RNA Hydrolysis by the Combination of the Lanthanum(III) Ion and Hydrogen Peroxide

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Diribonucleotides are efficiently hydrolyzed by the combination of the lanthanum(III) ion and hydrogen peroxide at pH 7.2 and 30 °C. The rate constant for the hydrolysis of adenylyl(3'-5')adenosine by the mixture of $La(ClO_4)_3$ (10 mmol dm^{-3}) and H_2O_2 (100 mmol dm^{-3}) is 7.7 x 10^{-2} min^{-1} (half-life 9 min), corresponding to a 460 fold cooperative promotion of the catalysis.

Hydrolysis of phosphodiester linkages in DNA and RNA has been attracting much interest, and varieties of catalysts were proposed.[1-3] However, molecular design of still more active catalysts is crucially required for further development of the field. Recently, Takasaki and Chin elegantly demonstrated that the hydrolysis of bis(4-nitrophenyl)phosphate (BNPP) by the lanthanum(III) ion is notably accelerated by hydrogen peroxide.[4] 2',3'-Cyclic monophosphate of adenosine was also hydrolyzed. According to the authors, however, the catalysis is only applicable to the substrates having good leaving groups or being activated by strain, and is virtually nil for non-activated phosphodiester linkages in biomaterials.

We report here that diribonucleotides, adenylyl(3'-5')adenosine (ApA) and cytidylyl(3'-5')cytidine (CpC), are efficiently hydrolyzed by La(III)/H_2O_2 combinations under the physiological conditions. The catalysis is 100-500 fold promoted by the cooperation of the components, resulting in an enormously fast RNA hydrolysis. The hydrolysis rate is drastically dependent on the catalyst concentration, and thus is hardly detectable under the conditions employed previously.[4]

As depicted in Figure 1 (a), ApA is promptly hydrolyzed by the combination of $La(ClO_4)_3$ (10 *mM*: *mM* = mmol dm^{-3}) and hydrogen peroxide (100 *mM*) at pH 7.2 (Hepes buffer) and 30 °C. The products are adenosine (Ado) and its 2'- and 3'-monophosphates (2'A and 3'A). No other products are formed, confirming that the scission proceeds via the hydrolysis of the phosphodiester linkage. 2',3'-Cyclic monophosphate of adenosine as the hydrolysis intermediate is rapidly hydrolyzed to 2'A or 3'A, and is hardly accumulated in the mixture.

Quite significantly, the ApA hydrolysis by the $La(ClO_4)_3/H_2O_2$ combination is much faster than that by $La(ClO_4)_3$ (compare Figure 1 (a) with (b)). The pseudo first-order rate constant for the combination is 7.7 x 10^{-2} min^{-1} (the half-life is only 9 min), whereas the value by $La(ClO_4)_3$ is 1.7 x 10^{-4} min^{-1}. Hydrogen peroxide is inactive for the hydrolysis when used alone (c). Thus, the catalytic activity of the combination is 460 fold greater than the sum of the activities of the metal ion and of hydrogen peroxide. Similarly, the combination hydrolyzes CpC by 100 fold faster than does the La(III) ion. It is noteworthy that the activity of the combination is comparable to that of the Tm(III) ion, which is one of the most active catalysts ever reported.[3] Similar cooperative hydrolysis of ApA by the metal ion/H_2O_2 system was also observed with other lanthanide ions such as Pr(III) and Nd(III). However, the hydrolysis was hardly accelerated by H_2O_2 when Lu(III) was used in place of La(III).

In contrast, 2'-deoxyadenylyl(3'-5')-2'-deoxyadenosine was not hydrolyzed at all by the La(III)/H_2O_2 combination. Apparently, the RNA hydrolysis proceeds via intramolecular attack by the 2'-OH toward the phosphorus atom. A direct nucleophilic attack by metal-bound hydrogen peroxide or by metal-bound hydroxide toward the phosphorus atom is ruled out.

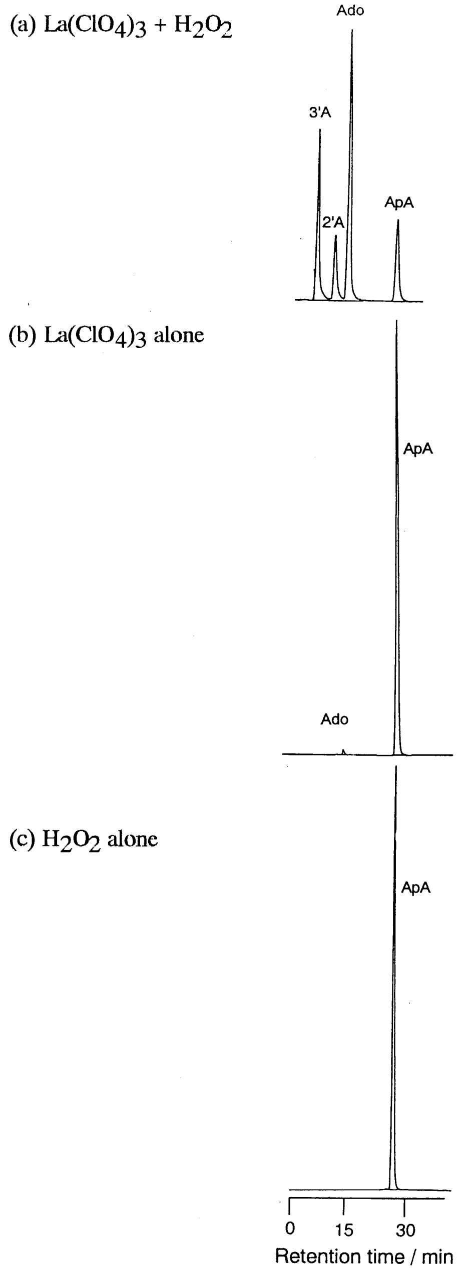

Figure 1. Reversed-phase HPLC profiles for the hydrolysis of ApA at pH 7.2 and 30 °C for 30 min: (a) by $La(ClO_4)_3$ (10 *mM*) + H_2O_2 (100 *mM*), (b) $La(ClO_4)_3$ (10 *mM*), and (c) H_2O_2 (100 *mM*).

As evidenced previously,[4] the hydrolysis of BNPP is catalyzed by a dinuclear complex composed of two lanthanum(III) ions and two hydrogen peroxides. In the present diribonucleotide hydrolysis, however, the dependence of rate on the La(III) concentration is far more drastic than expected from the mechanism. The rate constant is $6.2 \times 10^{-3}\ \mathrm{min}^{-1}$ when $[\mathrm{La(III)}]_0 = 5\ mM$, and is $2.8 \times 10^{-4}\ \mathrm{min}^{-1}$ at 2 *mM* ($[\mathrm{H_2O_2}]_0$ is kept constant at 100 *mM*). If the hydrolysis were proceeding via the same mechanism as the BNPP hydrolysis, the rate should be decreased only by 2 and 6 fold, respectively, with respect to the value ($7.7 \times 10^{-2}\ \mathrm{min}^{-1}$) at $[\mathrm{La(III)}]_0 = 10\ mM$.

These results strongly indicate that aggregates of more than three La(III) ions and hydrogen peroxides are responsible for the catalysis.[5] Cooperation of these metal ions is required for the hydrolysis of the non-activated phosphodiester linkages in dinucleotides. Both a general base catalyst (for the intramolecular nucleophilic attack by the 2'-OH toward the phosphate) and a general acid catalyst (for the decomposition of the pentacoordinated intermediate) should be provided for RNA hydrolysis.[6] The acid catalysis is indispensable, since the departure of the 5'-hydroxyl residue, a poor leaving group, from the intermediate is rate-limiting.[7,8]

In conclusion, the combination of lanthanum(III) ion and hydrogen peroxide effectively hydrolyzes diribonucleotides under the physiological conditions. A detailed kinetic study, as well as spectroscopic study on the La(III)/$\mathrm{H_2O_2}$ system, is currently under way.

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4 B. K. Takasaki and J. Chin, *J. Am. Chem. Soc.*, **115**, 9337 (1993) and **117**, 8582 (1995). For the BNPP hydrolysis, $[\mathrm{La(III)}]_0$ was changed from 0 to 2 *mM*.

5 The potentiometric titration in Ref. 4 is consistent with the present argument, since it simply indicated that "at least a dimer or higher order aggregates are formed".

6 A similar cooperation of metal ions for RNA hydrolysis has been reported: M. Yashiro, A. Ishikubo, and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, **1995**, 1793 and references cited therein.

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8 The dinuclear complex is inactive for RNA hydrolysis, probably because it can function only as a base catalyst. Thus the catalysis is restricted to the hydrolysis of activated substrates in which the formation of pentacoordinated intermediates is rate-limiting.