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Lanthanide ions for the hydrolysis of 5′-mononucleotides and 3′-mononucleotides

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Abstract—Cleavage of adenosine-5′-monophosphate (5′-AMP), guanosine-5′-monophosphate (5′-GMP), adenosine-3′-monophosphate (3′-AMP) and guanosine-3′-monophosphate (3′-GMP) by lanthanides was investigated by NMR and the method of measuring the liberated phosphates. Rapid cleavage of both 5′-mononucleotides and 3′-mononucleotides by Ce^{III} and Ce^{IV} under air at pH 9 and 37°C was observed. Other lanthanides showed less efficiency for hydrolyzing 5′-mononucleotides but 3′-mononucleotides were catalyzed by a range of lanthanide ions. The mechanism for hydrolyzing 3′-mononucleotides by lanthanides was investigated. The notable difference in reactivity between Ce^{III} and the other lanthanide ions under air was further studied showing that the cleavage is enhanced with increasing molar fraction of Ce^{IV}. The fast cleavage of mononucleotides by Ce^{III} under air at pH 9 is ascribed to the resultant Ce^{IV} in the reaction mixture. © 1997 Elsevier Science Ltd

Keywords: artificial restriction enzyme; hydrolytic cleavage; 5′-mononucleotides; 3′-mononucleotides; $Ce(NH_4)_2(NO_3)_6$; lanthanide ions.

Recently artificial nucleases for the oxidative of nucleic acids primarily at the ribose residues have attracted much interest [1]. However, artificial systems involving fission of the phosphodiester linkage are scarce [2], the greatest obstacle being the lack of appropriate catalytic residue for the purpose. Early studies by Bamann [3–5] indicated the effectiveness of lanthanide ions in the hydrolysis of glycerol phosphates. These and transition metal ions have also been used as catalysts for the cleavage of ribose phosphates but, the catalytic mechanism is not distinct. Recently, Komiyama *et al.* succeeded in the first nonenzymatic hydrolysis of linear DNAs by using lanthanides and the catalytic activity of $CeCl_3$ was especially high, which was ascribed to the intramolecular attack by the Ce^{IV}-bound hydroxide ion [6–8]. This proposal has urged us to study the hydrolysis mechanism more in detail and indicate the tetravalent positive charge of the Ce^{4+} effect exists in the hydrolysis of the phosphodiester bonds of mononucleotides. In addition, the notable difference in the degree of hydrolysis between 5′-mononucleotides and 3′-mononucleotides by lanthanides was studied in detail.

EXPERIMENTAL

Reagents

5′-AMP, 5′-GMP, 3′-AMP and 3′-GMP were obtained from the Sigma Company and the purities of all nucleotides and nucleosides were identified as a single peak by HPLC. $(NH_4)_2Ce(NO_3)_6$ was obtained from the Aldrich Chemical Company. $Ce(SO_4)_2$ solution was prepared by dissolving CeO_2 in H_2SO_4. The concentration of Ce^{IV} was determined by titration with $Fe(SO_4)(NH_4)_2$. $CeCl_3$ was prepared by firing 99.99% cerium oxalate and dissolving it in HCl with the elimination of excess acid. Lanthanide chlorides were prepared from their oxides (99.99%). The concentration was determined by EDTA. All other reagents were of analytical grade.

Apparatus

HPLC was performed on a HITACHI 638-50 at 254 nm. A Spherisorb C_{18} column was used. ^1H NMR and ^{31}P NMR spectra were obtained on a Varian UNITY 400 NMR spectrometer at 37°C and ^{31}P

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NMR spectra were taken at 161.9 MHz. 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) and 85% phosphoric acid were used as internal reference and external reference, respectively. The amount of Ce^{IV} was determined by spectrophotometric measurement in absorbance at 340 nm in 0.5 mol dm^{-3} H_2SO_4 and 99.99% $Ce(NH_4)_2(NO_3)_6$ was used as the standard.

Measurement of nucleotides hydrolysis

All dephosphorylation experiments were carried out at 37°C and the pH was adjusted with NaOH or HCl with a glass stick (the change in volume was negligible). The concentration of liberated phosphate from [5′-AMP] and [3′-AMP] was determined with molybdate reagent. [5′-AMP] or [3′-AMP] at time *t* are given by $[5'\text{-NMP}]_t = [5'\text{-NMP}]_0\text{-}[PO_4]_t$, where $[5'\text{-NMP}]_0$ is the initial concentration of 5′-AMP and $[5'\text{-NTP}]_t$ and $[PO_4]_t$ are at time *t*. The free phosphate initially present (1–2%) was taken into account.

RESULTS AND DISCUSSION

Figure 1 shows that various lanthanides (La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+}, Lu^{3+}, Sc^{3+}, Y^{3+} and Ce^{4+}) accelerate the cleavage of 3′-AMP and 3′-GMP at pH 9, 37°C, 10 h. 3′-Mononucleotides were catalyzed by a range of lanthanide ions, both Ce^{3+} (initial valence) and Ce^{4+} showed high activities for hydrolyzing 3′-mononucleotides. In addition, the hydrolysis of 5′-AMP and 5′-GMP by lanthanides were also studied, it is interesting that lanthanides show nonactivities for hydrolyzing 5′-AMP and 5′-GMP, except for Ce^{3+} and Ce^{4+}. This reflects the high efficiency for hydrolyzing nucleotides by Ce^{4+} and preferential scission of the P—O(3′) bond over the P—O(5′) bond by lanthanides.

Cleavage of 3′-AMP to adenosine (A) and inorganic phosphate by $CeCl_3$ were clearly evidenced by ^1H NMR and ^{31}P NMR (Fig. 2). The signals for 3′-AMP (the C_1'—H protons of 3′-AMP and the chemical shift of phosphate of 3′-AMP at 6.18, 6.20 and

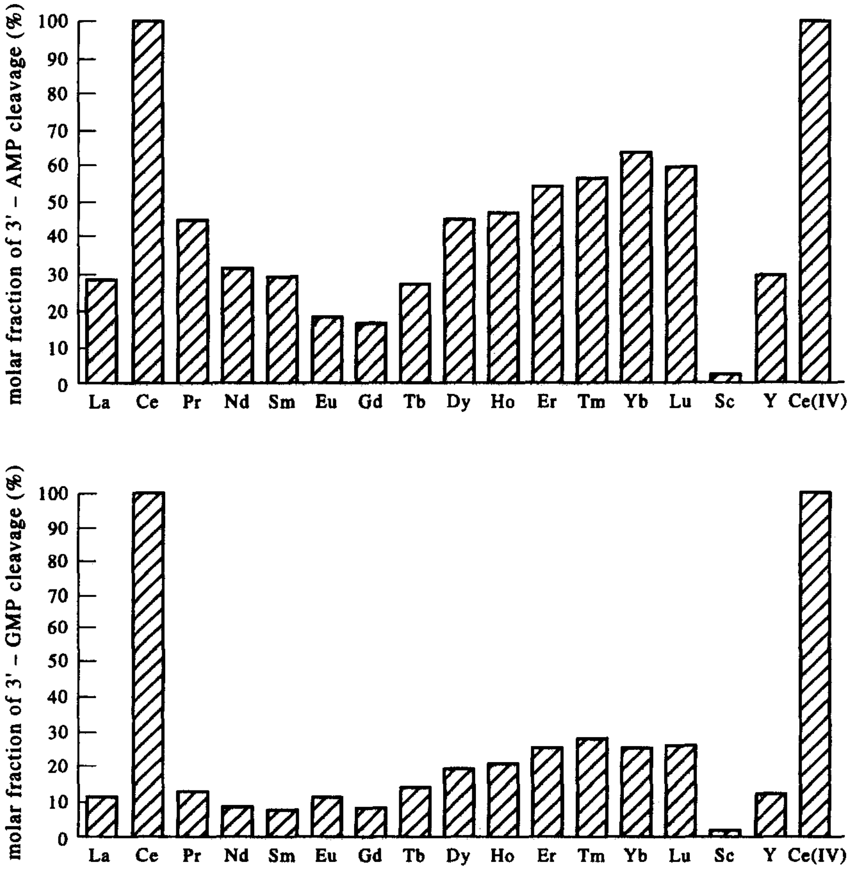

Fig. 1. Effects of various $LnCl_3$ (9.6 mmol dm^{-3}) under air on the cleavage of 3′-AMP (0.2 mmol dm^{-3}) and 3′-GMP (0.2 mmol dm^{-3}) at pH 9, 37°C and 10 h.

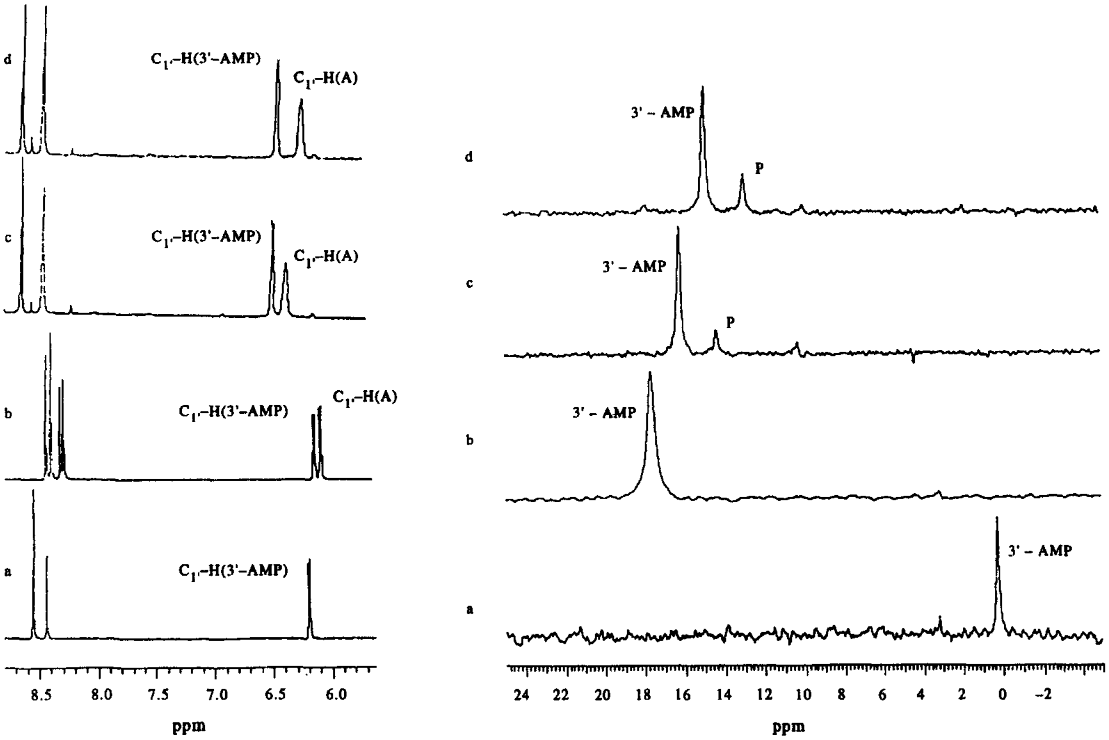

Fig. 2. I: ^{1}H NMR spectra for the cleavage of 3′-AMP (0.01 mol dm^{-3}) by Ce^{3+} (0.2 mol dm^{-3}) under air at pH 9 and 37°C: (a) pure sample of 3′-AMP, (b) mix A to the solution of 3′-AMP, (c) 8 h and (d) 16 h. II: ^{31}P NMR spectra for the cleavage of 3′-AMP (0.01 mol dm^{-3}) by Ce^{3+} (0.2 mol dm^{-3}) under air at pH 9 and 37°C: (a) 0, (b), 2h, (c) 8 h and (d) 16 h.

0.34) [9] decrease accompanied by an increase of C'_1—H signals and chemical shift or inorganic phosphate for adenosine (A) and free phosphates at 6.41, 6.39 and 14.43. In order to verify our results, cleavage of 3′-AMP by Ce^{3+} (initial valence) was further investigated by HPLC which by comparison of their retention time to those of standards, the formation definite amount of A was determined, and no other products were detected.

The fact mentioned above further proved the results of the method of measuring the liberated phosphates and indicated that Ce^{3+} hydrolyzed 3′-AMP to A and free phosphate. The same results were also observed for hydrolyzing other nucleotides by Ce^{3+}.

Table 1. The influence of the lanthanum (III) ion (0.1 mol dm^{-3}) on the ^{31}P NMR chemical shift of 3′-AMP (0.01 mol dm^{-3}) at pH 9

Lanthanide ions	pH	P	Δδ
—	9	4.39	
Sc^{3+}	9	4.37	0.02
Y^{3+}	9	4.35	0.04
La^{3+}	9	4.31	0.08

We investigated the notable differences in reactivity between 5′-mononucleotides and 3′-mononucleotides by lanthanides. We noticed that lanthanides showed great activities for hydrolyzing 3′-AMP and 3′-GMP except for Sc^{3+}. The Sc^{3+} complex with OH^- is quite stable [10] and the larger stability for the Sc^{3+} complex with OH^- results in the lesser activity of the Sc^{3+} complex with the phosphate group of 3′-AMP under basic conditions. Furthermore, we studied the effects of Sc^{3+}, Y^{3+} and La^{3+} on the chemical shift of phosphate of 3′-AMP (Table 1). The upfield shift of signals of phosphate was obvious and the most active species for the $LnCl_3$-induced shift of 3′-AMP is La^{3+}, which is more active than Y^{3+} and the least active species is Sc^{3+}. In addition, we investigated the hydrolysis of deoxyadenosine-3′-monophosphate (3′-dAMP) by lanthanides. The fact of less activity for cleavage of 3′-dAMP by lanthanides indicated that cleavage of 3′-AMP is related to the 2′-hydroxide of 3′-AMP. It was reported by Bamann *et al.* that the hydroxides of La^{3+} and Ce^{3+} promoted the hydrolysis of nucleotides and indicated that cleavage depends on the function of neutralization of positive charge of Ln^{3+} to weaken the electron density of the hydrolysis-susceptible bonds. The cleavage of nucleotides should be great in acidic condition and it has been known that cleavage of nucleotides by lanthanide ions only occurred in basic conditions. The possible mechanism for the cleavage of 3′-mononucleotides by lanthanide ion is

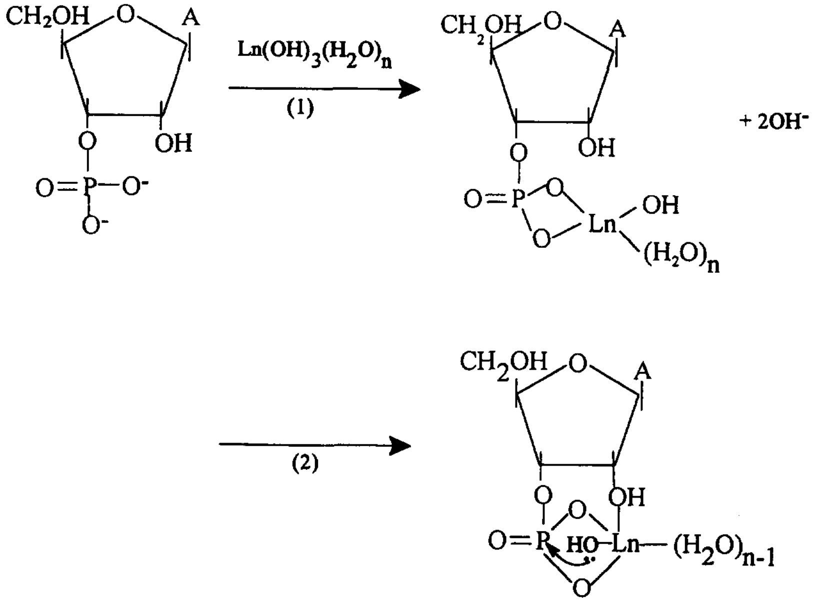

Fig. 3. Proposed mechanism for the 3′-AMP cleavage by $LnCl_3$ at pH 9.

depicted in Fig. 3. The lanthanide ions complex with the phosphate group as well as to the 2′-hydroxyl group and form an unstable seven-membered structure; cleavage is ascribed to the intramolecular attack by the Ln^{3+}-bound hydroxide ion.

In addition, we investigated the notable difference in reactivity between Ce^{3+} and the other lanthanides under air. Cerium is the only lanthanide which can be oxidized to the tetravalent state under the reaction conditions. Interestingly, in contrast to the rapid 5′-AMP cleavage by $CeCl_3$ at pH 9 under air, when the oxygen was gradually removed from the Ce^{3+} reaction medium by degassing the solvent with nitrogen gas, the cleavage became slow, which indicated the reaction is related to the oxygen in the solution. We also determined the amount of Ce^{4+} in the reaction mixture, which is shown in Table 2. The cleavage rate of 5′-AMP is enhanced with increasing the molar fraction of Ce^{4+} in the reaction mixture. In order to avoid the oxidation of Ce^{3+}, K_2SO_3 was added to the reaction mixture, resulting in a decrease in the cleavage rate. Fast cleavage of 5′-AMP by pure Ce^{4+} at pH 9 and 37°C is also observed. In conclusion, 5′-AMP cleavage by Ce^{3+} under air is much faster than that by any other lanthanides because of the presence of Ce^{4+}. It was reported by Komiyama *et al.* that thymidy(3′-5′)thymidyl (TpT) hydrolysis involved intramolecular attack by the Ce^{IV}-bound hydroxide ion towards the phosphate TpT [7]. However, we have shown that the hydrolysis of nucleotides by Ce^{4+} also occurred in acidic conditions.

The cleavage of 5′-GMP to guanosine (G) and inorganic phosphate by Ce^{4+} under acidic conditions, at 37°C were clearly evidenced by 400 MHz ^1H NMR and 161.9 MHz ^{31}P NMR (Fig. 4). The signals for 5′-GMP the C'_1—H protons of the 5′-GMP and the chemical shift of the phosphate of 5′-GMP at 6.05, 6.04 and −0.87 [11] decrease accompanied by an increase of the C'_1—H signals and a chemical shift of inorganic phosphate for free G and free phosphate at 5.64 and 2.45. The nonactivity for hydrolyzing 5′-GMP by 1 M H_2SO_4 rule out the possibility of acid hydrolysis.

The Ce^{4+} complex with SO_4^{2-} is quite stable [12], so the concentration of free Ce^{IV} decreases due to the addition of Na_2SO_4. The degree of hydrolysis of 5′-AMP decreases with increasing concentration of Na_2SO_4. It is obvious that Ce^{IV} in solution is responsible for the hydrolysis of 5′-AMP and 5′-GMP.

Furthermore, we studied the influence of concentrations of various acids on cleaving 5′-AMP by $Ce(SO_4)_2$. The concentration of SO_4^{2-} in a solution of

Table 2. Effect of cerium on the cleavage of 5′-AMP and the amount of Ce^{IV} in the reaction mixture

	Under air				Degassing the solvent by N_2			
Reaction time (h)	0	2	6	10	0	2	6	10
Molar fraction of 5′-AMP cleavage (%)	0.0	12.0	30.3	64.5	0.0	2.3	4.5	6.0
Molar fraction of Ce^{IV} (%)	0.0	15.2	35.7	58.5	0.0	3.1	4.6	7.8

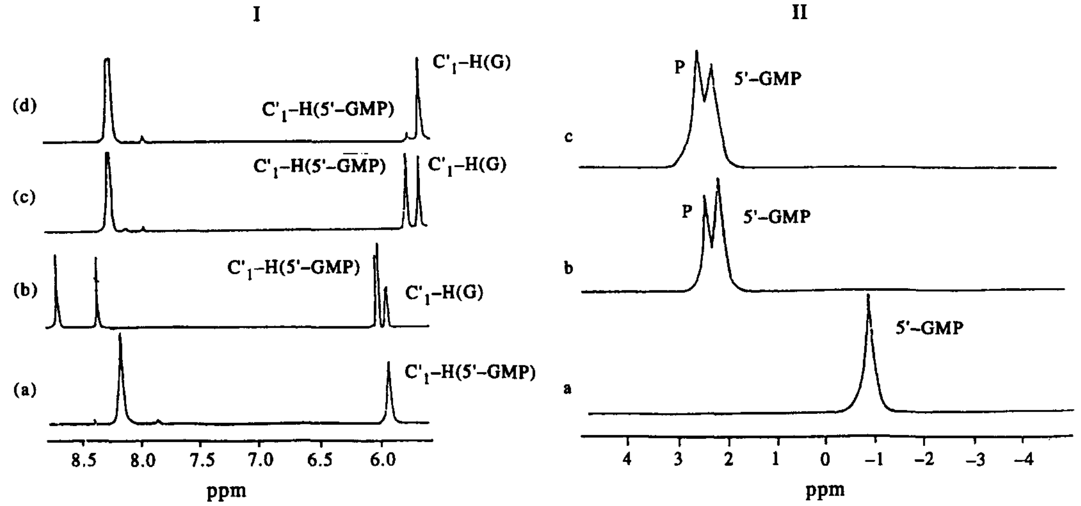

Fig. 4. I: ^{1}H NMR spectra for the cleavage of 5′-GMP (18 mmol dm^{-3}) by $Ce(SO_4)_2$ (241 mmol dm^{-3}). Acidity: H_2SO_4 (1 mol dm^{-3}) at 37°C in D_2O: (a) pure sample of 5′-GMP, (b) mix G to the solution of 5′-GMP, (c) 4 and (d) 16 h. II: ^{31}H NMR spectra for the cleavage of 5′-GMP (18 mmol dm^{-3}) by $Ce(SO_4)_2$ (241 mmol dm^{-3}). Acidity: H_2SO_4 (1 mol dm^{-3}) at 37°C. (a) pure sample of 5′-GMP in a solution of H_2SO_4 (1 mol dm^{-3}), (b) 16 and (c) 30 h.

$Ce(SO_4)_2$ increases with an increase in the concentration of H_2SO_4, so the concentration of free Ce^{IV} decreases. The decreased concentration of Ce^{IV} results in the low degree of hydrolysis of 5′-AMP. However, the hydrolysis of 5′-AMP is promoted by the addition of HCl and $HClO_4$. Both of them are weak complexing agents, therefore, the concentration of Ce^{IV} in a solution of $Ce(SO_4)_2$ increases with an increase in the concentration of H^+. It appears that a major factor in hydrolyzing 5′-AMP is the concentration of Ce^{IV} in the solution of $Ce(SO_4)_2$.

In order to verify our results, we studied the difference between $(NH_4)_2Ce(NO_3)_6$ and $Ce(SO_4)_2$ in hydrolyzing 5′-AMP and found that the hydrolysis of 5′-AMP by $Ce(NH_4)_2(NO_3)_6$ was stronger than by $Ce(SO_4)_2$. This results from the fact that the concentration of Ce^{IV} in a solution of $Ce(NH_4)_2(NO_3)_6$ is higher than that of $Ce(SO_4)_2$ due to the larger stability constant of Ce^{IV} complex with SO_4^{2-}.

Thus the free Ce^{IV} in a solution of $Ce(SO_4)_2$ is the actual reactive species for hydrolyzing mononucleotides in acidic condition. The tetravalent positive charge of Ce^{IV} neutralizes the negative charge of oxygen of the PO_4^- and weakens the P—O bond. This results in the cleavage of the phosphate diester linkage of mononucleotides. In addition, we compare the rate of cleavage of 5′-AMP and 5′-GMP by Ce^{4+} both in basic and acidic conditions. Results showed that the degree of hydrolysis of 5′-AMP in basic conditions was larger than that in acidic condition. These results combined with the fact of other lanthanide ions showing less efficiency for hydrolyzing 5′-AMP and 5′-GMP in both conditions indicated that in addition to the mechanism of the intramolecular attack by the Ce^{IV}-bonded hydroxide ion for supporting the high activity of Ce^{4+}, the function of neutralization of tetravalent positive charge of Ce^{4+} is also important in the hydrolysis of mononucleotides in basic conditions.

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