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Enormously Fast RNA Hydrolysis by Lanthanide(III) Ions under Physiological Conditions: Eminent Candidates for Novel Tools of Biotechnology[1]

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Lanthanide(III) ions have shown enormous catalyses for the hydrolysis of the phosphodiester linkages in RNA, indicating their high potential for versatile applications to biotechnology and molecular biology. The activity monotonically increases with increasing atomic number in the lanthanide series, the last three ions (Tm^{3+}, Yb^{3+}, and Lu^{3+}) being the most active. Non-lanthanide metal ions are virtually inactive. The pseudo first-order rate constant for the hydrolysis of adenylyl(3′-5′)adenosine (ApA) by $LuCl_3$ (5 mmol·dm^{-3}) at pH 7.2 and 30°C is 1.9×10^{-1} min^{-1} (the half-life is only 3.6 min), corresponding to 10^8-fold acceleration. The product is an equimolar mixture of adenosine and its 2′- or 3′-monophosphate without any byproducts. The 2′,3′-cyclic monophosphate of adenosine is not accumulated much in the reaction mixture. Lanthanide ions also efficiently hydrolyze oligoribonucleotides without a specific base-preference. In ApA hydrolysis by $NdCl_3$ and $GdCl_3$, the dependence of the hydrolysis rate on either the pH or concentration of the metal salt coincides fairly well with the corresponding profile of the equilibrium concentration of the bimetallic hydroxo-cluster $[M_2(OH)_2]^{4+}$ (M=metal ion). Both the formation of the pentacoordinated intermediate and its decomposition are greatly promoted by lanthanide ions. A catalytic mechanism in which two metal ions (or their coordination water) in these tetracationic hydroxo-clusters show acid/base cooperation is proposed.

Key words: lanthanide ion, lutetium(III) ion, metal hydroxo-cluster, RNA hydrolysis, ytterbium(III) ion.

Non-enzymatic hydrolysis of RNA is the subject of growing interest (*1-3*). To date, a number of organic and inorganic catalysts have been reported (*1-30*). However, most of them are not active enough to hydrolyze RNA under physiological conditions (in the absence of natural enzymes, RNA is quite stable and resistant to hydrolysis: *vide infra*). The designing of highly active catalysts is crucially important for further application of non-enzymatic RNA hydrolysis to biotechnology, molecular biology, and therapy.

In a preliminary communication (*10*), the present authors reported that lanthanide ions are enormously active as to RNA hydrolysis. The phosphodiester linkages are hydrolyzed within an hour at pH 7 and 30°C. The last three lanthanide ions (Tm^{3+}, Yb^{3+}, and Lu^{3+}) are especially eminent. Macrocyclic lanthanide complexes for RNA hydrolysis were also reported by Morrow *et al.* (*11*). Subsequently, lanthanide ions and their complexes are widely used for the hydrolysis of a variety of phosphoesters such as RNA (*12, 22, 26, 28, 29*), DNA (*31-36*), the 3′,5′-cyclic monophosphate of adenosine (*37, 38*), phosphatidyl inositol (*39*), and others (*40, 41*). Furthermore, sequence-selective artificial ribonucleases, which cut RNA at the desired sites, were prepared by attaching lanthanide complexes to DNA oligomers as sequence-recognizing moieties (*42-44*).

In spite of the remarkable catalytic activities of lanthanide ions as to RNA hydrolysis and their successful application to various purposes, the mechanism of RNA hydrolysis by these metal ions has not yet been sufficiently clarified. Even the catalytically active species have not been characterized. Recently, Chin *et al.* (*29*) proposed that $[La_2(OH)_5]^+$ is responsible for RNA hydrolysis by La^{3+} (the first in the lanthanide series). However, no kinetic studies have been performed on the catalysis by other lanthanide ions, which are far more active than La^{3+} [the above proposal of La^{3+}-induced RNA hydrolysis is not consistent with our present kinetic results, as described later]. It is not clear (i) why the last three lanthanide ions are the most effective for RNA hydrolysis, or (ii) why lanthanide ions are overwhelmingly superior to non-lanthanide ions in the activity. These two points must be addressed for the designing of still more active catalysts for RNA hydrolysis.

Here we report the results of detailed and systematic studies on lanthanide ion-induced RNA hydrolysis. The

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Abbreviations: ApA, adenylyl(3′-5′)adenosine; A2′p, adenosine 2′-monophosphate; A3′p, adenosine 3′-monophosphate; A, adenosine; A>p, 2′,3′-cyclic monophosphate of adenosine; Apϕ, phenyl ester of adenosine 3′-monophosphate.

dependence of the rate of RNA hydrolysis on either the pH or metal salt concentration is presented. The kinetic results are analyzed in terms of the equilibrium concentrations of metallic species (monomeric ion and metal hydroxo-clusters) in the reaction mixtures. Furthermore, the phenyl ester of adenosine 3′-monophosphate (Apϕ), in which the leaving group in ApA (the 5′-alkoxide ion of A) is replaced by phenolate, was used as a probe to determine the rate-limiting step of the reaction. On the basis of this kinetic evidence, a novel catalytic mechanism for RNA hydrolysis is proposed.

MATERIALS AND METHODS

Materials—Lanthanide metal salts (except for $LaCl_3$ from Nacalai) were purchased from Soekawa. ApA was obtained from Seikagaku Industries, and other diribonucleotides were from Sigma. Apϕ was prepared from A>p and phenol, as described previously (*45*). A 39-mer RNA (5′-AUG CUU CCA GGG CUC UAG UCU AGG AUC UAC UGG CUC CAU-3′) was synthesized with an automated synthesizer, and then ^{32}P-labeled at the 5′-end using [γ-^{32}P]ATP (from Amersham) and T4 kinase (from Sigma). The choice of sequence is rather arbitrary. The labeled oligomer was purified by polyacrylamide gel electrophoresis followed by ethanol precipitation.

All the buffers were prepared with ion-exchanged water, which was purified with a Millipore purification system (Milli-XQ; specific resistance of the water > 18.3 MΩ·cm). These buffers and the reaction vessels were sufficiently sterilized in an autoclave immediately before use. Special care was taken throughout the present experiments to avoid contamination by natural ribonucleases and other metal ions.

Hydrolysis of Diribonucleotides—The hydrolysis of diribonucleotides was achieved at 30°C, and was analyzed by reversed-phase HPLC: Merck LiChrosphere RP-18(e) ODS column; water/acetonitrile = 93 : 7 to 97 : 3 (v/v), depending on the substrate. The reaction mixtures were prepared by adding a lanthanide salt (chloride salt unless otherwise noted) to Hepes buffer solutions [I = 0.1 M ($NaClO_4$)], followed by pH adjustment with a small amount of an NaOH solution (M = mol·dm^{-3}). The reaction was initiated by the addition of a small amount of the stock solution of the substrate (in water) to the mixture. The initial concentration of the substrate was 10^{-4} M.

At appropriate intervals, a specimen was taken from the reaction mixture and subjected to HPLC analysis. The HPLC peaks were unequivocally assigned by coinjection of authentic samples. The reactions were followed for 2-3 half-lives, where fair pseudo first-order kinetics were always observed. The change in pH during the reactions was less than 0.2 unit.

All the rate constants presented here are the averages of the results of duplicate or triplicate runs, which coincided with each other within 10%. The absence of contamination by natural enzymes was confirmed by repeated and careful control experiments.

Hydrolysis of Oligoribonucleotides—The hydrolysis of the 39-mer RNA, ^{32}P-labeled at the 5′-end, was achieved at pH 7.5 or 8.0 (100 mM Tris buffer) and 30°C with an initial concentration of the oligonucleotide of 5×10^{-7} M. The products were directly subjected to denaturing 20% polyacrylamide gel electrophoresis. The scission sites were determined using the fragments obtained on alkaline hydrolysis.

RESULTS

Hydrolysis of Diribonucleotides by $LuCl_3$—Figure 1 depicts a typical HPLC pattern for the hydrolysis of ApA by $LuCl_3$ (5 mM) at pH 7.2 and 30°C. ApA is promptly and stoichiometrically converted to a mixture of adenosine (A) and its 2′- or 3′-monophosphate (A2′p or A3′p). The ratio of the sum of A2′p and A3′p to A is virtually 1:1. The hydrolysis of A2′p and A3′p to A is much slower than the ApA hydrolysis (the validity of this argument has been further confirmed by independent experiments involving authentic samples of these monophosphates). No other byproducts are formed.

The rate constant for ApA hydrolysis is 1.9×10^{-1} min^{-1} (the half-life is only 3.6 min). Thus, more than 10^8-fold acceleration has been achieved with the metal ion [the intrinsic half-life of the phosphodiester linkage in diribonucleotides under physiological conditions is estimated to be 800 years, using the data in a previous paper (*9*)]. This is the fastest RNA hydrolysis ever reported at a physiological pH.

The 2′,3′-cyclic monophosphate of adenosine (A>p: the intermediate for ApA hydrolysis) was rapidly hydrolyzed to A2′p and A3′p, and was accumulated in the mixture in only a marginal amount. The ratio of A2′p to A3′p in the products was about 1/2, which is almost identical with the value for the corresponding alkaline hydrolysis. This result substantiates the absence of contamination by natural ribonucleases in the present reactions, since such enzymatic reactions, if any, should selectively provide the 3′-monophosphate. The activation energy of the ApA hydrolysis (estimated at 30-60°C) is 40 ± 5 kJ·mol^{-1}.

In contrast with the notable hydrolysis of ApA, 2′-deoxyadenylyl(3′-5′)2′-deoxyadenosine was not hydrolyzed to a

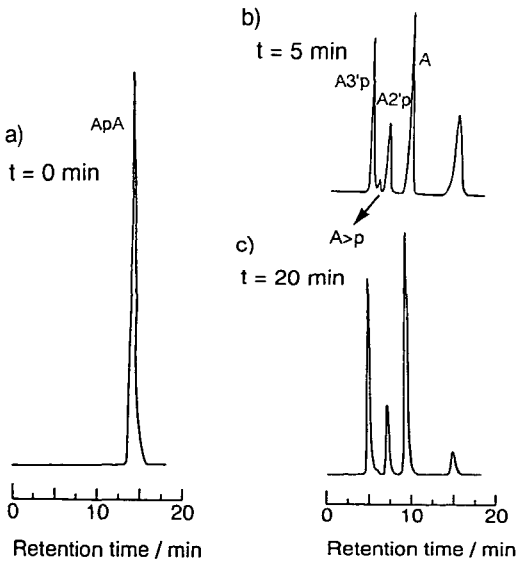

Fig. 1. **HPLC patterns of ApA hydrolysis by $LuCl_3$ (5 mM) at pH 7.2 (50 mM Hepes buffer) and 30°C.** (a) t = 0 min, (b) t = 5 min, and (c) t = 20 min.

measurable extent under the conditions examined. Undoubtedly, the RNA hydrolysis by $LuCl_3$ proceeds *via* an intramolecular attack by the 2′-OH towards the phosphorus atom.

In the ApA hydrolysis by $LuCl_3$, the isomerization of ApA to adenylyl(2′-5′)adenosine did not take place to a measurable extent (the isomer, if any, should be observed at the retention time of 9.4 min on HPLC). The pentacoordinated intermediate, formed on the intramolecular attack by the 2′-OH, is promptly converted to the products before pseudo-rotation at the phosphorus atom occurs. The possibility that the 2′-5′ isomer is formed but not accumulated in the mixtures (due to rapid hydrolysis) is ruled out, since an authentic sample of the isomer is hydrolyzed by $LuCl_3$ at a comparable rate, as is ApA.

Catalytic Activities of Various Lanthanide(III) Ions and Non-Lanthanide Metal Ions—All the lanthanide(III) ions are active as to ApA hydrolysis. As depicted in Fig. 2, the catalytic activity at pH 7.2 monotonically increases with an increase in the atomic number. The activity of Tm^{3+} is about 1/2 that of Lu^{3+}, whereas Yb^{3+} is as active as Lu^{3+}; the half-lives of ApA with these metal ions (5 mM) are 6.4 and 3.3 min, respectively. The Sc^{3+} and Y^{3+} ions are also effective (the rate constants are 1.5×10^{-3} and 4.6×10^{-3} min^{-1}). In contrast, non-rare earth metal ions (Zn^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, and Fe^{3+}) showed no measurable activities as to RNA hydrolysis under the conditions employed. Only rare-earth metal ions are highly effective for RNA hydrolysis.

The rate constants for ApA hydrolysis by $La(ClO_4)_3$ and $La(NO_3)_3$ (5 mM) at pH 7.2 and 30°C are 6.0×10^{-5} and 5.6×10^{-5} min^{-1}, which are almost identical with the corresponding value (7.0×10^{-5} min^{-1}) for $LaCl_3$. The counter ions show no significant effects on the catalytic activities of the lanthanide ions.

A Ce^{4+} salt, $Ce(NH_4)_2(NO_3)_6$, is also active as to RNA hydrolysis. However, its activity is far lower than those of Tm^{3+}, Yb^{3+}, and Lu^{3+}, and is only comparable with that of the Tb^{3+} ion (the rate constant of Ce^{4+} is 1.8×10^{-2} min^{-1}). This is in high contrast with the result for DNA hydrolysis, where Ce^{4+} is overwhelmingly more active than other lanthanide(III) ions and non-lanthanide ions (*33*).

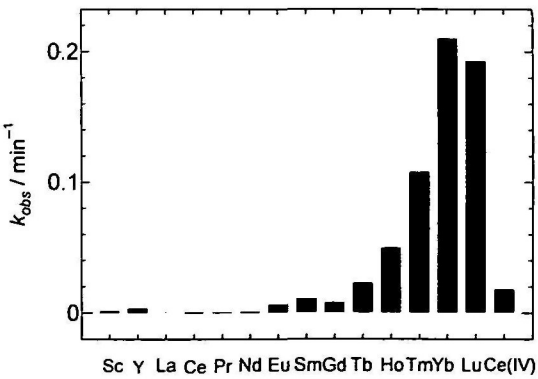

Fig. 2. **Catalytic activities of various rare earth metal ions as to ApA hydrolysis at pH 7.2 and 30°C.** The activities are expressed in terms of the rate constants for the reactions with 5 mM metal ions. All the metal ions [except for $Ce(NH_4)_2(NO_3)_6$] were used as trichlorides. The activities of non-rare earth metal ions (Zn^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, and Fe^{3+}) were too small to be depicted here.

Hydrolysis of Oligoribonucleotides—Lanthanide ions are also effective for the hydrolysis of oligoribonucleotides. As shown in lanes 1-3 and 5 in Fig. 3, $LuCl_3$ (1 mM) hydrolyzes the 39-mer RNA at all the phosphodiester linkages. No specific base-preference for the scission is observed. Totally consistently, the rates of hydrolysis of six diribonucleotides (UpG, CpC, GpA, ApU, UpA, and ApA) by $LuCl_3$ are not much different from each other. The most reactive UpG is hydrolyzed only 2.2 times as fast as the least reactive ApA.

All the bands in Fig. 3 comigrated with those on alkaline hydrolysis (the OH lane). Thus, the 3′-termini of the fragments are 2′- and 3′-monophosphates, rather than the 2′,3′-cyclic monophosphates (note that these fragments are ^{32}P-labeled at the 5′-ends). This result is in fair agreement with those for diribonucleotide hydrolysis, where the main products are the 2′- and 3′-monophosphates of ribonucleosides (together with the ribonucleosides on the 3′-sides), and the cyclic monophosphates are not accumulated much (see Fig. 1). When $[LuCl_3]_0$ was increased to 10 mM, the oligonucleotide was almost completely degraded into smaller fragments within 1 h at either pH 7.5 or 8.0 (see lanes 4 and 6 in Fig. 3).

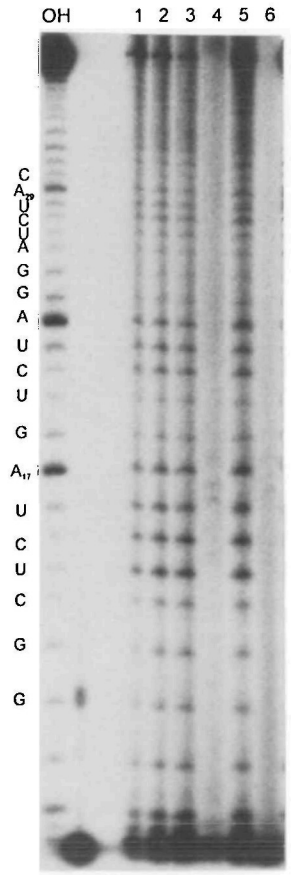

Fig. 3. **Polyacrylamide gel electrophoresis patterns of the hydrolysis of a 39-mer RNA by $LuCl_3$ at 30°C.** Lanes 1-3, $[LuCl_3]_0=1$ mM, pH 7.5, and $t=1$ h (lane 1), $t=3$ h (lane 2), and $t=6$ h (lane 3). Lane 4, $[LuCl_3]_0=10$ mM, pH 7.5, and $t=1$ h. Lane 5, $[LuCl_3]_0=1$ mM, pH 8.0, and $t=1$ h. Lane 6, $[LuCl_3]_0=10$ mM, pH 8.0, and $t=1$ h. The OH lane contains the products obtained on alkaline hydrolysis. The sequence of the RNA is presented under "MATERIALS AND METHODS."

pH Dependence of the Rate of ApA Hydrolysis—Figure 4 shows the pH-rate constant profiles for ApA hydrolysis by various lanthanide ions. For all the metal ions, the rate constant (k_{obs}) steeply increases with increasing pH up to a certain value, and then attains a plateau above that value. The slope in the steep region is about 2 for La^{3+} and Nd^{3+}, and is slightly greater (about 2.5) for Tb^{3+} and Lu^{3+}. As evidenced below, these pH dependencies reflect the formation of bimetallic hydroxide-clusters which are the catalytically active species. The cluster formation is more predominant at higher pH.

As the atomic number of the lanthanide ion increases, the steep straight line gradually shifts towards the lower pH side. For La^{3+}-induced RNA hydrolysis, for example, the steep straight line and the horizontal line (at a higher pH) intersect around pH 9. However, the intersections are located near pH 8.2 for Nd^{3+}, pH 7.7 for Tb^{3+}, and pH 7.5 for Lu^{3+}. As a result, the concentration of the active species at pH 7.0 is greater for a lanthanide ion with a greater atomic number. As to the last three lanthanide ions (Tm^{3+}, Yb^{3+}, and Lu^{3+}), significant portions of them exist as the active species at a physiological pH, and thus they are quite superb for RNA hydrolysis. In contrast, La^{3+} is poor in the activity, since the concentration of the active species at pH 7.0 is marginal (its activity is 10^3-fold lower than that of Lu^{3+}). Only in highly alkaline solutions, where its active species are abundant, does the La^{3+} show sufficient activity (*29*).

Quantitative Analysis of the pH-Rate Constant Profiles—Detailed analysis of the pH-rate constant profiles in Fig. 4 requires quantitative information on the solvolysis of the metal salts. Since the solvolysis of the Nd^{3+} and Gd^{3+} ions is rather well documented in the literature (*46, 47*), the pH-rate constant profiles for the ApA hydrolysis by these metal ions have been precisely investigated. No data are available on the solvolysis of Tm^{3+}, Yb^{3+}, and Lu^{3+}, which are the most effective for RNA hydrolysis. On the other hand, previous data on the solvolysis of other metal ions are not completely consistent with each other (*48*).

In solutions containing $NdCl_3$, the following two equilibria hold (*46*). Thus, the equilibrium concentrations of the free metal ion ($[Nd^{3+}]$), the metal ion carrying a hydroxide ion ($[Nd(OH)]^{2+}$), and the bimetallic hydroxo-cluster ($[Nd_2(OH)_2]^{4+}$) in the reaction mixtures are calculated by using the values of K_1 and K_{22}.

$$Nd^{3+} + H_2O \overset{K_1}{\rightleftharpoons} [Nd(OH)]^{2+} + H^+ \quad pK_1 = 9.4 \qquad (1)$$

$$2Nd^{3+} + 2H_2O \overset{K_{22}}{\rightleftharpoons} [Nd_2(OH)_2]^{4+} + 2H^+ \quad pK_{22} = 13.9 \qquad (2)$$

As depicted in Fig. 5a, the pH-rate constant profile for Nd^{3+}-induced ApA hydrolysis fairly well fits the theoretical line (the solid one), which represents the pH dependence of the equilibrium concentration of $[Nd_2(OH)_2]^{4+}$. The corresponding curves for $[Nd^{3+}]$ and $[Nd(OH)]^{2+}$ (the dotted ones) are far from the experimental results. It is strongly indicated that the tetracationic bimetallic hydroxo-cluster ($[Nd_2(OH)_2]^{4+}$) is responsible for the remarkable catalysis of RNA hydrolysis by $NdCl_3$.

Similarly, the pH-rate constant profile for the ApA hydrolysis by $GdCl_3$ satisfactorily coincides with the pH dependence of the equilibrium concentration of $[Gd_2(OH)_2]^{4+}$ (Fig. 5b). The equilibria for the solvolysis of $GdCl_3$ are similar to those for $NdCl_3$: $pK_1 = 9.2$ and $pK_{22} = 14.2$ (*47*).

Dependence of the Rate of ApA Hydrolysis on the Concentration of Lanthanide Ions—When the pH was kept constant at 7.5, the rate constant for the $NdCl_3$-induced ApA hydrolysis monotonically increased with an increase in the charged concentration of $NdCl_3$ ($[NdCl_3]_0$). However, the increase was not linear. Rather, the log-log plot of the

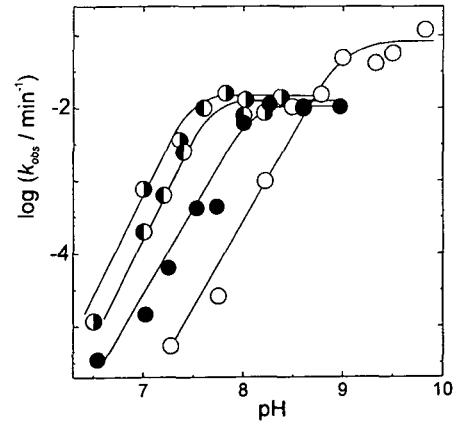

Fig. 4. **pH-rate constant profiles of ApA hydrolysis by various lanthanide ions at 30°C.** La^{3+}, ○; Nd^{3+}, ●; Tb^{3+}, ◐; Lu^{3+}, ◑. The concentration of the metal salt was kept constant at 1 mM. Only the results for typical metal ions are presented here to make the figure clearer, since the profile monotonically changes with an increase in the atomic number.

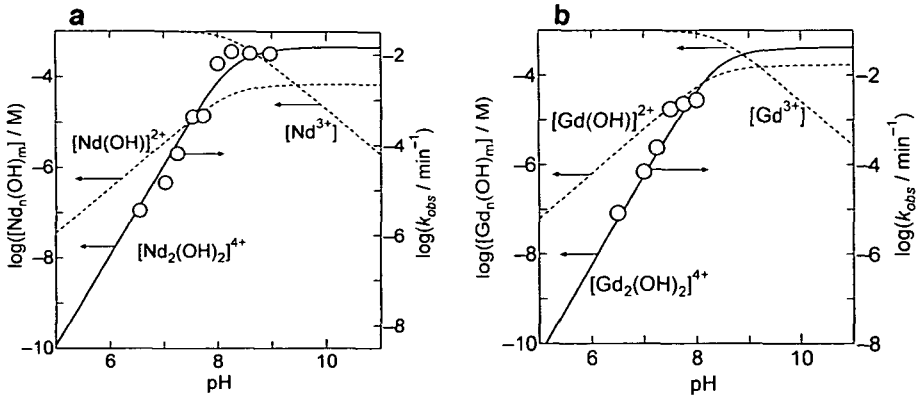

Fig. 5. **Quantitative analysis of the pH-rate constant profiles of ApA hydrolysis at 30°C by (a) $NdCl_3$ and (b) $GdCl_3$.** The solid line is the theoretical one showing the concentration of $[M_2(OH)_2]^{4+}$, whereas the dotted lines are the ones for $[M^{3+}]$ and $[M(OH)]^{2+}$ (M = metal ion). All the theoretical lines were calculated using the corresponding K_1 and K_{22} values. Only the data in the region where the solutions are virtually homogeneous are used here (the solutions are turbid at quite a high pH).

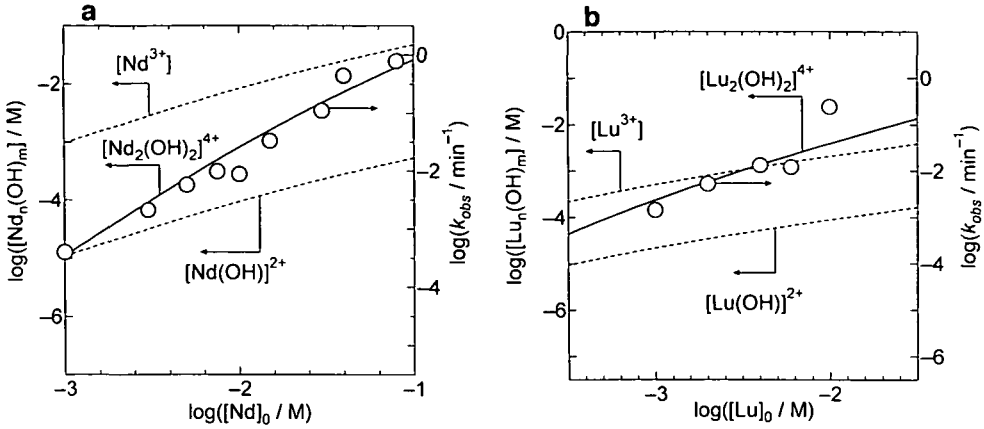

Fig. 6. **Log-log plots of the pseudo first-order rate constant (k_{obs}) *vs.* (a) $[NdCl_3]_0$ and (b) $[LuCl_3]_0$ for ApA hydrolysis at 30°C.** The pH is 7.5 in (a) and 6.8 in (b). The solid line is the theoretical one showing the concentration of $[M_2(OH)_2]^{4+}$, whereas the dotted lines are the ones for $[M^{3+}]$ and $[M(OH)]^{2+}$. The lines in (b) were calculated with the assumptions described in the text.

rate *vs.* $[NdCl_3]_0$ could satisfactorily be superimposed on the theoretical line showing the equilibrium concentration of $[Nd_2(OH)_2]^{4+}$ (the solid line in Fig. 6a). The equilibrium concentrations were calculated using Eqs. 1 and 2. The experimental points never fitted the lines for the equilibrium concentration of either $[Nd^{3+}]$ or $[Nd(OH)]^{2+}$, as was the case in Fig. 5a. The validity of the mechanism involving $[Nd_2(OH)_2]^{4+}$ as the active species was further confirmed.

The corresponding log-log plot for $LuCl_3$, which is the most active among the lanthanide ions, is depicted in Fig. 6b. Here data on the solvolysis of the metal salt were not available, and so the theoretical line (the solid one) was obtained with the following two assumptions: (i) The equilibria for the solvolysis of Lu^{3+} are identical with those for Nd^{3+} and Gd^{3+}, and (ii) the K_{22} value in the equilibria is identical with that for $NdCl_3$. The second assumption is based on the fact that K_{22} is almost identical for all the lanthanide ions from Nd^{3+} to Er^{3+} (*48*). The K_1 value was estimated from the first dissociation constant of the Lu^{3+}-bound water ($10^{-8.17}$) in the literature (*49*). The experimental points satisfactorily fit the theoretical line, indicating that the bimetallic hydroxo-cluster is also an active species here.

Apϕ Hydrolysis by Lanthanide Ions—In order to shed light on the reaction mechanism, Apϕ was hydrolyzed with lanthanide ions. In Apϕ, the leaving group in ApA (the 5′-alkoxide ion of A) is replaced by phenolate as a better leaving group (the pK_a of phenol is 9.8, whereas that of the 5′-OH is around 15). In the absence of metal ions, the pH-rate constant profile for Apϕ hydrolysis is a straight line of slope 1.0 down to pH 6. Alkaline hydrolysis is predominantly taking place. The hydrolysis rate is 10^5-fold faster than that of ApA (the rate constants at pH 8.0 are 7×10^{-3} and 7×10^{-8} min^{-1}, respectively). The products are A>p and phenol (A>p is then slowly hydrolyzed to A2′p and A3′p). The hydrolysis of Apϕ proceeds *via* the same pathway as RNA hydrolysis does (the intramolecular attack by the 2′-OH of ribose toward the phosphorus atom).

It is noteworthy that Apϕ hydrolysis is accelerated only marginally by lanthanide ions: the rate constant at pH 8.0 in the presence of Tm^{3+} (5 mM) is 2×10^{-1} min^{-1}, which is only 3 times greater than the value in the absence of the metal ion. This result is in marked contrast with the enormous acceleration (10^8-fold) of ApA hydrolysis by the metal ion. Apparently, the rate-limiting step in RNA hydrolysis is different from that in Apϕ hydrolysis, and the lanthanide ions effectively accelerate the rate-limiting step for RNA hydrolysis.

DISCUSSION

RNA Hydrolysis by Lanthanide Ions—Lanthanide ions are characterized by their enormous activities as to RNA hydrolysis. The phosphodiester linkages, which are highly stable (as long as ribonucleases are absent), are efficiently hydrolyzed under physiological conditions. Their activities are far greater than those of other catalysts ever reported. The potential of lanthanide ions for versatile applications is strongly indicated.

As clearly evidenced in the present paper, tetracationic bimetallic hydroxo-clusters ($[M_2(OH)_2]^{4+}$; M=metal ion) are responsible for the catalysis by Nd^{3+} and Gd^{3+} (Figs. 5 and 6a). The dependence of the rate of RNA hydrolysis on $[LuCl_3]_0$ is also consistent with the catalysis by the bimetallic species (Fig. 6b). In the corresponding pH-rate constant profile in Fig. 4, the slope from pH 6.5 to 7.5 is slightly greater than 2. Probably, the $[Lu_2(OH)_3]^{3+}$ species, in addition to $[Lu_2(OH)_2]^{4+}$, participates in the catalysis to some extent. The greater acidity of Lu^{3+} facilitates the deprotonation of the water bound to the cluster. It is assumed that the catalysis by other lanthanide ions is also associated with the bimetallic hydroxo-clusters, although the participation of aggregates involving more than two metal ions is plausible. Direct assignment of the active species for these metal ions was unsuccessful, because of the lack of concrete data on their solvolysis.

In the pH-rate constant profile for $LaCl_3$-induced ApA hydrolysis, the slope in the pH 7.2-9.0 region is around 2 (see Fig. 4). This value is far smaller than the one (5.0) reported by Chin *et al.* (*29*). On the basis of the slope of 5, these authors proposed that the active species is $[La_2(OH)_5]^+$. In their study, however, the slope of 5 was evaluated using only three points in a narrow pH range (from 8.4 to 8.8). Thus, we believe that our value, which was determined very carefully by using many experimental points in a wider pH range, is correct. The active species should be $[La_2(OH)_2]^{4+}$ (or higher aggregates having two OH^-), rather than $[La_2(OH)_5]^+$.

The mechanism involving the bimetallic hydroxo-cluster is further supported by the gradual increase in the catalytic activity with increasing atomic number (Fig. 2). The steep straight line in the pH-rate constant profile, which reflects

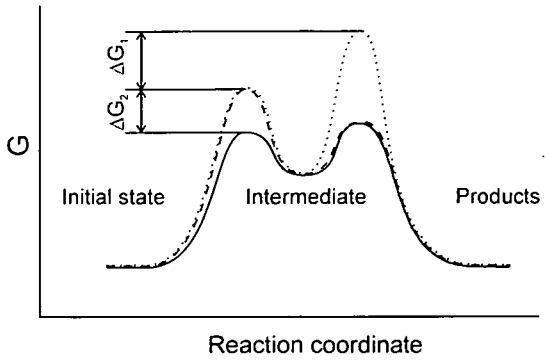

Fig. 7. **Energy profiles of lanthanide ion-induced hydrolysis of RNA (the solid line).** The profiles for the alkaline hydrolysis of RNA (the dotted line) and 5′O→S substituted RNA (the broken line), which are essentially identical with those in Ref. *50*, are also presented.

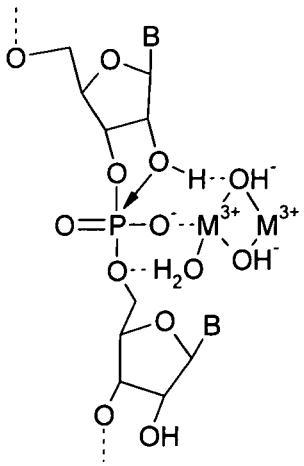

Fig. 8. **Proposed reaction mechanism for RNA hydrolysis by the bimetallic hydroxo-cluster.**

the promoted formation of the active species at higher pH, shifts towards the lower pH side in this order (see Fig. 4). Thus, the last three ions (Tm^{3+}, Yb^{3+}, and Lu^{3+}) are the most active for RNA hydrolysis under physiological conditions.

Assignment of the Step Which Is Susceptible to the Catalysis by Lanthanide Ions—The energy profile for alkaline hydrolysis of RNA is presented as a dotted line in Fig. 7 (*50*). Here, the departure of the 5′-OH from the phosphorus atom, rather than the intramolecular nucleophilic attack by the 2′-OH towards the phosphorus atom, is rate-limiting. This proposal is based on the finding that the substitution of 5′-oxygen with sulfur in RNA promotes the hydrolysis of the corresponding phosphodiester linkage by 10^4-10^6-fold (*50, 51*). The pK_a of a thiol (~10.5) is more than 5 units smaller than that of an alcohol. The decrease in the activation free energy for RNA hydrolysis is designated as ΔG_1 in Fig. 7.

The argument is further substantiated by the fact that the *S*-substitution of non-bridging oxygens in the phosphodiester linkages causes no notable acceleration (*52*). Although the *S*-substitution of 3′-oxygen also accelerates RNA hydrolysis, it is probably associated with the relief of the strain in the five-membered ring (formed through the nucleophilic attack by 2′-OH), as indicated by the authors of the paper (*53*). Consistently, alkaline hydrolysis of Apϕ, which has a better leaving group, is 10^5-fold faster than that of ApA.

In the presence of lanthanide ions, however, Apϕ is hydrolyzed at comparable rates as is ApA: *e.g.*, the difference is only 3-fold when $[TmCl_3]_0 = 5$ mM. Thus, the decomposition of the intermediate is not totally rate-limiting any more in lanthanide ion-induced RNA hydrolysis. It is concluded that the catalysis by these metal ions is primarily due to the promotion of decomposition of the intermediate.

However, it is noteworthy that the magnitude of the acceleration of dinucleotide hydrolysis by Tm^{3+}, Yb^{3+}, and Lu^{3+} (10^8-fold) is much greater than the corresponding value (10^4-10^6-fold) observed on the 5′O→S substitution in RNA. Thus, these metal ions catalyze the formation of a pentacoordinated intermediate as well. This catalysis further decreases the activation free energy for RNA hydrolysis by the amount of ΔG_2 (compare the solid line in Fig. 7 with the broken one). Because of these duplicate catalyses of both the formation of the intermediate and its decomposition, RNA is promptly hydrolyzed. The decrease in activation free energy caused by lanthanide ions is mostly ascribed to a decrease in activation enthalpy. The activation enthalpy for alkaline hydrolysis of ApA is 78 ± 8 kJ·mol^{-1} (estimated at 30-60°C and pH 13), while the corresponding value for Lu^{3+}-catalyzed hydrolysis is 40 kJ·mol^{-1} (as described above). The difference between these two values (38 kJ·mol^{-1}) is close to the decrease in activation free energy (46 kJ·mol^{-1}) caused by Lu^{3+}, which was estimated from the magnitude of its acceleration (10^8-fold).

Proposed Mechanism for Lanthanide Ion-Induced RNA Hydrolysis—The mechanism of RNA hydrolysis by bimetallic hydroxo-clusters is schematically depicted in Fig. 8. First, the phosphodiester linkage in RNA is coordinated to one of the metal ions in the cluster. This complex formation increases the electrophilicity of the phosphorus atom. Then, the hydroxide ion bound to the cluster activates the 2′-OH of RNA as a general base catalyst and promotes its intramolecular nucleophilic attack towards the phosphorus atom. The decrease in the energy barrier for the formation of the pentacoordinated intermediate (ΔG_2 in Fig. 7) is mainly due to these factors. On decomposition of the resultant intermediate to the products, the coordination water on one of the metal ions (or the metal ion itself) functions as a general acid catalyst and stabilizes the leaving group. The 5′-alkoxide ion of ribose is otherwise too unstable to be removed. It is also plausible that the acid catalysis and the base catalysis take place concertedly, rather than in a stepwise manner, as described above.

The proposed mechanism is consistent with the recent finding of the cooperation of two metal ions in phosphoester hydrolysis (*18, 20, 22-24, 54-59*). Here, each of the two metal ions plays a role as an acid catalyst or a base catalyst. Ribozymes also take advantage of the cooperation of two Mg^{2+} ions (*60*). Furthermore, this bimetallic cooperation is reminiscent of the reaction mechanisms observed for some natural enzymes (*61*).

The enormous catalyses by lanthanide ions, in comparison with those by non-lanthanide ions, are ascribed to the following factors: (i) Bimetallic hydroxo-clusters (or

higher-ordered aggregates) are efficiently formed under physiological conditions, and two (or more) metal ions in them are oriented appropriately for acid/base cooperative catalysis. Otherwise, the cooperation would be much less efficient. (ii) The metal ions and/or their coordination water are eminent as acid and base catalysts. (iii) The large positive charges on the metal ions electrostatically stabilize the negatively charged transition state of RNA hydrolysis (this effect is magnified in the hydroxo-clusters). (iv) The complex formation between the phosphodiester linkage in RNA and the metal ions is efficient, due to the hard characters of the metal ions.

In conclusion, non-enzymatic hydrolysis of the phosphodiester linkages in RNA has been successfully achieved with lanthanide ions under physiological conditions. The scission is totally hydrolytic, and no specific base-preference exists. The high potential of lanthanide ions as the catalytic center of artificial ribonucleases is evidenced (*42-44*). Preparation of lanthanide complexes which are even more active for RNA hydrolysis and are applicable to various purposes is currently under way in our laboratory.

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