Efficient RNA hydrolysis by lanthanide(III)–hydrogen peroxide combinations. Novel aggregates as the catalytic species[1]

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Combinations of lanthanum(III) ion and hydrogen peroxide efficiently hydrolyze RNA under physiological conditions, because of a synergetic cooperation. The rate constant for the hydrolysis of adenylyl(3′-5′)adenosine at pH 7.2 and 30 °C is 7.7×10^{-2} min^{-1}, when $[La^{III}]_0 = 10$ and $[H_2O_2]_0 = 100$ mM. This value is 460 times as great as that for the ApA hydrolysis by La^{III} alone (1.7×10^{-4} min^{-1}). Hydrogen peroxide is inactive when used separately. A similar synergism operates between Nd^{III} and H_2O_2. According to the kinetic analysis and the potentiometric titration, a trimeric aggregate of $[La(O–O)_3La]$ complex is responsible for the RNA hydrolysis. This result is in contrast with the previous proposal on the hydrolysis of bis(4-nitrophenyl)phosphate that monomeric species of $[La(O–O)_2La]^{2+}$ is the active species (B. K. Takasaki and J. Chin, *J. Am. Chem. Soc.*, 1995, 117, 8582). The discrepancy is ascribed to the difference in the basicities of the leaving groups in the substrates.

Introduction

There has been increasing interest in non-enzymatic hydrolysis of RNA, and varieties of organic and inorganic catalysts for phosphoester hydrolysis have been reported.[2–9] Of these catalysts, lanthanide(III) ions are characterized by enormously high catalytic activities, as reported in 1992 by ourselves[3a] and Morrow *et al.*[3b] The last three lanthanide ions (Tm, Yb and Lu) are especially eminent, and rapidly hydrolyze RNA under physiological conditions. However, still more active catalysts, if available, should be useful for further development of the field. Fundamental information on the catalytic mechanism for RNA hydrolysis is also important.

Recently, Takasaki and Chin demonstrated that the hydrolysis of bis(4-nitrophenyl)phosphate (BNPP) by the La^{III} ion is notably promoted by hydrogen peroxide.[10] The activated phosphodiester linkage in 2′,3′-cyclic monophosphate of adenosine has also been hydrolyzed. The catalysis was ascribed to a dinuclear complex ($[La(O–O)_2La]^{2+}$) which is formed from two La^{III} ions and two hydrogen peroxide molecules.[11]

We report here that diribonucleotides are efficiently hydrolyzed by the combinations of various lanthanide(III) ions and H_2O_2 under physiological conditions. A significant dependency of the rate of RNA hydrolysis on the catalyst concentration, which is far more drastic than was reported for the BNPP hydrolysis in ref. 10, is shown. Detailed kinetic analysis and titration study have been carried out, and a novel mechanism involving a trimeric aggregate of dinuclear La^{III} complex is proposed on the basis of these results.

Experimental

Materials

Adenylyl(3′-5′)adenosine (ApA) was obtained from Seikagaku Kogyo and other dinucleotides were from Sigma. Lanthanide(III) salts were purchased from Soekawa Chemicals. The concentration of H_2O_2 in the aqueous solution of hydrogen peroxide (from Takahashi Pure Chemicals) was determined by redox titration with $KMnO_4$. Both the reaction vessels and highly purified water (the specific resistance > 18.3 MΩ cm^{-1}) were sterilized immediately before use. Throughout this study, great care was taken to avoid contamination by natural enzymes and other metal ions.

Kinetic analysis of hydrolysis of diribonucleotides

The hydrolysis of diribonucleotides was initiated by adding a stock solution of the substrate in water to 50 mM HEPES buffer, which contained the required amount of lanthanide(III) salt and H_2O_2. The reaction was carried out at 30 °C and pH 7.2 unless otherwise noted. The initial concentration of the substrate was 0.1 mM. At appropriate intervals, a small portion of the mixture was taken, and 10% aqueous solution of H_3PO_4 was added. The specimen was analyzed by reversed-phase HPLC [a Merck LiChrospher RP-18(e) ODS column; water–acetonitrile = 92 : 8 (v/v)]. All the reactions satisfactorily showed pseudo-first-order kinetics. The assignment of the HPLC peaks was achieved by coinjection with authentic samples. Most of the reactions were followed for 2–3 half-lives. At low pH or low metal concentration, however, the reactions were slow so they were followed for a few hours. During this period of time, 1–50% of ApA was hydrolyzed. The pH change in the reactions was less than 0.1 unit.

Potentiometric titration on La^{III}–H_2O_2 systems

In a typical run, aqueous solutions containing $La(ClO_4)_3$ (5 mM), H_2O_2 (100 mM) and $NaClO_4$ (100 mM) were potentiometrically titrated against an aqueous solution of NaOH (1.0 M). Both the sample solutions and the NaOH solutions were prepared immediately before use.

Results

Hydrolysis of diribonucleotides by combinations of La^{III} and H_2O_2

As depicted in Fig. 1(*a*), ApA is promptly hydrolyzed by the combination of $La(ClO_4)_3$ (10 mM) and H_2O_2 (100 mM) at pH 7.2 (HEPES buffer) and 30 °C.[12] The products are adenosine (Ado) and its 2′- and 3′-monophosphates (2′A and 3′A). No other byproducts are formed, confirming that the scission proceeds *via* hydrolysis of the phosphodiester linkage. 2′,3′-Cyclic monophosphate of adenosine (A > p) as the hydrolysis intermediate is rapidly hydrolyzed to the final product (either 2′A or 3′A) also by the La^{III}–H_2O_2 combination, and thus is hardly accumulated in the mixture.[13] The 2′A : 3′A ratio in the product, which reflects the ratio of the scission rates of the P–O(2′) and P–O(3′) linkages in A > p, is *ca.* 0.5.

Quite significantly, the ApA hydrolysis by the $La(ClO_4)_3$–H_2O_2 combination is overwhelmingly faster than that by

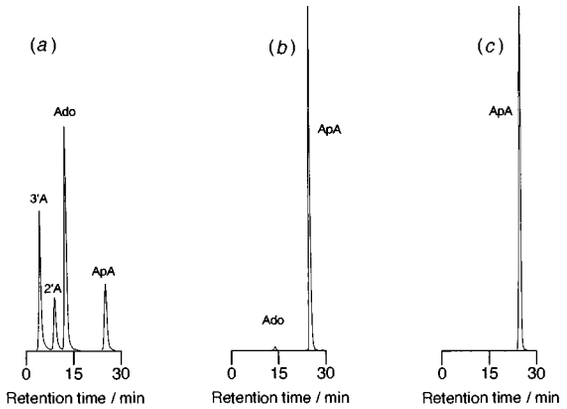

Fig. 1 Reversed-phase HPLC profiles for the hydrolysis of ApA (*a*) by $La(ClO_4)_3$ (10 mM) + H_2O_2 (100 mM), (*b*) $La(ClO_4)_3$ (10 mM) and (*c*) H_2O_2 (100 mM) at pH 7.2 and 30 °C for 30 min

$La(ClO_4)_3$ alone [compare Fig. 1(*a*) with (*b*)]. The pseudo-first-order rate constant for the combination is 7.7×10^{-2} min^{-1} (the half-life of ApA is 9 min). This corresponds to 5×10^7-fold acceleration with respect to the value in the absence of the $La(ClO_4)_3$–H_2O_2 combination (the intrinsic half-life of the phosphodiester linkage in RNA under the conditions employed is estimated to be 800 years from the data in ref. 5*c*). However, the rate constant for the ApA hydrolysis by $La(ClO_4)_3$ alone is 1.7×10^{-4} min^{-1}, whereas H_2O_2 is inactive when used alone [see Fig. 1(*c*)]. Thus, the catalytic activity of the La^{III} ion is promoted by 460-fold by the cooperation with H_2O_2. The rate constant of the ApA hydrolysis by the $La(ClO_4)_3$–H_2O_2 combination is about $\frac{1}{3}$ times as great as the value (21×10^{-2} min^{-1}) for the Tm^{III} ion (10 mM), which is one of the most active catalysts for RNA hydrolysis ever reported.[3a,c] Notable cooperation of the La^{III} ion with H_2O_2 for RNA hydrolysis is evidenced.

Cytidylyl (3′-5′)cytidine is also efficiently hydrolysed by the $La(ClO_4)_3$–H_2O_2 combination; the rate constant (7.7×10^{-2} min^{-1}) is identical with the value for the ApA hydrolysis by the combination. Here, the synergetic cooperation between $La(ClO_4)_3$ and H_2O_2 causes 100-fold acceleration. The rate constant for the hydrolysis by the combination of $LaCl_3$ (5 mM) and H_2O_2 (100 mM) is 5.6×10^{-3} min^{-1}, which is close to the corresponding value (6.2×10^{-3} min^{-1}) for the $La(ClO_4)_3$–H_2O_2 combination. Neither the nucleic acid bases in the substrates nor the counter anion of the La^{III} ion show significant effects on the present RNA hydrolysis.

In contrast with the remarkable RNA hydrolysis, 2′-deoxyadenylyl(3′-5′)2′-deoxyadenosine was not hydrolyzed at all by the La^{III}–H_2O_2 combination. Apparently, the present RNA hydrolysis proceeds *via* the intramolecular attack by the 2′-OH of the ribose toward the phosphorus atom.

RNA hydrolysis by the combination of various lanthanide(III) ions and H_2O_2

Table 1 shows the rate constants for the ApA hydrolysis by the combinations of various lanthanide(III) ions and H_2O_2. For the purpose of comparison, the values for the catalysis by the metal ions (in the absence of H_2O_2) are also presented. All the lanthanide ions are used as the chloride salts.

The activities of Nd^{III} and Sm^{III} are also promoted by H_2O_2; the magnitudes of synergetic acceleration are 13- and 1.5-fold, respectively. However, the cooperation becomes gradually less efficient as the atomic number of lanthanide ion increases. The rate of hydrolysis by Gd^{III}–H_2O_2 combination is almost identical with the value by the metal ion alone, whereas the RNA hydrolysis by the other lanthanide ions is suppressed by H_2O_2. As a result, the activities of lanthanide(III)–H_2O_2 combinations are not very dependent on the kind of lanthanide(III) ion. The rate of RNA hydrolysis by the lanthanide ions themselves, however, monotonically and significantly increases with the increasing atomic number.[3c]

Table 1 Pseudo-first-order rate constants for the hydrolysis of ApA by lanthanide ions in the presence and the absence of H_2O_2 at 30 °C and pH 7.2[a]

	$k_{obs}/10^{-3}$ min^{-1}	
Lanthanide ion	Metal ion–H_2O_2	Metal ion[b]
La^{III}	5.6	0.07
Nd^{III}	9.5	0.73
Sm^{III}	9.2	6.2
Eu^{III}	7.1	11
Gd^{III}	8.1	8.1
Tb^{III}	11	23
Ho^{III}	9.7	50
Tm^{III}	8.0	110
Yb^{III}	7.1	210
Lu^{III}	7.9	190

[a] [Lanthanide chloride]$_0$ = 5 mM and [H_2O_2] = 100 mM. [b] Data from ref. 3*c*.

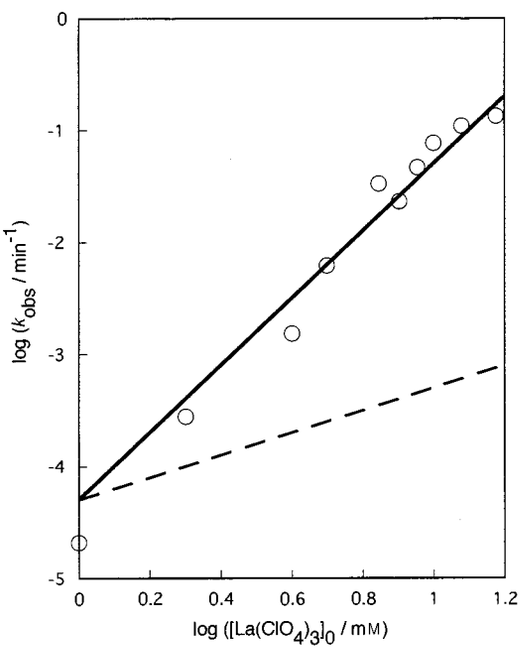

Fig. 2 Log–log plot of the pseudo-first-order rate constant *vs.* [$La(ClO_4)_3$]$_0$ for the ApA hydrolysis by $La(ClO_4)_3$–H_2O_2 combinations at pH 7.2 and 30 °C: [H_2O_2]$_0$ is kept constant at 100 mM. The solid line indicates the calculation using the eqn. (5), in which [$(La(O–O)_3La)_3$] is the active species for the catalysis. The broken line represents the assumption that [$La(O–O)_3La$] is the active species.

Kinetic analysis on the RNA hydrolysis by La^{III}–H_2O_2 combinations

The rate constant (k_{obs}) of ApA hydrolysis at pH 7.2 by $La(ClO_4)_3$–H_2O_2 combinations drastically increases with the increase in the concentration of $La(ClO_4)_3$. The slope in the corresponding log–log plot is almost 3 (Fig. 2). Thus, the increase in [$La(ClO_4)_3$]$_0$ by 10-fold results in almost a 1000-fold acceleration of ApA hydrolysis. This strongly indicates that the catalytically active species for the RNA hydrolysis are formed from a number of La^{III} ions. In Fig. 2, the concentration of $La(ClO_4)_3$ is varied from 1 to 15 mM, whereas [H_2O_2]$_0$ is kept constant at 100 mM.[14] These conditions ([H_2O_2]$_0$ ≫ [$La(ClO_4)_3$]$_0$) are chosen to make the kinetic analysis simpler and more straightforward.

The pH–rate constant profile for the ApA hydrolysis by the $La(ClO_4)_3$–H_2O_2 combination is represented by the open circles in Fig. 3. The rate constant steeply increases with increasing pH at pH 5.8–6.5. When pH > 6.5, however, the rate constant

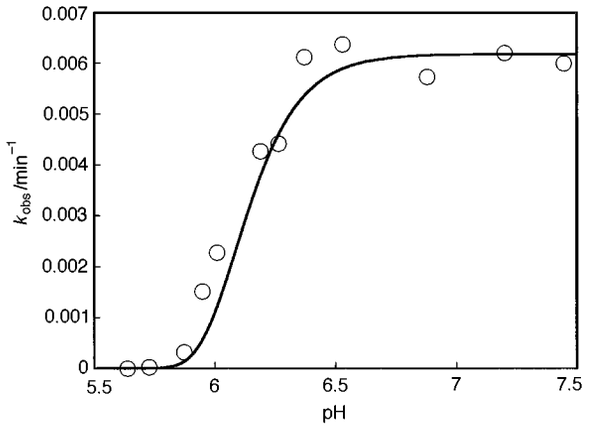

Fig. 3 pH-Rate constant profile for the ApA hydrolysis by $La(ClO_4)_3$–H_2O_2 combinations at 30 °C: $[La(ClO_4)_3]_0 = 5$ mM and $[H_2O_2]_0 = 100$ mM. The solid line is theoretical and is obtained from eqn. (3), in which $[(La(O–O)_3La)_3]$ is the active species.

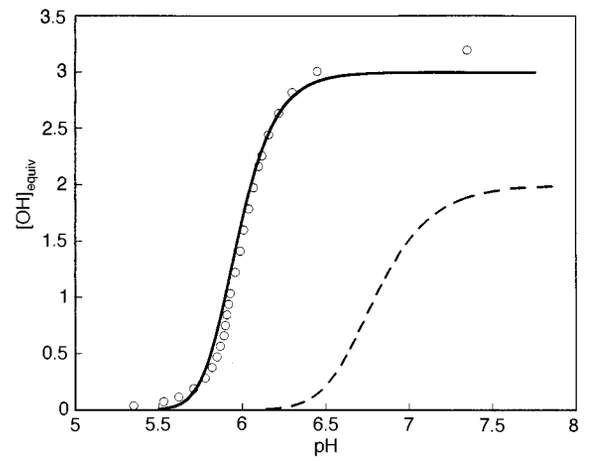

Fig. 4 Typical titration curve for the $La(ClO_4)_3$–H_2O_2 system: $[La(ClO_4)_3]_0 = 5$ mM and $[H_2O_2]_0 = 100$ mM. The solid line is theoretical and is obtained using the eqn. (1) ($K_1 = 3.3 \times 10^{-31}$ M^2). Release of three protons per La^{III} ion under the conditions for RNA hydrolysis is conclusive. The broken line is based on the data in ref. 10, which claimed that two protons are released per La^{III} ion and that $[La(O–O)_2La]^{2+}$ is formed in the mixture (see also ref. 16).

attains a plateau. As discussed below, both the plots in Figs. 2 and 3 are satisfactorily interpreted in terms of the mechanism involving trimeric aggregate of $[La(O–O)_3La]$ as the catalytic species (the solid lines in these figures are the theoretical ones based on this mechanism).

Potentiometric titration on the La^{III}–H_2O_2 systems

Fig. 4 depicts a typical titration curve on La^{III}–H_2O_2 system. Quite a steep increase in the pH 5.5–6.2 region is followed by a plateau at the higher pH. On the completion of the complex formation, three protons are released per La^{III} ion.[15] Consistently, all the experimental points fairly fit the solid line, which is calculated by using eqn. (1) ($K_1 = 3.3 \times 10^{-31}$ M^2). Formation of the dinuclear La^{III} complex $[La(O–O)_3La]$ in the mixtures is conclusive.[16]

$$2La^{3+} + 3H_2O_2 \overset{K_1}{\rightleftharpoons} [La(O–O)_3La] + 6H^+ \qquad (1)$$

Discussion

Active species for RNA hydrolysis by La^{III}–H_2O_2 combinations

According to Takasaki and Chin,[10] the hydrolysis of BNPP by the $La(ClO_4)_3$–H_2O_2 combination is ascribed to $[La(O–O)_2La]^{2+}$, which is formed as shown in eqn. (6) in ref. 16. However, the rate of ApA hydrolysis by the combination depends on $[La(ClO_4)_3]_0$ far more drastically than expected from this mechanism, *e.g.* the rate constant increases by more than 400-fold (from 2.8×10^{-4} to 1.3×10^{-1} min^{-1}) when $[La^{III}]_0$ is increased from 2 to 15 mM ($[H_2O_2]_0$ is kept constant at 100 mM). If $[La(O–O)_2La]^{2+}$ was really the active species, the rate of RNA hydrolysis should be virtually unchanged.[17] Apparently, the present RNA hydrolysis involves higher ordered aggregates than was proposed in the BNPP hydrolysis.

In the most plausible mechanism, a trimeric aggregate is formed in equilibrium from three molecules of the $[La(O–O)_3La]$ complex [eqn. (2)], and this aggregate $[(La(O–O)_3La)_3]$ is

$$3[La(O–O)_3La] \overset{K_2}{\rightleftharpoons} [(La(O–O)_3La)_3] \qquad (2)$$

the active species for the RNA hydrolysis.[18] Formation of $[La(O–O)_3La]$ (and not of $[La(O–O)_2La]^{2+}$) in the present reaction mixtures has been proven quantitatively by potentiometric titration (Fig. 4).[16] When the equilibrium constant K_2 is sufficiently small, eqn. (3) holds, and thus the rate constant of

$$\text{Rate constant} = k_c[(La(O–O)_3La)_3] = k_cK_2[La(O–O)_3La]^3 \qquad (3)$$

RNA hydrolysis is proportional to $[La(O–O)_3La]^3$ where k_c is the catalytic rate constant for the $[La(O–O)_3La]_3$ species. The pH–rate constant profile in Fig. 3 fits the theoretical line well (the solid one: $[La(O–O)_3La]^3$ *vs.* pH), which has been calculated using the K_1 value (3.3×10^{-31} M^2) determined by the potentiometric titration.

The arguments are further supported by the dependence of the hydrolysis rate on $[La(ClO_4)_3]_0$ in Fig. 2. Here, the RNA hydrolysis has been achieved at pH 7.2, and $[H_2O_2]_0$ is far greater than $[La(ClO_4)_3]_0$. Under these conditions, the equilibrium in eqn. (1) virtually completely shifts toward the right-hand side (all the three protons for the complex formation between $La(ClO_4)_3$ and H_2O_2 are released at pH 5.6–6.2, and the titration curve shows a plateau above pH 6.2, see Fig. 4). Thus eqn. (4) results and eqn. (3) is reduced to eqn. (5). Eqn. (5)

$$[La(O–O)_3La] = [La(ClO_4)_3]_0/2 \qquad (4)$$

$$\text{Rate constant} = (k_cK_2[La(ClO_4)_3]_0^3)/8 \qquad (5)$$

is in reasonable agreement with the rate dependency on $[La(ClO_4)_3]_0$ in Fig. 2. If $[La(O–O)_3La]$ was the active species for the hydrolysis of ApA, the slope of the plot of log [rate] *vs.* log $[La^{III}]_0$ should be 1 (broken line); the rate should be proportional to $[La(O–O)_3La]$ (all the La^{III} ions are converted to $[La(O–O)_3La]$). Apparently, this is not the case and the trimer of $[La(O–O)_3La]$ including six La^{III} ions is the active species for RNA hydrolysis.

Why does RNA hydrolysis require higher aggregates than BNPP hydrolysis?

In the hydrolysis of BNPP, the leaving group (4-nitrophenolate) is so good that the pentacoordinated intermediate, formed by nucleophilic attack at the phosphorus atom is promptly decomposed to the products. Thus, the reaction is promoted by the catalysts which facilitate nucleophilic attack (or provide the nucleophile) for the formation of the intermediate. Consistently, the $^-O–O^-$ residue in the dinuclear complex $[La(O–O)_2La]^{2+}$ functions as the nucleophile in BNPP hydrolysis by $La(ClO_4)_3$–H_2O_2.[10] The nucleophilicity (not basicity) of the H_2O_2-derived species is enhanced by α-effect.[19]

The leaving group in RNA hydrolysis (the 5′-alkoxide ion of ribose) is much poorer than that in BNPP hydrolysis (the pK_a of the 5′-OH is *ca.* 14, whereas that of 4-nitrophenol is 7). Here, the removal of the leaving group from the pentacoordinated intermediate is at least partially rate-limiting.[20] In order to efficiently accelerate the reaction, general acid catalysts for the decomposition of the intermediate are necessary, in addition to

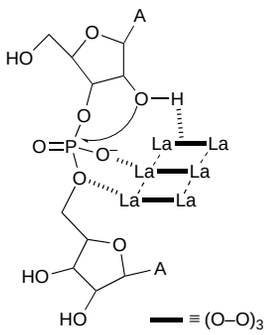

Fig. 5 Proposed mechanism for ApA hydrolysis by $La(ClO_4)_3$–H_2O_2 combinations. Cooperation of a number of La^{III} ions is emphasized (the structure of $[(La(O–O)_3La)_3]$ is not yet conclusive).

general base catalysts for its formation. Acid catalysts can further promote the nucleophilic attack for the formation of the intermediate. The remarkable catalysis by the $[(La(O–O)_3La)_3]$ aggregate is ascribed to the cooperation of many metal ions. The proposed mechanism of the RNA hydrolysis by $La(ClO_4)_3$–H_2O_2 combinations is schematically depicted in Fig. 5. First, the phosphate residue of RNA is coordinated to one of the metal ions in the aggregate. Then a pentacoordinated intermediate is formed by the general base catalysis, probably by the $^-O–O^-$ in the aggregate. When the 5′-OH residue of the ribose departs from the phosphorus atom in the intermediate, other metal ions function as acid catalysts. The reaction can proceed in either a concerted or a stepwise manner. The proposed mechanism is reminiscent of the cooperation of two or three metal ions at the active sites of natural phosphoesterases.[21] The di- or tri-nuclear metal complexes, which are active for the hydrolysis of phosphate esters, also take advantage of multimetallic synergism.[4b,5f–h,22]

Totally consistently, the active species for lanthanide(III) ion-induced RNA hydrolysis are dimeric metal hydroxide clusters.[3c] The last three metal ions in the lanthanide series are the most active at pH 7, since they predominantly exist as the dimeric clusters in neutral solutions. The addition of H_2O_2 perturbs the intrinsic aggregation of these metal ions, resulting in the suppression of their catalytic activities (see Table 1). In contrast, La^{III} mostly exists as monomeric ion at pH 7. Thus, the activity of La^{III} for RNA hydrolysis is greatly promoted when a number of the metal ions are integrated by the H_2O_2 added [eqns. (1) and (2)].

In conclusion, combinations of lanthanide(III) ion and hydrogen peroxide effectively hydrolyze diribonucleotides. A considerable number of metal ions must aggregate together for catalysis, since the cooperation of two or more metal ions is required for efficient catalysis. The present finding should be useful for designing the catalysts which are still more active for RNA hydrolysis.

Acknowledgements

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References

1 A preliminary communication: M. Komiyama, J. Kamitani, J. Sumaoka and H. Asanuma, *Chem. Lett.*, 1996, 869.
2 Reviews: (*a*) R. Breslow, *Acc. Chem. Res.*, 1995, **28**, 146; (*b*) E. Kimura and T. Koike, *Adv. Inorg. Chem.*, 1997, **44**, 229; (*c*) M. Komiyama, *J. Biochem.*, 1995, **118**, 665; (*d*) D. M. Perreault and E. V. Anslyn, *Angew. Chem.*, *Int. Ed. Engl.*, 1997, **36**, 432.
3 (*a*) M. Komiyama, K. Matsumura and Y. Matsumoto, *J. Chem. Soc.*, *Chem. Commun.*, 1992, 640; (*b*) J. R. Morrow, L. A. Buttrey, V. M. Shelton and K. A. Berback, *J. Am. Chem. Soc.*, 1992, **114**, 1903; (*c*) K. Matsumura and M. Komiyama, *J. Biochem.*, 1997, **122**, 387.
4 (*a*) R. Breslow and D.-L. Huang, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 4080; (*b*) M. Irisawa and M. Komiyama, *J. Biochem.*, 1995, **117**, 465; (*c*) M. Yashiro, A. Ishikubo and M. Komiyama, *J. Biochem.*, 1996, **120**, 1067; (*d*) P. Hurst, B. K. Takasaki and J. Chin, *J. Am. Chem. Soc.*, 1996, **118**, 9982.
5 (*a*) J. J. Butzow and G. L. Eichhorn, *Biochemistry*, 1971, **10**, 2019; (*b*) J. Ciesiolka, T. Marciniec and W. J. Krzyzosiak, *Eur. J. Biochem.*, 1989, **182**, 445; (*c*) Y. Matsumoto and M. Komiyama, *J. Chem. Soc.*, *Chem. Commun.*, 1990, 1050; (*d*) S. Kuusela and H. Lönnberg, *J. Phys. Org. Chem.*, 1993, **6**, 347; (*e*) F. Chu, J. Smith, V. M. Lynch and E. V. Anslyn, *Inorg. Chem.*, 1995, **34**, 5689; (*f*) M. Yashiro, A. Ishikubo and M. Komiyama, *J. Chem. Soc.*, *Chem. Commun.*, 1995, 1793; (*g*) M. Irisawa, N. Takeda and M. Komiyama, *J. Chem. Soc.*, *Chem. Commun.*, 1995, 1221; (*h*) A. Ishikubo, M. Yashiro and M. Komiyama, *Nucleic Acids Symp. Ser.*, 1995, **34**, 85; (*i*) B. Linkletter and J. Chin, *Angew. Chem.*, *Int. Ed. Engl.*, 1995, **34**, 472; (*j*) S. Kuusela, A. Guzaev and H. Lönnberg, *J. Chem. Soc.*, *Perkin Trans. 2*, 1996, 1895; (*k*) M. J. Young and J. Chin, *J. Am. Chem. Soc.*, 1995, **117**, 10 577.
6 Artificial enzymes for sequence-selective RNA scission were prepared by attaching lanthanide complexes to DNA oligomers: (*a*) K. Matsumura, M. Endo and M. Komiyama, *J. Chem. Soc.*, *Chem. Commun.*, 1994, 2019; (*b*) D. Magda, R. A. Miller, J. L. Sessler and B. L. Iverson, *J. Am. Chem. Soc.*, 1994, **116**, 7439; (*c*) J. Hall, D. Hüsken and R. Häner, *Nucleic Acid Res.*, 1996, **24**, 3522.
7 Non-enzymatic hydrolysis of DNA was also achieved by lanthanide ions: (*a*) Y. Matsumoto and M. Komiyama, *Nucleic Acids Symp. Ser.*, 1992, **27**, 33; (*b*) M. Komiyama, K. Matsumura, K. Yonezawa and Y. Matsumoto, *Chem. Express*, 1993, **8**, 85; (*c*) T. Shiiba, K. Yonezawa, N. Takeda, Y. Matsumoto, M. Yashiro and M. Komiyama, *J. Mol. Catal.*, 1993, **84**, L21; (*d*) B. K. Takasaki and J. Chin, *J. Am. Chem. Soc.*, 1994, **116**, 1121; (*e*) M. Komiyama, T. Shiiba, T. Kodama, N. Takeda, J. Sumaoka and M. Yashiro, *Chem. Lett.*, 1994, 1025; (*f*) M. Komiyama, N. Takeda, Y. Takahashi, H. Uchida, T. Shiiba, T. Kodama and M. Yashiro, *J. Chem. Soc.*, *Perkin Trans. 2*, 1995, 269; (*g*) J. Rammo, R. Hettich, A. Roigk and H.-J. Schneider, *J. Chem. Soc.*, *Chem. Commun.*, 1996, 105; (*h*) S. Hashimoto and Y. Nakamura, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 2623.
8 Hydrolysis of other phosphodiesters: (*a*) K. Matsumura and M. Komiyama, *J. Inorg. Biochem.*, 1994, **55**, 153; (*b*) Ref. 2.
9 Phosphotriester hydrolysis: R. W. Hay and N. Govan, *J. Chem. Soc.*, *Chem. Commun.*, 1990, 714.
10 B. K. Takasaki and J. Chin, *J. Am. Chem. Soc.*, 1993, **115**, 9337; 1995, **117**, 8582.
11 The $La(ClO_4)_3$–H_2O_2 combination was used as the catalytic site in an enyme model: R. Breslow and B. Zhang, *J. Am. Chem. Soc.*, 1994, **116**, 7893.
12 Takasaki and Chin noted in ref. 10 that 'the dinuclear La^{III} complex is unstable and loses activity after about 30 min'. In our experiments, however, no significant deterioration of the catalyst was observed even after 100 min of pre-incubation. As noted in the Experimental section, fairly good pseudo-first-order kinetics was obtained. The reason for the discrepancy is not clear.
13 The pseudo-first-order rate constant of the hydrolysis of authentic sample of A > p by $La(ClO_4)_3$ (10 mM) and H_2O_2 (100 mM) at pH 7 and 30 °C is > 0.3 s^{-1}.
14 In ref. 10, Takasaki and Chin examined the hydrolysis of BNPP with rather small La^{III} concentrations (0.6–1.6 mM). However, Breslow *et al.* (ref. 11) reexamined the reaction in larger La^{III} concentrations (0.1–4 mM), and obtained a similar result to Takasaki and Chin. Therefore, notable differences in the kinetic features between RNA hydrolysis and BNPP hydrolysis really come from the differences in the leaving group of substrates, and not simply from the differences in the reaction conditions employed.
15 The possibility that either $(OH)_2La(O–O)La(OH)_2$ or $(OH)La(O–O)_2La(OH)$ is formed is ruled out by the following pH titration. When $[La^{III}]_0/[H_2O_2]_0 = 1$, two protons (per La^{III} ion) are released to the aqueous phase. This is contrast with three-proton release at $[La^{III}]_0 : [H_2O_2]_0$ ratio of 0.05. Thus, all the protons are from hydrogen peroxide, and not from water molecules.
16 The titration profile in Fig. 4 is significantly different from that reported by Takasaki and Chin (ref. 10). According to these authors, two protons were released on completion of complex formation. Based on this result, it was claimed that $[La(O–O)_2La]^{2+}$ is formed in the mixtures, as expressed by eqn. (6) ($K' = 1.4 \times 10^{-23}$ M).

$$2La^{3+} + 2H_2O_2 \overset{K'}{\rightleftharpoons} [La(O–O)_2La]^{2+} + 4H^+ \quad (6)$$

In contrast, our titration has shown absolutely that $[La(O–O)_3La]$, rather than $[La(O–O)_2La]^{2+}$, is formed in the solutions, at least under the conditions used for the present RNA hydrolysis. Probably, $[La(O–O)_2La]^{2+}$ further reacts with another H_2O_2 molecule to form $[La(O–O)_3La]$, since the concentrations of both the La^{III} salt and H_2O_2 are sufficiently great. The experimental points in Fig. 4 are far from the theoretical line (the broken one), which was calculated by using the parameters reported by Takasaki and Chin.

17 This conclusion was obtained under the hypothesis that eqn. (6) (for the formation of the dinuclear complex $[La(O–O)_2La]^{2+}$) is the sole equilibrium in the mixture (this hypothesis is ruled out by the potentiometric titration). As estimated by using $K' = 1.4 \times 10^{-23}$ M, the equilibrium lies mostly in the right-hand side, so that the increase in $[La^{III}]_0$ causes only a small increase in the concentration of the dinuclear complex.

18 Tetrameric (or even greater) aggregates of $[La(O–O)_3La]$, in addition to $[(La(O–O)_3La)_3]$, might be making some contributions in the catalysis.

19 M. L. Bender, R. J. Bergeron and M. Komiyama, *The Bioorganic Chemistry of Enzymatic Catalysis*, Wiley-Interscience, New York, 1984, p. 133.

20 The rate-limiting step in RNA hydrolysis was discussed in detail in ref. 3*c*. The decomposition of the pentacoordinated intermediates would be (at least partially) rate-limiting, since the substitution of 5′-O atom in RNA by 5′-S (replacement of the 5′-OH with 5′-SH as a better leaving group) causes 10^4–10^6-fold acceleration of the hydrolysis of the corresponding linkage.

21 N. Sträter, W. N. Lipscomb, T. Klabunde and B. Krebs, *Angew. Chem.*, *Int. Ed. Engl.*, 1996, **35**, 2024.

22 (*a*) R. G. Clewley, R. S. Brown and H. Slebockatilk, *Inorg. Chim. Acta*, 1989, **157**, 223; (*b*) Y. Chung, E. U. Akkaya, T. K. Venkatachalam and A. W. Czarnik, *Tetrahedron Lett.*, 1990, **31**, 5413; (*c*) S. Hikichi, M. Tanaka, Y. Moro-oka and N. Kitajima, *J. Chem. Soc.*, *Chem. Commun.*, 1992, 814; (*d*) D. H. Vance and A. W. Czarnik, *J. Am. Chem. Soc.*, 1993, **115**, 12 165; (*e*) N. Takeda, M. Irisawa and M. Komiyama, *J. Chem. Soc.*, *Chem. Commun.*, 1994, 2773; (*f*) J. A. Connolly, M. Banaszczyk, R. C. Hynes and J. Chin, *Inorg. Chem.*, 1994, **33**, 665; (*g*) T. Koike, M. Inoue, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1996, **118**, 3091.

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