Articles

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Catalytic Transesterification and Hydrolysis of RNA by Zinc(II) Complexes

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The pseudo-first-order rate constants (k_{obs}) for transesterification of adenylyl-3′,5′-uridine 3′-monophosphate (ApUp) by Zn([9]aneN$_3$)$^{2+}$ (**1**), Zn([12]aneN$_3$)$^{2+}$ (**2**), or Zn(cyclam)$^{2+}$ (**3**) in water at 64 °C, pH = 7.6, follow the order **1** ≈ **2** > **3** ([9]aneN$_3$ = 1,4,7-triazacyclononane, [12]aneN$_3$ = 1,5,9-triazacyclododecane, cyclam = 1,4,8,11-tetraazacyclotetradecane). Catalytic behavior is observed for transesterification of ApUp by **1**; five turnovers of **1** were observed with no decrease in rate under conditions of excess ApUp. The pH–rate profile for the transesterification of ApUp by **1** is consistent with Zn([9]aneN$_3$)(OH)$^+$ or its kinetic equivalent being the active catalyst. Transesterification of ApUp by **1** is inhibited at concentrations of **1** greater than 3.29×10^{-4} M, where higher order complexes between **1** and ApUp may form. Consistent with these results, studies by Job's method indicate that a 1:1 complex between **1** and ApUp is the most reactive species. At 64 °C and pH = 7.6, **1** catalyzes the hydrolysis of 2′,3′-cAMP less rapidly than **1** catalyzes the transesterification of ApUp.

Introduction

Metal ions catalyze many biologically important substitution reactions at phosphorus(V).[1] Metal ion promoted hydrolysis at phosphorus(V) is one type of substitution reaction that has been extensively studied; examples include ATP hydrolysis[2] and phosphate diester[3,4] and monoester[5] hydrolysis. Transesterification of phosphate esters is a second type of phosphorus(V) substitution reaction promoted by metal ions that has been less intensively studied from a mechanistic viewpoint than hydrolysis. Metal ion promoted cleavage of RNA, the most prominent example of phosphate ester transesterification, proceeds by attack of the 2′-hydroxyl group of ribose to produce a 2′,3′-cyclic phosphate ester with concomitant cleavage of the RNA strand. In hydrolysis or transesterification reactions, the metal ion may bind to the phosphate ester to activate the phosphorus center to nucleophilic attack or may bind to the leaving group to assist leaving group departure. The obvious difference between hydrolysis and transesterification at phosphorus(V) lies in the attacking nucleophile. Hydrolysis catalysts that have large rate accelerations bind phosphorus(V) compound and hydroxide in a cis orientation and promote intramolecular attack of hydroxide at phosphorus. Thus, hydrolysis catalysts generally have two available coordination sites in a cis orientation.[3,5] In the transesterification of RNA, where the attacking nucleophile is the 2′-hydroxyl of ribose, a metal hydroxide will not play the same role as it does in hydrolysis. Hence, there should be a distinction made between metal ion hydrolysis catalysts and metal ion transesterification catalysts because the two types of catalysts may have very different characteristics. Here, we report on a metal complex that is both a transesterification and an hydrolysis catalyst; however, there is at least one example of a metal complex, Cu(trpy)$^{2+}$ (trpy = 2,2′,6′,2″-terpyridine), that promotes transesterification of RNA[6] but does not promote phosphate diester hydrolysis.[3a] It has been proposed that Cu(trpy)$^{2+}$ may act both as a general base and as a general acid to catalyze transesterification of RNA by a bifunctional mechanism.[6]

Many divalent and trivalent metal salts have been shown to promote transesterification of RNA including those of La^{3+},[7] Eu^{3+},[8] Bi^{3+},[9] Al^{3+},[9] Cd^{2+},[9] Ce^{3+},[7b] Pb^{2+},[10] Zn^{2+},[6,9,11,12] Cu^{2+},[6,12] Ni^{2+},[6,12] Co^{2+},[12] Mn^{2+},[12,13] and Mg^{2+}.[14] Recent interest has focused on the use of metal complexes of polydentate ligands to cleave RNA by transesterification.[6,15,16] The polydentate ligand may modulate the efficiency of metal ion catalyzed cleavage and act to maintain the metal ion in solution by preventing formation of precipitates of metal hydroxide or metal nucleotide complexes. In addition a polydentate ligand may serve as a handle to couple metal ion catalysts to recognition agents such as oligodeoxynucleotides or mononucleotides.[17] Other efforts have centered on accelerating metal ion catalysis because cleavage is slow at 37 °C. Bifunctional catalysis by zinc complexes containing a pendent general base achieves moderately higher rates of transesterification of a phosphate ester in a model RNA compound.[18]

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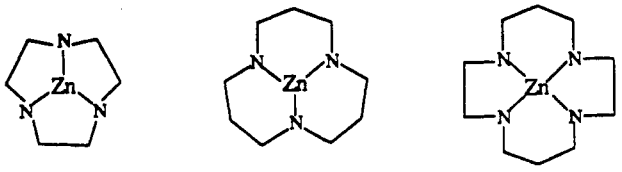
Similarly, 10 mM imidazole and 1 mM in zinc together effect higher rates of dinucleoside cleavage at 80 °C than either 1 mM zinc or 10 mM imidazole alone.[19] Motivation for the study of metal transesterification catalysts lies in the development of molecules that catalyze sequence-specific cleavage of RNA. Generally such molecules consist of a cleaving agent attached to a sequence-recognition molecule such as an oligodeoxynucleotide. Transesterification catalysts are potentially superior to metal complexes that promote oxidative cleavage of nucleic acids because transesterification is selective for RNA over DNA;[20] hence, the sequence-specific cleaving molecule will not destroy itself if transesterification catalysts are used. An important application for sequence-specific cleaving molecules for RNA is the selective inhibition of gene expression at the messenger RNA level.[21] Turnover of the transesterification catalyst will be crucial to this strategy, yet to date there have been no studies to demonstrate catalytic behavior in metal complexes that promote transesterification of RNA.

As part of our efforts to design transesterification catalysts based on metal ions that are not redox active, we have investigated three macrocyclic amine complexes of zinc(II) (**1**–**3**) as catalysts for the cleavage of adenylyl-3′,5′-uridine 3′-monophosphate (ApUp). Macrocyclic amine ligands were chosen because of their known ability to maintain zinc(II) in solution at neutral to mildly alkaline pH. Kinetic studies were performed to compare the three zinc complexes and to elucidate the mechanism of transesterification of ApUp by **1**. The catalytic behavior of **1** in the transesterification of a dinucleotide of RNA and in the hydrolysis of one of the transesterification products, adenosine 2′,3′-cyclic monophosphate (2′,3′-cAMP) was examined. To our knowledge, **1** is the first complex to exhibit catalytic behavior in the transesterification of a phosphate ester or in the hydrolysis of 2′,3′-cAMP. A zinc(II) complex of a macrocycle containing unsaturated nitrogen donors was recently shown to cleave RNA; however, no kinetic data was given nor was the catalytic behavior of the complex assessed.[6]



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Experimental Section

Materials. The sodium salt of adenylyl-3′,5′-uridine 3′-monophosphate (ApUp), the sodium salt of adenosine 2′,3′-cyclic monophosphate (2′,3′-cAMP), the acid form of adenosine 3′-monophosphate (3′-AMP), and the acid form of adenosine 2′-monophosphate (2′-AMP) were purchased from Sigma Chemicals and used as received. Macrocyclic amine ligands 1,4,7-triazacyclododecane trihydrochloride, 1,5,9-triazacyclododecane trihydrobromide, and 1,4,8,11-tetraazacyclotetradecane were purchased from Aldrich and used as received. Reagent grade buffers HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid) and CHES (2-(cyclohexylamino)ethanesulfonic acid) were purchased from Fisher Scientific. Reagent grade zinc nitrate was purchased from Fisher Scientific; the concentration of zinc(II) was determined by titration against EDTA with Eriochrome black T as indicator. Solutions were made with either Milli-Q purified water or Fisher HPLC grade water. All water was boiled, and all glassware was acid-washed and oven-dried at 200 °C prior to use, as precautionary measures to avoid contamination by ribonucleases. Plasticware was used without special treatment and gloves were worn in all stages of preparation of solutions.

Instrumentation. High-performance liquid chromatography (HPLC) was performed on a Water's 600E equipped with a 490E programmable multiwavelength detector. An Optima II C_{18} column (250 mm by 4.5 mm) was used. A Hewlett-Packard diode array 8452A spectrophotometer was employed for UV–vis spectra. An Orion 510 research digital ion analyzer equipped with temperature compensation probe was used for all pH measurements.

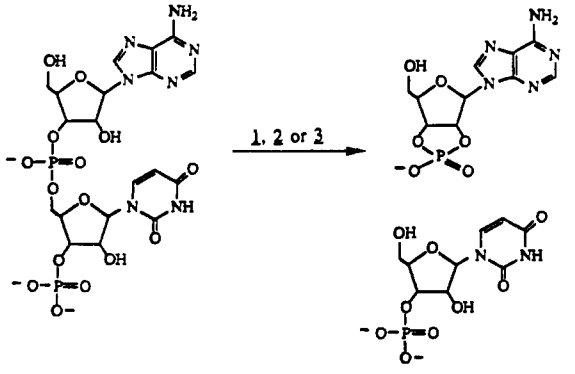
Kinetics. Solutions were prepared with a 10% excess of amine ligand over $Zn(NO_3)_2$. Solutions contained 4.322×10^{-3} M internal standard (*p*-toluenesulfonic acid) and 0.005M buffer. Reported pHs are those at 64 °C. The concentrations of ApUp and 2′,3′-cAMP ranged from approximately 1×10^{-5} to 2×10^{-4} M and were determined by HPLC as described below. Controls containing buffer, internal standard and ApUp or 2′,3′-cAMP were run alongside of reactions with zinc complexes. Solutions were incubated in microcentrifuge tubes at 64 °C and the reaction was quenched at 0 °C or by addition of EDTA. No reaction of ApUp or 2′,3′-cAMP was observed in the presence of zinc complexes and EDTA. Macrocyclic ligands alone (1 mM) had no affect on cleavage of ApUp or hydrolysis of 2′,3′-cAMP under conditions where zinc complex catalysis was observed.

Samples were analyzed by reversed-phase HPLC, and products were identified by comparison of their retention times to those of standards. An isocratic gradient (A = 10 mM KH_2PO_4; B = methanol–water 3:2) of 80% A and 20% B was used. The concentration of ApUp, 2′,3′-cAMP, 3′-AMP, or 2′-AMP was determined by use of a calibration curve with *p*-toluenesulfonic acid as internal standard. Compounds were detected at either 254 or 260 nm. Extinction coefficients of 23 700 M^{-1} cm^{-1} at 260 nm (ApUp), 13 400 M^{-1} cm^{-1} at 254 nm (3′-AMP), 12 200 at 254 nm (2′-AMP), and 270.4 M^{-1} cm^{-1} at 260 nm or 239 M^{-1} cm^{-1} at 254 nm (internal standard) were used.

The rates of transesterification were measured by monitoring the disappearance of ApUp, and the rates of hydrolysis of 2′,3′-cAMP were measured by monitoring the disappearance of 2′,3′-cAMP. Good pseudo-first-order kinetics were observed to greater than three half-lives with **1** or **2** as transesterification catalysts. The transesterification of ApUp by **3** and the hydrolysis of 2′,3′-cAMP by **1** were determined by the method of initial rates because the reactions were slow. Values of the pseudo-first-order rate constant, k_{obs}, were calculated by using a least-squares program. The slope of a least-squares fit line of log k_{obs} vs log [**1**] (concentration of **1** ranged from 8×10^{-5} to 3×10^{-4} M) was used to determine reaction order in **1**. Rate constants had an average reproducibility of ±10%. All rate constants and catalytic turnover determinations were corrected, where not negligible, for reaction of the control. Experiments demonstrating catalytic turnover in **1** were run at pH 7.6 with the following initial concentrations: [**1**] = 6.00×10^{-6} M and [ApUp] = 1.18×10^{-4} M or [**1**] = 2.67×10^{-5} M and [2′,3′-cAMP] = 1.52×10^{-4} M. After 96 h, $[ApUp]_{initial} - [ApUp]_{final}$ was 3.03×10^{-5} M for the catalytic transesterification experiment. (Theoretical value for a perfect catalyst is 2.89×10^{-5} M ApUp.) After 191 h, $[2',3'\text{-}cAMP]_{initial} - [2',3'\text{-}cAMP]_{final}$ was 6.43×10^{-5} M for the catalytic hydrolysis experiment. (Theoretical value is 7.76×10^{-5} M 2′,3′-cAMP.) The number of catalytic turnovers was determined by measuring the amount of substrate that had reacted by HPLC as described above and dividing by the concentration of the zinc complex. Catalytic turnovers calculated from the amount of product were within experimental error of those calculated on the basis of reacted substrate.

Results

The compounds adenylyl-3′,5′-uridine 3′-monophosphate (ApUp), adenosine 2′,3′-cyclic monophosphate (2′,3′-cAMP), uridine 3′-monophosphate (3′-UMP), adenosine 2′-monophosphate (2′-AMP), and adenosine 3′-monophosphate (3′-AMP) were resolved by reversed-phase high-performance liquid chromatography and detected at either 254 or 260 nm. 2′,3′-cAMP and 3′-UMP were the initial products (eq 1) from transesterification of ApUp by macrocyclic amine complexes of zinc(II). Both 2′-AMP and 3′-AMP were detected toward the end of the kinetic experiments. Neither adenosine nor uridine were detected in reaction mixtures.



(1)

Pseudo-first-order rate constants (k_{obs}) for the transesterification of ApUp by three macrocyclic amine complexes of zinc(II) at 64

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Table I. First-Order Rate Constants for the Transesterification of ApUp in Aqueous Solution at 64 °C, pH 7.6[a]

catalyst	k_{obs}, h^{-1}	substrate
$Zn([9]aneN_3)^{2+}$	0.081[b]	ApUp
$Zn([12]aneN_3)^{2+}$	0.086[b]	ApUp
$Zn(cyclam)^{2+}$	0.0071[b]	ApUp
$Zn([9]aneN_3)^{2+}$	0.019[c]	cAMP

[a] 5 mM HEPES buffer, [9]aneN$_3$ is 1,4,7-triazacyclononane, [12]-aneN$_3$ is 1,5,9-triazacyclododecane, and cyclam is 1,4,8,11-tetraazacyclotetradecane. [b] [catalyst] = 2.0×10^{-4} M. [c] [catalyst] = 1.5×10^{-4} M.

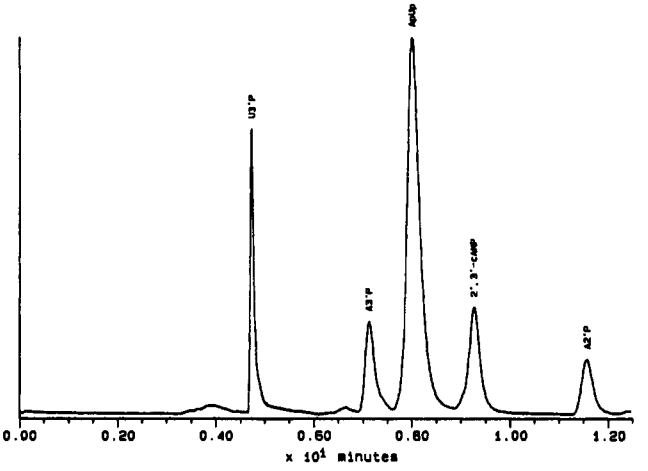


Figure 1. Representative HPLC trace for the transesterification of ApUp by **1** after 4 days. Initial concentrations were [ApUp] = 1.18×10^{-4} and [**1**] = 6.00×10^{-6} at pH 7.6.

°C at pH 7.6 are listed in Table I. A 10% excess of amine ligand was used (zinc:L = 1:1.1). All rate constants were corrected, where not negligible, for transesterification of the control. Under similar conditions, the initial rate of transesterification of ApUp by 0.150 mM $Zn(NO_3)_2$ was twice as fast as that for 0.200 mM **1**. However, precipitates were formed in reaction mixtures of ApUp and $Zn(NO_3)_2$ over time, and simple kinetics were not observed. In contrast, both triamine complexes of zinc(II) (**1** and **2**) showed good pseudo-first-order kinetics for the transesterification of ApUp to greater than three half-lives. Five catalytic turnovers of **1** were observed without any decrease in rate under conditions of excess ApUp. A representative HPLC trace from a typical experiment with excess ApUp and catalytic amounts of **1** is shown in Figure 1.

The terminal phosphate ester of a dinucleotide facilitates transesterification by zinc(II) complexes (**1**–**3**); under similar conditions, adenylyl-3′,5′-uridine did not react. This appears to be a general feature of metal ion catalyzed transesterification of RNA; transesterification of adenylyl-3′,5′-adenosine 3′-monophosphate by zinc nitrate is approximately 100 times faster than transesterification of adenylyl-3′,5′-adenosine by zinc nitrate.[12b] In addition, the rate of transesterification of RNA by metal ions increases with increasing RNA chain length.[11b] Zinc(II)-promoted transesterification of oligomers of adenylic acid p(A_{12}–A_{18}) occurs under milder conditions than here probably because of the longer length of RNA employed in these studies.[6]

A pH–rate profile for the transesterification of ApUp by **1** is shown in Figure 2. An unusual dependence of k_{obs} on pH is observed: from pH 6.7 to pH 7.3, k_{obs} increases with increasing pH; at a pH of 7.6, k_{obs} reaches an optimal value and decreases with further increase in pH. Generally, if a metal-bound hydroxide is the active catalyst, a plateau is observed in the pH–rate profile. However, the reaction order in **1** for concentrations of **1** ranging from 8.0×10^{-5} to 3.0×10^{-4} M that was 0.80 in **1** at pH 7.0 decreased at alkaline pH; at pH 8.3, the reaction order in **1** was only 0.33. This decrease in reaction order may be attributed to the formation of new zinc(II) species at high pH that are inactive as catalysts. These might be formed from oligomerization of

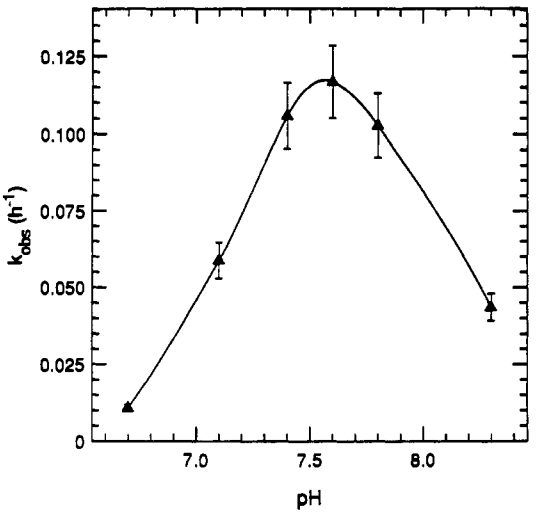


Figure 2. Dependence of k_{obs} on pH for the transesterification of ApUp by **1** in water at 64 °C. [**1**] = 4.00×10^{-4} M; [ApUp] = 1.00×10^{-5} M.

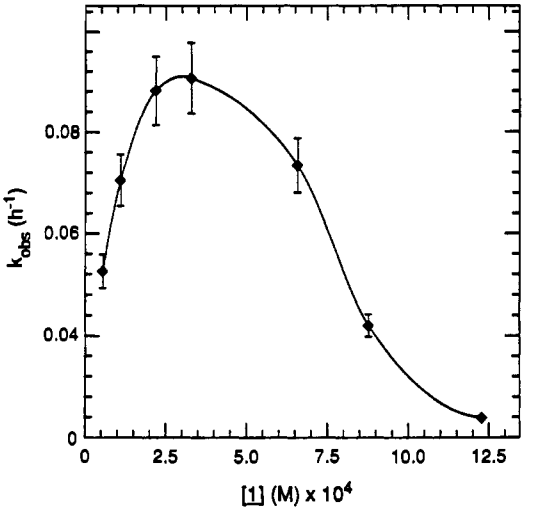


Figure 3. Dependence of k_{obs} on [**1**] for the transesterification of ApUp by **1** in water at 64 °C; pH 7.6. [ApUp] = 1.00×10^{-5} M.

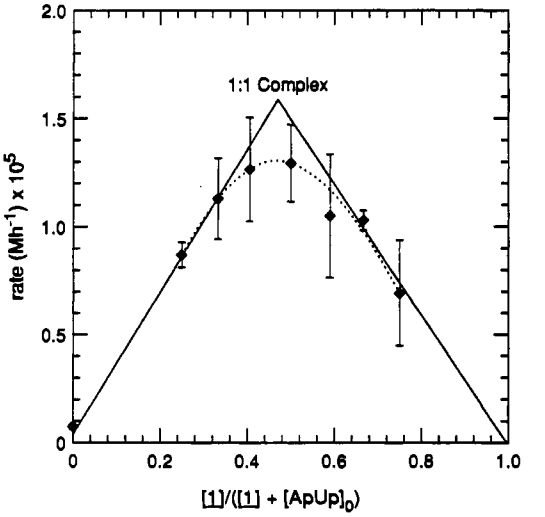


Figure 4. Job's series of continuous variation for ApUp and **1** at 64 °C, pH 7.6. [**1**] + [ApUp] = constant = 3.00×10^{-4} M.

{Zn([9]aneN$_3$)(OH)}$^+$ or may involve zinc(II) nucleotide complexes.[19]

At concentrations of **1** in the millimolar range, transesterification of ApUp by **1** is less rapid than it is with 10-fold less catalyst (Figure 3). To obtain an estimate of the composition

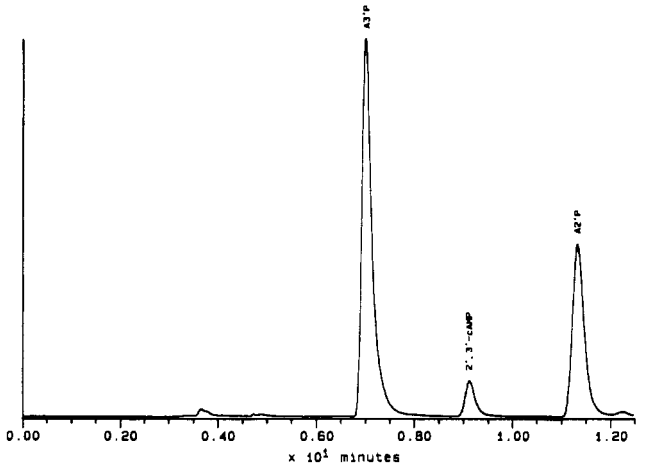
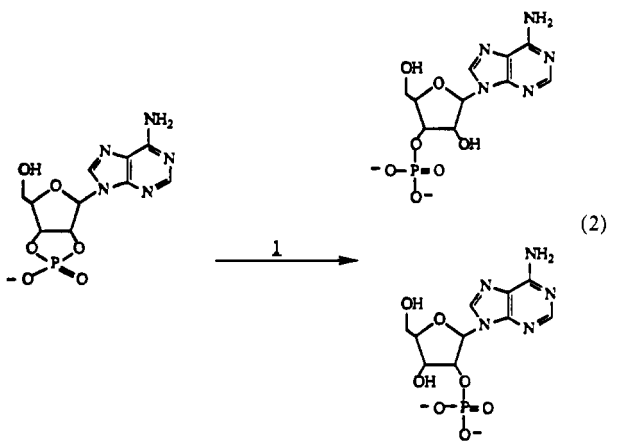


Figure 5. Representative HPLC trace for the hydrolysis of 2',3'-cAMP by **1** after 8 days. Initial concentrations were [2',3'-cAMP] = 1.52×10^{-4} M and [**1**] = 2.67×10^{-5} M at pH 7.6.

of the reactive complex, Job's method[22] of continuous variation was used (Figure 4). The total concentration of **1** and ApUp was kept constant at 3.00×10^{-4} M. Note that a maximal rate at 0.33, 0.50, or 0.67 suggests reactive species with a stoichiometry in **1**:ApUp of 1:2, 1:1, or 2:1, respectively. Our data indicate that a 1:1 complex is probably the most reactive species at pH 7.6.

Complex **1** catalyzed the hydrolysis of 2',3'-cAMP at pH 7.6 (eq 2). The first-order-rate constant for hydrolysis is 0.019 h^{-1} for 0.15 mM **1** (Table I). Greater than two turnovers of **1** were observed when catalytic amounts of **1** were used (Figure 5). The products, adenosine 2'- and 3'-monophosphate were resolved under our HPLC conditions (Figure 5) and were quantitated. A ratio of 2.3:1 (3'-AMP:2'-AMP) was observed after one turnover of **1**.

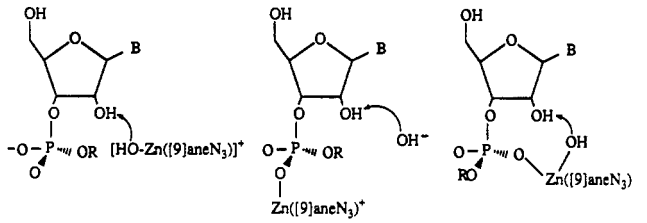


(2)

Discussion

Macrocyclic amine ligands maintain the zinc(II) catalyst in solution; several catalytic turnovers are observed for **1**. In contrast, reactions with $Zn(NO_3)_2$ and ApUp were characterized by precipitates and simple kinetics were not observed. Because **1** is a catalyst, ApUp is cleaved to a larger extent after several hours with the zinc(II) complex compared to zinc(II) salts. Thus, metal complexes may be more useful reagents for RNA cleavage than metal salts even though cleavage may be initially more rapid with a metal salt than with a metal complex. Similarly, a copper(II) complex promoted a greater extent of cleavage of RNA oligomers compared to cleavage promoted by a copper(II) salt.[6] This is consistent with either a higher rate of transesterification by the copper(II) complex compared to the copper(II) salt or with inactivation of the copper(II) salt over time by precipitation of copper–nucleotide complexes. An efficient stoichiometric reagent for RNA cleavage is (triethylenetetramine)cobalt(III);[16] catalytic behavior is not observed because adenosine and an inert cobalt phosphate complex are produced.

Scheme I



a b c

The mechanism of metal ion catalyzed transesterification probably involves electrophilic activation of the phosphorus center by binding of the metal to the phosphate ester; hence, Lewis acidity of the metal center may be important in catalytic transesterification. Polydentate amine ligands may slow down the rate of transesterification by zinc(II) because of a decrease in the Lewis acidity of zinc(II) upon replacement of water ligands by amine ligands. In addition, coordination sites normally available for binding the phosphate ester of ApUp are occupied by amine ligands, and this may result in a decrease in the rate of catalytic transesterification. From Table I, it appears that the higher dentate the amine ligand, the poorer the catalyst. The zinc(II) complex of the tetradentate macrocycle, cyclam, is a much poorer catalyst than the zinc(II) complexes of either of the tridentate macrocycles. The macrocyclic amine complexes Zn[*N*-methyl-(CR)]$^{2+}$ and Zn(CR)$^{2+}$ (*N*-methyl-(CR) = 7-(*N*-methyl)-2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1-(17),2,11,13,15-pentaene; CR = 2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),2,11,13,15-pentaene) promote cleavage of oligomers of adenylic acid[6] and an RNA model compound,[18] respectively. Consistent with our observations on ligand denticity, the zinc complex of the tetradentate CR ligand promotes transesterification of an RNA model compound 63-fold more slowly than does **2**.[18] Macrocyclic ring size appears to have little effect on catalytic transesterification by zinc(II); little difference is observed in the first-order rate constants for **1** compared to **2**.

The pH–rate profile for the transesterification of RNA by metal salts[12b,23] generally shows an optimum in k_{obs} at a pH close to the pK_a of a metal-bound water ligand. The increase in k_{obs} with increasing pH may track the formation of a metal hydroxide that is the active catalyst. The decrease in k_{obs} at pHs greater than this pK_a is attributed to precipitation of the catalyst. To obtain further information about the mechanism of metal ion catalyzed transesterification, the pH dependence of transesterification by the soluble metal complex **1** was investigated. The shape of the pH–rate profile (Figure 2) suggests that Zn([9]aneN$_3$)(OH)$^+$ or its kinetic equivalent is the active catalyst. An optimum in k_{obs} at a pH of 7.6 is observed in the pH region where the water ligand bound to **1** is probably substantially in the hydroxide form ((Zn([11]aneN$_3$),$^{2+}$ pK_a = 8.2; Zn([12]aneN$_3$),$^{2+}$ pK_a = 7.5).[24] Zn([9]aneN$_3$)(OH)$^+$ may catalyze transesterification by acting as a general base to deprotonate the 2'-hydroxyl group (Scheme Ia). A kinetically equivalent mechanism is shown in Scheme Ib. Alternately, Zn([9]aneN$_3$)(OH)$^+$ may catalyze transesterification by acting both as a Lewis acid and as a general base (Scheme Ic). Path c requires that Zn([9]aneN$_3$)(OH)$^+$ become five-coordinate. Although we cannot distinguish between these three possibilities, we favor path c over path a because **1** is a much more effective catalyst than are organic bases with similar pK_as.[25] If a zinc hydroxide complex is the more active catalytic species in

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the transesterification of ApUp, then the inability of $Zn(CR)^{2+}$ (pK_a = 8.69) or 3 (pK_a = 9.77) to form an hydroxide complex at pH 7.6 may be the reason that these complexes are poorer catalysts. Two other metal hydroxide complexes,[6,16] Cu(trpy)-$(OH)^+$ and $Co(trien)(OH)^{2+}$ (trpy = 2,2':6',2''-terpyridine; trien = triethylenetetramine), are implicated as the active catalytic species in the transesterification of RNA.

A decrease in k_{obs} with increasing [1] is found for concentrations of 1 greater than 3.29×10^{-4} M. A similar observation[12b] was reported for the cleavage of dinucleotides of RNA by $Zn(NO_3)_2$. Neither saturation in 1 nor dimerization of 1 would account for the observed kinetics. There are multiple metal binding sites on ApUp, including two phosphate esters, adenosine and, at alkaline pH, uridine. Higher order complexes between 1 and ApUp, formed by occupation of multiple binding sites at high ratios of 1:ApUp, may not readily undergo transesterification. Consistent with this proposal is the 1:1 stoichiometry of the reactive complex as determined by use of Job's method of continuous variation. The shallow curve of Figure 4 is consistent[26] with the formation of a weak complex between 1 and ApUp. At higher or lower ratios of 1 to ApUp, the rate of transesterification decreases because the concentration of the 1:1 reactive complex decreases. There are multiple binding sites on ApUp that could be occupied by 1 at high ratios of 1:ApUp; however, these studies indicate that higher order complexes undergo transesterification more slowly than does the 1:1 complex.

RNA end groups produced by metal ion catalyzed cleavage will depend on the relative rates of transesterification of the RNA strand and subsequent hydrolysis of these products. Hydrolysis of unactivated phosphate diesters is notoriously slow. However, phosphate diesters containing cyclic groups such as 2',3'-cAMP usually hydrolyze more readily than simple phosphate diesters.[27]

Zinc(II) complex 1 catalyzes the hydrolysis of 2',3'-cAMP more slowly than it catalyzes transesterification of ApUp. Because phosphate ester hydrolysis is generally promoted by metal hydroxide complexes[3,5] and at pH 7.6 a substantial proportion of 1 is present in hydroxide form,[24] $Zn([9ane]N_3(OH)^+$ may be the active catalyst in the hydrolysis of 2',3'-cAMP. Both 3',5'-cAMP[3d] and 2',3'-cAMP[16] are hydrolyzed by cobalt(III) complexes at 50 °C, but catalytic behavior is not observed.

The observed (2.3:1) 3'-AMP:2'-AMP ratio is close to the 3:1 ratio observed for hydrolysis of 2',3'-cAMP by $Zn(NO_3)_2$.[11b] In contrast, base-catalyzed hydrolysis gives close to equal amounts of adenosine 2'- and 3'-monophosphates.[28] Regioselective hydrolysis of 2',3'-cAMP by 1 may be related to the nature of the complex between 1 and 2',3'-cAMP. From molecular models, coordination of 1 to both adenosine and phosphate ester does not appear likely. In ATP hydrolysis[2a] by metal ions, the reactive complex has two metal ions and two ATPs with adenine bases stacked and metal ion coordination to an adenine and to a phosphate of different ATP molecules. Formation of a similar reactive dimeric complex of the 2',3'-cAMP complex of 1 could account for preferential formation of a single isomer of AMP. Further investigations are underway to determine the origin of regioselective hydrolysis of 2',3'-cyclic nucleotides by metal complexes.

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Selective Hydrolysis of Unactivated Peptide Bonds, Promoted by Platinum(II) Complexes Anchored to Amino Acid Side Chains†

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Certain platinum(II) complexes attached to the sulfur atom of cysteine, *S*-methylcysteine, and methionine in peptides and other amino acid derivatives promote, under relatively mild conditions, hydrolysis of unactivated amide bonds involving the platinated amino acid. Kinetics of hydrolysis was studied with the substrates *N*-acetyl-L-cysteine, *S*-methyl-L-cysteine, *N*-acetyl-*S*-methyl-DL-cysteine, *N*-(2-mercaptopropionyl)glycine, *N*-acetylmethionylglycine, leucylglycine, reduced glutathione, *S*-methylglutathione, and oxidized glutathione and with complexes of platinum(II) and platinum(IV) containing chloro, aqua, iodo, ethylenediamine, 2,2'-bipyridine, and 2,2':6',2''-terpyridine ligands. When the substrates and platinum promoters are matched so as to aid hydrolysis, the observed rate constant varies between 2.3×10^{-4} and 7.4×10^{-3} min^{-1} at 40 °C, depending on the substrate, promoter, pH, ionic strength, and chloride concentration. Unplatinated (free) substrates and substrates platinated with complexes designed to hinder hydrolysis do not hydrolyze at a detectable rate under identical conditions. The mechanism involves initial aquation of the platinum(II) complex attached to the substrate and a subsequent rate-determining step within the platinated substrate; details of the mechanism are discussed in terms of kinetic evidence and precedents. Hydrolysis is regioselective—it occurs preferably at the amide bond involving the carboxylic group of the platinated amino acid. This study may point the way toward new methods for selective, perhaps even catalytic, cleavage of peptides and proteins with metal complexes.

Introduction

Many biological processes involve hydrolysis of proteins and peptides, but relatively little is known about the mechanism of this reaction. The amide bond is extremely unreactive;[1-3] the half-life for its hydrolysis in neutral solution is ca. 9 years.[4] Hydrolytic procedures in synthetic and biochemical work call for prolonged heating and high concentration of the strongest acids or bases. Kinetic and mechanistic studies have been done almost

† This paper is dedicated to Phan To Su (1956-1990), a graduate student who ably undertook an extension of this study and in her last days still hoped to return to research.

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