their mode of catalysis.[5] Interest in this topic derives from the numerous applications of metal ion promoted RNA cleavage that range

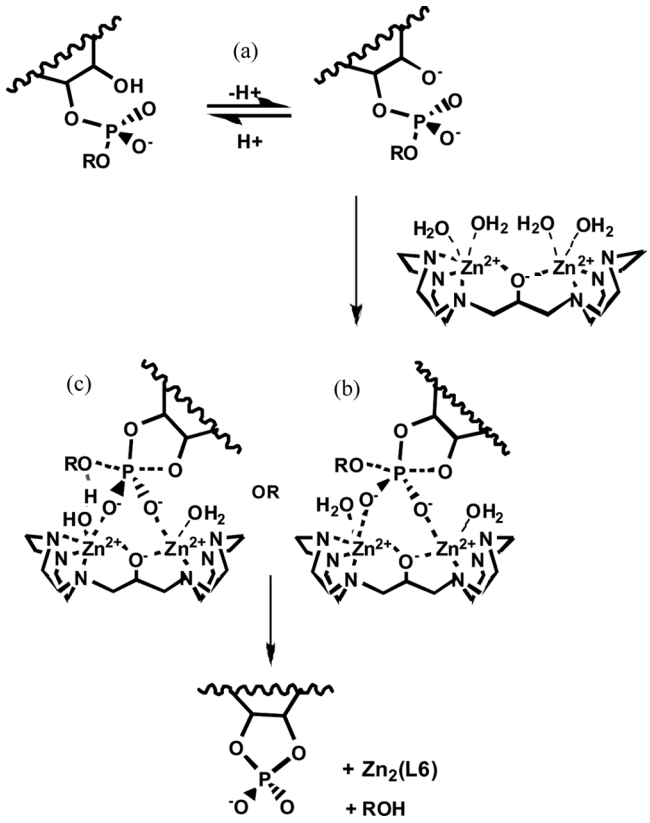
Address correspondence to Janet Morrow, 526 Natural Sciences Complex, Department of Chemistry, University at Buffalo, Buffalo, NY 14260-3000, USA. E-mail: jmorrow@buffalo.edu



Scheme 1. Catalysts and RNA analogs.

from the development of agents for the selective suppression of gene expression[6] to the design of in-vitro selected RNA cleaving DNAzymes for the detection of metal ions.[7,8]

Cleavage of RNA is facilitated by the proximity of the 2′-hydroxyl to the phosphate diester (Scheme 2). In the absence of catalyst at neutral pH and physiological temperatures, RNA phosphate diester bonds have a half-life of over 100 years at neutral pH and 23°C, with small variations that depend on RNA base sequence and structure.[9] This half-life is reduced to seconds by ribozymes, microseconds by ribonucleases,[10] or minutes by simple metal ion complexes.[3] Much discussion has focused on the mechanism of RNA or protein based enzyme catalyzed cleavage and whether there are certain associated "speed limits" that



Scheme 2. Proposed mechanism for RNA cleavage by a dinuclear Zn(II) catalyst.

depend on catalytic modes of cleavage.[10] By contrast, there is more limited mechanistic work on metal ion complex catalyzed cleavage of RNA. Such mechanistic information is critical to the design of more potent catalysts.[2,11,12]

In this article, I will summarize our recent work on metal ion catalysts for RNA cleavage and present our view of the mechanism of cleavage by these catalysts. An additional challenge is to promote the structure-specific cleavage of RNA through synthetic variations of ligands and recognition groups. A brief description of ongoing work on specific cleavage of RNA by small molecules catalysts in my laboratory will be given.

PROPOSED MECHANISM OF RNA CLEAVAGE BY METAL ION COMPLEXES

There are four major catalytic strategies by which metal ion complexes may promote RNA cleavage by phosphate ester transesterification, also termed "hydrolytic cleavage" of RNA (Scheme 2). Estimations of rate enhancements associated with these modes have been given.[3,13,14] These catalytic modes include: a) deprotonation of the 2′-hydroxyl to give a better nucleophile ($\approx 10^6$ RE (rate enhancement)); b) interaction with the non-bridging oxygen of the phosphate diester and stabilization of the phosphorane-like transition state ($\approx 10^5$ RE); and c) interaction with the leaving group ($\approx 10^6$ RE) (Scheme 1). A fourth catalytic mode utilized by RNA and protein based enzymes is to promote optimal alignment for in-line attack of the 2′-hydroxyl on the phosphate ester and has been estimated to give up to 100-fold enhancement.[10] This mode is important for structured RNA but not simple RNA analogs and will be discussed in the last section on cleavage of structured RNA.

An important step toward the design of better catalysts is to understand the relative importance of the different roles of the metal ion. As pointed out by Breaker, the best of nature's catalysts use a combination of catalytic strategies for RNA cleavage.[10,15] Estimates for the rate enhancement from each catalytic mode are useful to assess the benefits that might accrue if each catalytic mode was optimized.

ACTIVATION OF THE 2′-HYDROXYL GROUP (A)

All mechanistic schemes must account for a pH-rate profile that has a second-order rate constant (first-order in RNA and catalyst) that increases in a linear fashion with pH and shows a break at the $pH = pK_a$ of the metal ion water molecule,[16] as shown in Figure 1 for a series of mononuclear Zn(II) complexes.[17] Data is shown for the simple substrate HpPNP, but similar pH-rate profiles are observed for cleavage of RNA analogs with poor leaving groups such as UpU.[18] This type of pH dependence signifies the loss of a proton on going from ground to transition state but does not identify the origin of the proton. One option is that the proton is lost from the metal water ligand and the metal hydroxide complex that is formed acts as a general base in cleavage. This option is congruent with the fact that the pK_a of the metal complex water ligand , as measured by pH-potentiometric titrations, is identical within error to the pK_a obtained in the kinetic studies. However, this is not the

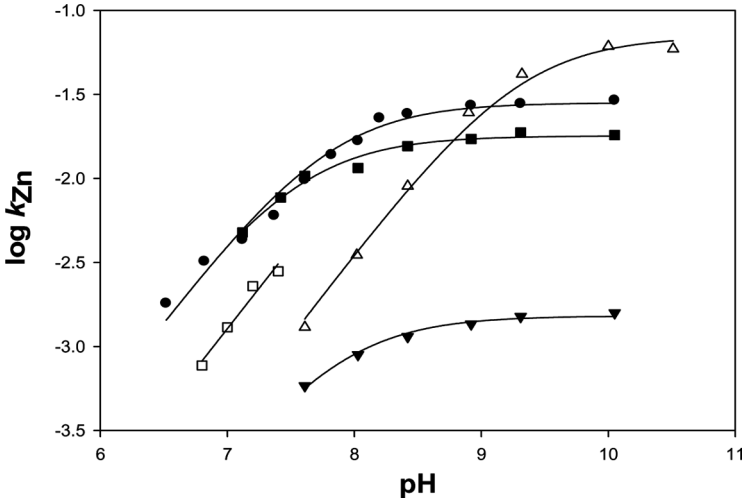


Figure 1. pH rate profiles of second-order rate constants $(k_{Zn})_{app}$ for cleavage of HpPNP catalyzed by several mononuclear Zn(II) complexes at 25°C, 20 mM buffer, $I = 0.100$ ($NaNO_3$). Key: Zn(L8) ▼, Zn(L9) ●, Zn(L10) ■, Zn(L7) △, $Zn(OH_2)_6$ □. Figure reproduced from reference 17 with permission from the Royal Society of Chemistry.

only option because there is a kinetically equivalent expression with a very different mechanism. This latter one involves the ionization of the 2′-hydroxyl in a pre-equilibrium step, followed by the rate-limiting interaction of the metal ion aqua complex with this ionized substrate to catalyze the formation of the phosphorane. In this latter case, the rate constant increases with pH as more of the 2′-alkoxide is formed, but levels out because the active metal aqua complex becomes converted into the inactive metal hydroxide complex.[19]

Solvent deuterium isotope studies on the Zn_2(L6) catalyzed cleavage of UpPNP were consistent with the second mechanism: catalysis by the aqua complex.[20] These studies showed a small inverse isotope effect at the pH-rate plateau. Thus there is no Bronsted acid/base catalysis in the rate-limiting step for cleavage of RNA analogs with good leaving groups. To accommodate this data, our mechanistic model includes a pre-equilbrium ionization of the 2′-hydroxyl group followed by catalysis of the formation of the phosphorane from the 2′-hydroxyl deprotonated species by the metal ion aqua complex. This result suggests that 2′-hydroxyl deprotonation (a) for cleavage of UpPNP by the Zn(II) complex is not part of the rate limiting step.

ELECTROPHILIC CATALYSIS BY PHOSPHORANE STABILIZATION (B)

Cationic metal ion complexes are well-suited for the mechanistic role of binding to and stabilizing the additional developing negative charge on the phosphorane transition state. This can be seen in the comparison of phosphate ester binding constants for monoanionic versus dianionic esters (Table 1). All Zn(II) and Eu(III) complexes bind more strongly to dianionic methyl phosphate than to monoanionic diethyl phosphate. The Eu(III) complexes bind the phosphate monoester 2 kcal/mol and the dinuclear Zn(II) complex 4.4 kcal/mol more strongly than the diester. This suggests that in catalysis, the weak interaction between substrate and catalyst strengthens as the additional negative charge of the phosphorane develops. Of course, MP is not a perfect transition state analog, but it does have an additional negative charge compared to substrate. MP has a different geometry at the phosphorus center and a different distribution of negative charge on the oxygens compared to a phosphorane. There may also be differences in the extent equilibrium solvation of charge for MP compared to the phosphorane transition state.[21]

Coordination chemistry properties of metal ion complexes are consistent with the mechanism shown in Scheme 2. Studies show that methyl phosphate binds preferentially to the metal aqua form of the catalyst. For

Table 1. Phosphate ester dissociation constants and rate enhancements for Eu(III) and Zn(II) complex catalyzed cleavage of UpU at 25°C, 20 mM buffer, $I = 0.10$ M ($NaNO_3$) at pH 7.6

Complex	K(MP, mM)[a] (ΔG_{MP}, kcal/mol)	K(DEP, mM)[a] (ΔG_{DEP}, kcal/mol)	k_{cat} (s^{-1}) UpU	k_{cat}/k_{un}[e]
Eu_2(L1)	0.025 (6.3)	0.43 (4.6)	1.5×10^{-5} [b]	3.4×10^4
Eu(L2)	0.28 (4.9)	7.5 (2.9)	3.3×10^{-6} [c]	7.5×10^3
Zn(L7)	14 (2.5)	94 (1.4)	–	–
Zn_2(L6)	0.010 (6.9)	16 (2.5)	1.6×10^{-5} [d]	5.8×10^4

[a]Dissociation constants and free energy (given parentheses) from data in references 16, 17, 18, 21, 27, 28. [b]$k_{cat} = k_2K_{UpU}$ calculated for k_2, second-order rate constant at pH 7.6 and K_{UpU}, UpU dissociation constant from reference 27. [c]$k_{cat} = k_2K_{DEP}$ calculated for k_2, second-order rate constant at pH 7.6 and K_{DEP}, DEP dissociation constant from reference 28. [d]$k_{cat} = k_2K_{DEP}$ for k_2, second-order rate constant at pH 7.4 and K_{DEP} from reference 16. [e] background rate constant (k_{un}) for cleavage of UpU from references 18 and 22.

example, for the highly active dinuclear Zn(II) catalyst, Zn_2(L6), the aqua complex species (Zn_2(L6)$(OH_2)_4$) but not the hydroxide species (Zn_2(L6)(OH)$(OH_2)_3$) binds to MP. [19,21] This result supports the mechanism that has active metal aqua complex catalyst interacting with the anionic phosphorane whereas the metal hydroxide complex is inactive as a catalyst. In this case, hydroxide is a good ligand and competes with the phosphate ester for binding to the catalyst. Note that such inhibition by hydroxide is problematic in catalyst design because many metal ions that are good Lewis acids readily form hydroxide complexes. Our analysis suggests that the catalyst should be designed to interact strongly with phosphate anions with minimal formation of hydroxide complexes. Ways of incorporating this specificity are discussed below.

LEAVING GROUP INTERACTIONS (C)

The final step in the catalytic cycle (Scheme 2) entails interaction of the catalyst with the leaving group. Does the metal ion catalyst facilitate its departure? Our studies show that the dinuclear Zn(II) catalyst, in comparison to background or hydroxide catalyzed cleavage, is more effective for promoting cleavage of a substrate with a poor leaving group (**UpU**) than it is for an analog with a good leaving group (UpPNP).[22] This is shown in Table 2 where the transition state binding energies are greater

Table 2. Rate constants for RNA and RNA analog cleavage by Eu(III) and Zn(II) complexes at 25°C, 20 mM buffer, $I = 0.10$ M ($NaNO_3$)

Complex	Substrate	k_2 ($M^{-1}s^{-1}$)[a]	k_T ($M^{-2}s^{-1}$)[b]	k_t/k_{OH} (M^{-1})[c]	$\Delta G_s^{\dagger}$(kcal/mol)[d]
Eu_2(L1)	HpPNP	2.4	8.7×10^6	8.7×10^7	−11
EU_2(L1)	UpU	0.021	2.2×10^4	2.0×10^7	−10
EU(L2)	HpPNP	0.042	1.2×10^5	1.2×10^6	−8.3
Zn(L9)	HpPNP	0.0013	3.8×10^3	3.8×10^4	−6.2
Zn_2(L6)	HpPNP	0.25	1.1×10^6	1.1×10^7	−9.6
Zn_2(L6)	UpPNP	200	3.2×10^8	2.0×10^5	−7.2
Zn_2(L6)	UpU	0.005	8×10^3	7.0×10^6	−9.3

[a]Second-order rate constant at pH 7.6 from references 16, 17, 18, 21, 27, 28, 62. [b]apparent third-order rate constant for the metal complex catalyzed reaction in the pH range where there is a first-order dependence on hydroxide. [c]Rate acceleration in the pH range where both metal complex catalyzed and background reaction are first order in hydroxide for $k_{OH} = 0.099\ M^{-1}s^{-1}$ (HpPNP), $k_{OH} = 1600\ M^{-1}s^{-1}$ (UpPNP), $0.0011\ M^{-1}s^{-1}$ (UpU). [d]Transition state binding energy as calculated by using Eq. 1.

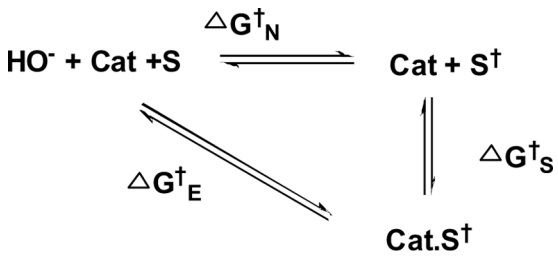
for UpU compared to UpPNP. These studies have recently been extended to examine a range of uridine derivatives having alkyl leaving groups with pK_as ranging from 12.2 to 16.0.[5,23] For these substrates, a substantially smaller leaving group dependence for Zn_2(L6) catalyzed cleavage was observed than for hydroxide catalyzed cleavage. One rationale for this is that the Zn(II) catalyst interacts with the leaving group through protonation by a water ligand or by direct coordination to facilitate its departure. An alternate explanation, however, is that the phosphorane transition state formed from phosphate esters with more basic leaving groups is more effectively stabilized by the Zn(II) catalyst than are analogs with less basic leaving groups. This would explain why cleavage of RNA models with poor leaving groups is more strongly catalyzed by the dinuclear Zn(II) catalyst. Similar leaving group effects are observed for free Zn(II) ion.[14]

OPTIMIZING CATALYSIS

All catalysts studied to date show pH-dependent cleavage of RNA over various pH ranges. This raises the question of the best way to compare their catalytic properties. One way is to compare second-order rate constants for cleavage at neutral pH (Table 2). This analysis shows that the dinuclear Eu(III) catalysts are better than all of the other complexes for cleavage of RNA analogs HpPNP and UpU. This is a practical comparison because these conditions are similar to physiological ones. A comparison that is grounded in mechanistic considerations uses the third-order rate constant (k_T) for the catalyzed reaction that represents the slope of the second-order rate constant as a function of pH at pH values that are less than the pK_a of the metal water ligand.[5,17,22,24] In this analysis we compare rate constants for the complexes that take into account a first-order dependence on hydroxide, catalyst and substrate. Division of this third-order rate constant by the background second-order

$$\Delta G_S^{\dagger} = \Delta G_E^{\dagger} - \Delta G_N^{\dagger} = -RT\ln\left[\frac{k_M K_a/K_w}{k_{HO}}\right] \qquad (1)$$

rate constant for hydroxide catalyzed cleavage (in absence of metal catalyst) gives a new constant (k_T/k_{OH}) with units of M^{-1}, the same units as a dissociation constant. In fact, this constant is related to the dissociation constant of the catalyst bound to altered substrate in the transition state, as reported for numerous enzymes.[25,26] The free energy calculated from



Scheme 3. Thermodynamic cycle to calculate the free energy of catalyst binding to the transition state.

this constant is related to the transition state stabilization energy of the catalyst ($\Delta G_s^{\ddagger}$, Scheme 3, derived for Eq. 1 with k_m = limiting second order rate constant on pH plateau). As shown in Table 2, these transition state stabilization energies reach large values for dinuclear Ln(III) and Zn(II) complexes for both activated HpPNP and inactivated RNA (UpU) analogs.

Lanthanide ion complexes of neutral macrocycles have some of the coordination properties that we seek. They bind strongly to phosphate esters ($K_d = 0.018$ mM for Eu(L5) and MP) but do not readily form hydroxide complexes ($pK_a >$ pH 9.0) as shown by kinetic studies and by direct excitation luminescence spectroscopy.[27,28] By contrast, mononuclear Zn(II) catalysts we have used have pK_a values of 7 to 9 and bind MP more weakly ($K_d \approx 1$ mM).[17,29] These mononuclear Zn(II) complexes are poorer catalysts than lanthanide ions with transition state stabilization energies that are 2 kcal/mol lower than mononuclear Eu(III) complexes (Table 2). In addition to weaker phosphate ester binding, Zn(II) complexes with low water pK_a values form hydroxide complexes more readily than do lanthanide ions. Thus, the Zn(II) catalysts reach their optimum rate constants at near neutral pH, close to their pK_a values (Figure 1). By contrast, the Eu(III) complexes of L2-L4 have pK_a values that are greater than 9.0 and this leads to a steady increase in the second-order rate constant over the pH range from 7 to 9 (Figure 2).

Aside from switching metal ions, there are other ways to increase catalysis. One method is the incorporation of hydrogen bonding groups that interact with bound phosphate esters.[19,30–35] Other workers have observed enormous rate enhancements for metal ion catalyzed cleavage of RNA analogs in organic solvent where cation-anion interactions are strong.[36–39] A fourth way to form complexes with increased affinity

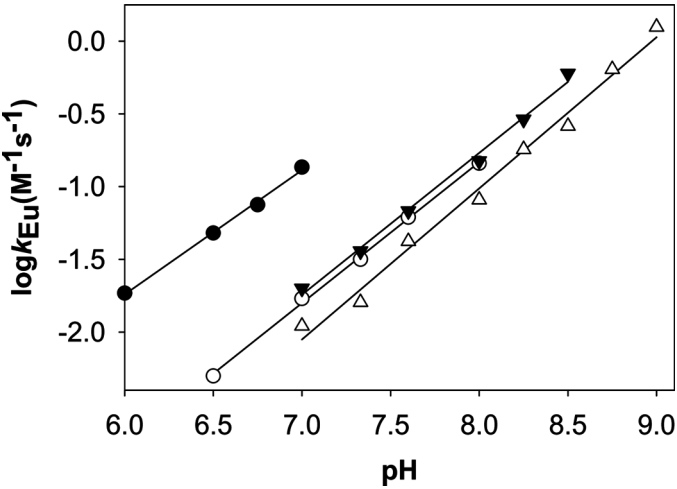


Figure 2. pH rate profiles of second-order rate constants (k_{Eu}) for cleavage of HpPNP catalyzed by several mononuclear Eu(II) complexes at 25°C, 20 mM buffer, $I = 0.100$ ($NaNO_3$). Key: Eu(L2) △, Eu(L3) ▼, Eu(L4) ○, Eu$(OH_2)_9$ ●. Figure reproduced from reference 28 with permission from the Royal Society Chemistry.

for phosphate anions and decreased affinity for hydroxide is to prepare dinuclear complexes. Dinuclear complexes have a large overall cationic charge that is spread over two metal ions. If the two metal ion centers are sufficiently far apart that the phosphate anions can bridge but hydroxide cannot, the dinuclear complex becomes a better phosphate anion receptor than the analogous mononuclear complex. A good example of this is the dinuclear complex Zn_2(L6), which has two Zn(II) ions at 3.6 Å apart, a distance suitable for binding a bridging phosphate diester.[16] The first pK_a for a water ligand is relatively high (8.0), indicative of a terminal hydroxide ligand.

DINUCLEAR CATALYSTS

Getting two metal ions to cooperate in catalysis is not always straight forward. For Zn(II) complexes, a bridging linker is a necessity for cooperative catalysis. Zn(II) complexes that lack this linker are barely more active than their mononuclear analogs.[40] For Zn_2(L6), a bridging alkoxide ligand serves to keep the two Zn(II) ions in close enough proximity for both ions to work cooperatively in catalysis as reflected

by the large free energy for transition state stabilization and for MP binding.[16,21] A caveat is that there must be sufficient coordination sites available for catalysis after the bridging ligand binds. Metal ions with lower numbers of coordination sites than Zn(II) such as Cu(II) form inactive dinuclear complexes with L6.[41]

For lanthanide ions, the preparation of dinuclear complexes with a bridging group built into the ligand is challenging and, to date, elusive. Hydroxide bridged complexes that form through self-assembly are common but, if they contain anionic ligands, are not exceptionally active.[24,42] An exception to this is a dinuclear La(III) complex that forms at high pH in the presence of buffer that gives rise to some of the fastest cleavage rates reported to date.[43]

In an attempt to prepare well-defined yet highly catalytically active lanthanide complexes, we prepared Eu(III) macrocyclic amide ligands tethered through an aromatic group (Eu_2(L1)).[27] These complexes are among the best catalysts for RNA cleavage reported to date in water at neutral pH despite the fact that there is no bridging donor group between lanthanide centers. How can this be? Each Eu(III) center has two coordination sites occupied by water as shown by time-resolved luminescence spectroscopy. Luminescence studies show that the phosphate monoester binds by bridging the two lanthanide centers, suggesting that the two Eu(III) centers may participate cooperatively in catalysis. Another source of the rate enhancement for these complexes is the pendent aromatic group. Comparison to a mononuclear analog with an aromatic group (Eu(L5)) shows that the aromatic group itself is sufficient to accelerate catalysis by 10-fold in comparison to a mononuclear complex lacking the aromatic group. Such substituent effects have been observed for transition metal ion and Zn(II) complexes, but not for lanthanide ions. There are clearly many opportunities to increase catalytic efficiency of lanthanide complexes, especially those with neutral ligands. Compared to transition elements and Zn(II), there are relatively few neutral ligands that have been shown to bind strongly to lanthanide ions in water.

CATALYTIC RATE ENHANCEMENTS AND SPEED LIMITS

Table 1 shows rate enhancements for the cleavage of a dinucleoside, **UpU**, by Eu(III) and Zn(II) complexes. In this analysis, k_{cat}, the maximum rate constant, is calculated by using the values of the second-order rate constants in Table 2 and dissociation constants from Table 1 ($k_{cat} = k_2K_d$).

This analysis uses the assumption that the Michaelis constant for formation of the catalyst-UpU complex is similar to the dissociation constant for the complex with the phosphate diester, DEP. The rate constant for the uncatalyzed reaction, (k_{un}) is calculated for the pH of interest from the second-order rate constant for hydroxide catalyzed cleavage of UpU.[22] The rate enhancements calculated in this manner show that the dinuclear catalysts approach a rate enhancement of 10^5. A 10^5 rate enhancement can be accounted for by one catalytic mode: stabilization of the phosphorane transition state. This compares to self-cleaving ribozymes that have k_{cat} values ranging from 0.005 to 0.17 s^{-1} with rate enhancements of 10^7 to nearly 10^9. Clearly the metal ion complexes are not yet on par with ribozymes. Ribozymes have been proposed to use at least two catalytic modes including optimizing in-line attack and by deprotonation of the 2′-hydroxyl through general base catalysis.[10,15]

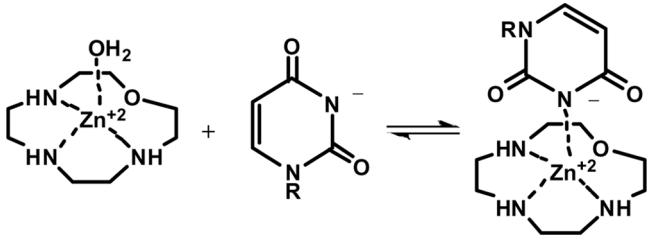
Are metal ion complexes relegated to perform at a low speed limit through a single catalytic mode? There may indeed be limitations for the cleavage of simple RNA analogs by metal ion complexes in water. It is certainly possible that metal ion complexes with a more complex array of catalytic functional groups will break these speed limits. A related question is whether the same speed limits hold in the cleavage of structured RNA by metal ions where there is precedence for higher rate enhancements. For example, cleavage of transfer RNA by Pb(II) ion occurs primarily at one site in the 75-nucleotide RNA with a maximum rate constant of $1\times10^{-3}\,s^{-1}$ or about 70-fold higher than the dinuclear Eu(III) complex with UpU.[44] In vitro-selected Pb(II) cleavage motifs have rate constants of about 2000-fold higher than the dinuclear Eu(III) complex.[45] These metal ion catalyzed cleavage reactions must utilize additional modes of catalysis. One important catalytic strategy used by ribozymes is alignment of the 2′-hydroxyl, a mode that is not available for simple RNA analogs. The fact that a single site is cleaved suggests that RNA folding is important to bind the metal ion and to activate selected phosphate ester bonds. This suggests that the speed limits for metal ion cleavage of simple RNA analogs such as HpPNP or UpU will be exceeded in more complex folded RNAs.

SEQUENCE AND STRUCTURE SPECIFIC CLEAVAGE

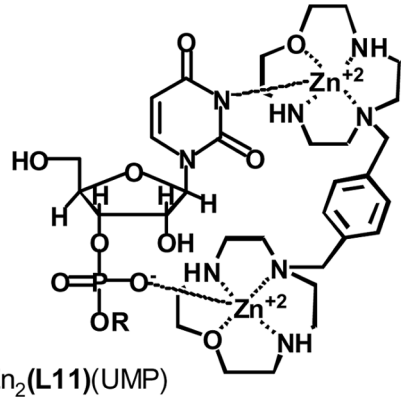
An important goal is to design metal ion catalysts for selective cleavage of a target RNA molecule. This has been accomplished by attaching a

metal ion catalyst to a complementary piece of DNA or RNA (antisense oligonucleotide, ASO) for base pairing to a single-stranded portion of the RNA.[11,46–49] ASO conjugates with metal ion catalysts promote RNA sequence-dependent cleavage and selectively suppress expression of a gene in cell culture.[6] Drawbacks to this approach include the cost of the antisense oligonucleotide, the difficulty of getting it into the cell and interference by competing RNA structure. To overcome these drawbacks, an alternative approach is the development of metal ion catalysts attached to small molecule recognition agents for RNA. There are few studies on this topic to date. The most common examples have attachment of catalysts to amino acids[50] or to aminoglycosides.[51,52]

One recognition module under development in our lab uses the binding properties of Zn(II) macrocyclic complexes.[29,53,54] Zn(II) macrocyclic complexes bind strongly to the deprotonated N7 of uridine or thymidine. Kimura's group showed that much smaller binding constants are found for guanosine, adenosine, and cytidine and developed 1,4,7,10-tetraazacyclododecane (cyclen) complexes of Zn(II).[55–57] Our work showed that many different types of Zn(II) macrocycles, in addition to Zn(cyclen), bound to uridine (Scheme 4).[53] The strength of binding to uridine is linearly correlated to the pK_a of the Zn(macrocycle) water ligand. Based on this work, we prepared a dinuclear Zn(II) complex of a triaazamacrocycle (Scheme 5) that is both a good binder for uridine and a good cleavage agent.[54] This dinuclear complex binds two uridines with dissociation constants of 4 and 6 mM at pH 7.0. Binding of this dinuclear catalyst to uridine and to phosphate diesters was partially reflected in the formation of a Michaelis complex with UpPNP, a uridine containing substrate. This led to more effective catalytic cleavage of UpPNP by the dinuclear catalyst (Zn_2(L11)) compared to mononuclear



Scheme 4. Zn(II) macrocycle binds to the N3 deprotonated form of uridine.



Scheme 5. Bifunctional dinuclear Zn(II) complex preferentially cleaves uridine containing substrates.

complex Zn(L9). However, it is clear from these studies that the binding of a single Zn(II) complex to uridine is not sufficiently strong at neutral pH to obtain a high degree of selectivity. Instead, three Zn(II) ions[58] or a Zn(II) macrocycle with pendent aromatic groups[56,57] are required to strengthen binding to uridine groups. Ongoing work in our laboratory is directed toward strengthening binding and improving cleavage specificity for structured RNA.

CONCLUSIONS

Kinetic and mechanistic studies show that metal ion catalysts for RNA cleavage function primarily by stabilization of the anionic phosphorane transition state (b in Scheme 2) and possibly by interacting with the leaving group (c in Scheme 2). In line with this charge stabilization role are studies showing that the best RNA cleavage catalysts bind tightly to dianionic phosphate ester ligands. Lanthanide ion complexes are among the best catalysts, in part because of their specific coordination properties for phosphate esters in solution. Linking different catalytic or functional groups to interact more strongly with phosphate esters is another approach toward better catalysts. An example of this is found in the catalytic power of dinuclear metal ion complexes. The two metal ion centers need appropriate bridging donor groups to facilitate interaction of both metal ions with the phosphate ester.

Our mechanistic work to date suggests that relatively few catalytic modes are used by metal ions in the cleavage of RNA in comparison to natural enzymes. Does this imply that metal ion complexes have a speed limit due to their limited number of catalytic strategies identified thus far? This argument may hold for simple RNA analogs as substrates and simple metal ion complexes as catalysts. However, simple RNA analogs, even dinucleotides such as UpU, do not reproduce the environment in structured RNA molecules. Certain RNA structures enhance metal ion catalyzed cleavage as shown by classic studies of Pb(II) or Eu(III) catalyzed cleavage of transfer RNA.[44,59] An important remaining challenge then is to prepare metal ion complexes that bind to and cleave a specific site in structured RNA molecules. Accepting this challenge entails conducting research in the design of small molecules for RNA recognition. RNA recognition is an area of intense interest given the increasing number of important roles of RNA in biology and the promise of therapeutic intervention upon targeting RNA.[60,61] Inorganic chemists in the field of artificial nucleases should consider contributing toward this important area of research.

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REFERENCES

1. Trawick, B. N., A. T. Daniher, and J. K. Bashkin, 1998. Inorganic mimics of ribonucleases and ribozymes: From random cleavage to sequence-specific chemistry to catalytic antisense drugs. *Chem. Rev.*, **98**, 939–960.
2. Niittymaki, T. and H. Lonnberg, 2006. Artificial ribonucleases. *Org. Biomol. Chem.*, **4**, 15–25.
3. Morrow, J. R. and O. Iranzo, 2004. Synthetic metallonucleases for RNA cleavage. *Curr. Opin. Chem. Biol.*, **8**, 192–200.
4. Williams, N. H., B. Takasaki, M. Wall, and J. Chin, 1999. Structure and nuclease activity of simple dinuclear metal complexes: quantitative dissection of the role of metal ions. *Acc. Chem. Res.*, **32**, 485–493.

5. Morrow, J. R., T. L. Amyes, and J. P. Richard, 2008. Phosphate binding energy and catalysis by small and large molecules. *Acc. Chem. Res.*, **41**, 539–548.
6. Baker, B. F., S. S. Lot, J. Kringel, S. Cheng-Flournoy, P. Villiet, H. M. Sasmor, A. M. Siwkowski, L. L. Chappell, and J. R. Morrow, 1999. Oligonucleotide-europium complex conjugate designed to cleave the 5′ cap structure of the ICAM-1 transcript potentiates antisense activity in cells. *Nucl. Acids Res.*, **27**, 1547–1551.
7. Lu, Y. 2002. New transition-metal-dependent DNAzymes as efficient endonucleases and as selective metal biosensors. *Chem. Eur. J.*, **8**, 4588–4596.
8. Lu, Y. and J. Liu, 2006. Functional DNA nanotechnology: Emerging applications of DNAzymes and aptamers. *Curr. Opin. Biotechnol.*, **17**, 580–588.
9. Li, Y. and R. R. Breaker, 1999. Kinetics of RNA degradation by specific base catalysis of transesterification involving the 2′-hydroxyl group. *J. Am. Chem. Soc.*, **121**, 5364–5372.
10. Breaker, R. R., G. M. Emilsson, D. Lazarev, S. Nakamura, I. J. Puskarz, A. Roth, and N. Sudarsan, 2003. A common speed limit for RNA-cleaving ribozymes and deoxyribozymes. *RNA*, **9**, 949–957.
11. Komiyama, M. 2003. Sequence-selective scission of DNA and RNA by lanthanide ions and their complexes. *Met. Ion Biol. Syst.*, **40**, 463–475.
12. Cowan, J. A. 2001. Chemical nucleases. *Curr. Opin. Chem. Biol.*, **5**, 634–642.
13. Kuusela, S. and H. Lonnberg, 1997. Metal ion dependent hydrolysis of RNA. *Curr. Topics Sol. Chem.*, **2**, 29–47.
14. Mikkola, S., E. Stenman, K. Nurmi, E. Yousefi-Salakdeh, R. Stromberg, and H. Lonnberg, 1999. The mechanism of the metal ion promoted cleavage of RNA phosphodiester bonds involves a general acid catalysis by the metal ion on the departure of the leaving group. *J. Chem. Soc., Perkin Trans.*, **2**, 1619–1625.
15. Emilsson, G. M., S. Nakamura, A. Roth, and R. R. Breaker, 2003. Ribozyme speed limits. *RNA*, **9**, 907–918.
16. Iranzo, O., A. Y. Kovalevsky, J. R. Morrow, and J. P. Richard, 2003. Physical and kinetic analysis of the cooperative role of metal ions in catalysis of phosphodiester cleavage by a dinuclear Zn(II) complex. *J. Am. Chem. Soc.*, **125**, 1988–1993.
17. Mathews, R. A., C. S. Rossiter, J. R. Morrow, and J. P. Richard, 2007. A minimalist approach to understanding the efficiency of mononuclear Zn(II) complexes as catalysts of cleavage of an RNA analog. *Dalton Trans.*, 3804–3811.
18. O'Donoghue, A., S. Y. Pyun, M. Y. Yang, J. R. Morrow, and J. P. Richard, 2006. Substrate specificity of an active dinuclear Zn(II) catalyst for cleavage of rna analogues and a dinucleoside. *J. Am. Chem. Soc.*, **128**, 1615–1621.

19. Feng, G., J. C. Mareque-Rivas, R. Martin de Rosales, and N. H. Williams, 2005. A highly reactive mononuclear Zn(II) complex for phosphodiester cleavage. *J. Am. Chem. Soc.*, **127**, 13470–13471.
20. Yang, M. Y., O. Iranzo, J. P. Richard, and J. R. Morrow, 2005. Solvent deuterium isotope effects on phosphodiester cleavage catalyzed by an extraordinarily active Zn(II) complex. *J. Am. Chem. Soc.*, **127**, 1064–1065.
21. Yang, M.-Y., J. R. Morrow, and J. P. Richard, 2007. A transition state analog for phosphate diester cleavage catalyzed by a small enzyme-like metal ion complex. *Bioorg. Chem.*, **35**, 366–374.
22. O'Donoghue, A., S. Y. Pyun, M. Y. Yang, J. R. Morrow, and J. P. Richard, 2006. Substrate specificity of an active dinuclear Zn(II) catalyst for cleavage of RNA analogues and a dinucleoside. *J. Am. Chem. Soc.*, **128**, 1615–1621.
23. Yang, M.-Y. 2007. *Ph.D. Thesis, University at Buffalo.*
24. Farquhar, E. R., J. P. Richard, and J. R. Morrow, 2007. Formation and stability of mononuclear and dinuclear Eu(III) complexes and their catalytic reactivity toward cleavage of an RNA analog. *Inorg. Chem.*, **46**, 7169–7177.
25. Wolfenden, R. 2006. Degrees of difficulty of water-consuming reactions in the absence of enzymes. *Chem. Rev.*, **106**, 3379–3396.
26. Wolfenden, R. and M. J. Snider, 2001. The depth of chemical time and the power of enzymes as catalysts. *Acc. Chem. Res.*, **34**, 938–945.
27. Nwe, K., C. Andolina, and J. R. Morrow, 2008. Tethered dinuclear Eu(III) macrocyclic catalysts for RNA cleavage. *J. Am. Chem. Soc.*, **130**, 14861–14871.
28. Nwe, K., J. P. Richard, and J. R. Morrow, 2007. Direct excitation luminescence spectroscopy of Eu(III) complexes of 1,4,7-tris(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane derivatives and kinetic studies of their catalytic cleavage of an RNA analog. *Dalton Trans.*, 5171–5178.
29. Rossiter, C. S., R. A. Mathews, and J. R. Morrow, 2007. Cleavage of an RNA analog by Zn(II) macrocyclic catalysts appended with a methyl or an acridine group. *J. Inorg. Biochem.*, **101**, 925–934.
30. Feng, G., J. C. Mareque-Rivas, and N. H. Williams, 2006. Comparing a mononuclear Zn(II) complex with hydrogen bond donors with a dinuclear Zn(II) complex for catalysing phosphate ester cleavage. *Chem. Commun.*, 1845–1847.
31. Feng, G., D. Natale, R. Prabaharan, J. C. Mareque-Rivas, and N. H. Williams, 2006. Efficient phosphodiester binding and cleavage by a Zn complex combining hydrogen-bonding interactions and double Lewis acid activation. *Angew. Chem. Int. Ed.*, **45**, 7056–7059.
32. Linjalahti, H., G. Feng, J. C. Mareque-Rivas, S. Mikkola, and N. H. Williams, 2008. Cleavage and isomerization of UpU promoted by dinuclear metal ion complexes. *J. Am. Chem. Soc.*, **130**, 4232–4233.

33. Ait-Haddou, H., J. Sumaoka, S. L. Wiskur, J. F. Folmer-Andersen, and E. V. Anslyn, 2002. Remarkable cooperativity between a Zn(II) ion and guanidinium/ammonium groups in the hydrolysis of RNA. *Angew. Chem. Int. Ed.*, **41**, 4014–4016.
34. Livieri, M., F. Mancin, G. Saielli, J. Chin, and U. Tonellato, 2007. Mimicking enzymes: cooperation between organic functional groups and metal ions in the cleavage of phosphate diesters. *Chem. Eur. J.*, **13**, 2246–2256.
35. Livieri, M., F. Mancin, U. Tonellato, and J. Chin, 2004. Multiple functional group cooperation in phosphate diester cleavage promoted by Zn(II) complexes. *Chem. Commun.*, 2862–2863.
36. Sanchez-Lombardo, I. and A. K. Yatsimirsky, 2008. Simplified speciation and improved phosphodiesterolytic activity of hydroxo complexes of trivalent lanthanides in aqueous DMSO. *Inorg. Chem.*, **47**, 2514–2525.
37. Bunn, S. E., C. T. Liu, Z.-L. Lu, A. A. Neverov, and R. S. Brown, 2007. The dinuclear Zn(II) complex catalyzed cyclization of a series of 2-hydroxypropyl aryl phosphate RNA models: progressive change in mechanism from rate-limiting P-O bond cleavage to substrate binding. *J. Am. Chem. Soc.*, **129**, 16238–16248.
38. Liu, C. T., A. A. Neverov, and R. S. Brown, 2007. A reductionist biomimetic model system that demonstrates highly effective Zn(II)-catalyzed cleavage of an RNA model. *Inorg. Chem.*, **46**, 1778–1788.
39. Lu, Z.-L., C. T. Liu, A. A. Neverov, and R. S. Brown, 2007. Rapid three-step cleavage of RNA and DNA model systems promoted by a dinuclear Cu(II) complex in methanol. Energetic origins of the catalytic efficacy. *J. Am. Chem. Soc.*, **129**, 11642–11652.
40. Iranzo, O., T. Elmer, J. P. Richard, and J. R. Morrow, 2003. Cooperativity between metal ions in the cleavage of phosphate diesters and RNA by dinuclear Zn(II) catalysts. *Inorg. Chem.*, **42**, 7737–7746.
41. Iranzo, O., J. P. Richard, and J. R. Morrow, 2004. Structure-activity studies on the cleavage of an RNA analogue by a potent dinuclear metal ion catalyst: effect of changing the metal ion. *Inorg. Chem.*, **43**, 1743–1750.
42. Aguilar-Perez, F., P. Gomez-Tagle, E. Collado-Fregoso, and A. K. Yatsimirsky, 2006. Phosphate ester hydrolysis by hydroxo complexes of trivalent lanthanides stabilized by 4-imidazolecarboxylate. *Inorg. Chem.*, **45**, 9502–9517.
43. Hurst, P., B. K. Takasaki, and J. Chin, 1996. Rapid cleavage of RNA with a La(III) dimer. *J. Am. Chem. Soc.*, **118**, 9982–9983.
44. Behlen, L. S., J. R. Sampson, A. B. DiRenzo, and O. C. Uhlenbeck, 1990. Lead-catalyzed cleavage of yeast $tRNA^{Phe}$ mutants. *Biochem.*, **29**, 2515–2523.
45. Pan, T., B. Dichtl, and O. C. Uhlenbeck, 1994. Properties of an *in vitro* Selected Pb^{2+} Cleavage Motif. *Biochem.*, **33**, 9561–9565.

46. Huang, L., L. L. Chappell, O. Iranzo, B. F. Baker, and J. R. Morrow, 2000. Oligonucleotide conjugates of Eu(III) tetraazamacrocycles with pendent alcohol and amide groups promote sequence-specific RNA cleavage. *J. Biol. Inorg. Chem.*, **5**, 85–92.
47. Magda, D., M. Wright, S. Crofts, A. Lin, and J. L. Sessler, 1997. Metal complex conjugates of antisense DNA which display ribozyme-like activity. *J. Am. Chem. Soc.*, **119**, 6947–6948.
48. Hall, J., D. Husken, U. Pieles, H. E. Moser, and R. Haner, 1994. Efficient sequence-specific cleavage of RNA using novel europium complexes conjugated to oligonucleotides. *Chem. Biol.*, **1**, 185–190.
49. Daniher, A. T. and J. K. Bashkin, 1998. Precise control of RNA cleavage by ribozyme mimics. *Chem. Commun.*, 1077–1078.
50. Michaelis, K. and M. Kalesse, 2001. Selective cleavage of unpaired uridines with a tyrosine-cyclen conjugate. *Chembiochem.*, **2**, 79–83.
51. Chen, C.-A. and J. A. Cowan, 2002. In vivo cleavage of a target RNA by copper kanamycin A. Direct observation by a fluorescence assay. *Chem. Commun.* (*Cambridge, United Kingdom*), 196–197.
52. Sreedhara, A. and J. A. Cowan, 2001. Targeted site-specific cleavage of HIV-1 viral rev responsive element by copper aminoglycosides. *J. Biol. Inorg. Chem.*, **6**, 166–172.
53. Rossiter, C. S., R. A. Mathews, and J. R. Morrow, 2005. Uridine binding by Zn(II) macrocyclic complexes: Diversion of RNA cleavage catalysts. *Inorg. Chem.*, **44**, 9397–9404.
54. Rossiter, C. S., R. A. Mathews, I. M. A. DelMundo, and J. R. Morrow, 2008. Cleavage of a RNA analog containing uridine by a bifunctional dinuclear Zn(II) catalyst. *J. Inorg. Biochem.*, October 9 online publication.
55. Aoki, S. and E. Kimura, 2000. Highly selective recognition of thymidine mono- and diphosphate nucleotides in aqueous solution by ditopic receptors zinc(II)-bis(cyclen) complexes (cyclen = 1,4,7,10-tetraazacyclododecane). *J. Am. Chem. Soc.*, **122**, 4542–4548.
56. Kimura, E., H. Kitamura, K. Ohtani, and T. Koike, 2000. Elaboration of selective and efficient recognition of thymine base in dinucleotides (TpT, ApT, CpT, and GpT), single-stranded d(GTGACGCC), and double-stranded d(CGCTAGCG)2 by Zn2+-Acridinylcyclen (Acridinylcyclen = (9-Acridinyl)methyl-1,4,7,10-tetraazacyclododecane). *J. Am. Chem. Soc.*, **122**, 4668–4677.
57. Aoki, S. and E. Kimura, 2004. Zinc-nucleic acid interaction. *Chem. Rev.*, **104**, 769–787.
58. Wang, Q. and Lonnberg, H. 2006. Simultaneous interaction with base and phosphate moieties modulates the phosphodiester cleavage of dinucleoside 3′,5′-monophosphates by dinuclear Zn^{2+} complexes of di(aza-crown) ligands *J. Am. Chem. Soc*, **128**, 10716–10728.

59. Rordorf, B. F. and D. R. Kearns, 1976. Effect of europium(III) on the thermal denaturation and cleavage of transfer ribonucleic acids. *Biopolymers*, **15**, 1491–1504.
60. Tor, Y., 2003. Targeting RNA with small molecules. *Chem. Bio. Chem.*, **4**, 998–1007.
61. Thomas, J. R. and P. J. Hergenrother, 2008. Targeting RNA with small molecules. *Chem. Rev.*, **108**, 1171–1224.