Unprecedentedly Fast DNA Hydrolysis by the Synergism of the Cerium(IV)-Praseodymium(III) and the Cerium(IV)-Neodymium(III) Combinations[1]

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(Received April 8, 1996)

The most active catalysts for DNA hydrolysis so far have been obtained by using the synergetic cooperation of the cerium(IV) ion with the praseodymium(III) ion. The pseudo first-order rate constant for the hydrolysis of thymidylyl(3'-5')thymidine by the 2:1 combination is 10 times as great as that by the cerium(IV) ion.

Non-enzymatic hydrolysis of DNA has been one of the most challenging topics.[2-4] However, the phosphodiester linkages in DNA are quite stable and thus their first successful hydrolysis was demonstrated only a few years ago.[3] The most active catalyst hitherto reported is the cerium(IV) ion.[4] Although notable cooperation of two metal ions for the hydrolysis of phosphate esters and their analogs was documented,[5] none of these combinations could exceed the Ce^{IV} ion in the activity for DNA hydrolysis.[6] Here we report novel bimetallic catalysts (the Ce^{IV}-Pr^{III} and the Ce^{IV}-Nd^{III} combinations), which are one order of magnitude more active than the Ce^{IV} ion.

By using the combination of $Ce(NH_4)_2(NO_3)_6$ and $PrCl_3$ (the molar ratio 2:1) at pH 7.0 and 50 °C, about 60% of thymidylyl(3'-5')thymidine (TpT) was hydrolyzed to thymidine (Thd) within 30 min (Figure 1 (a)). The hydrolysis is much faster than that by the Ce^{IV} ion (compare (a) and (b) in Figure 1). The bimetallic synergism is conclusive, since the Pr^{III} ion is inactive when used alone (Figure 1 (c)). The hydrolysis intermediates, thymidine 3'- and 5'-monophosphates (Tp and pT), were not accumulated much.[7] No by-products assignable to oxidative cleavage of the ribose were formed, confirming the hydrolytic character of the scission.

The reactions obeyed good first-order kinetics, although the mixtures were turbid because of the formation of metal hydroxide in the mixtures (*vide infra*). The first-order rate constants k_{obsd} by the bimetallic combination are 8-10 times as great as those for the Ce^{IV} ion (Table 1). The bimetallic cooperation was even more remarkable (15 fold) in the hydrolysis of thymidylyl(3'-5')2'-deoxycytidine. The hydrolysis rate by the Ce^{IV}-Pr^{III} combination is not much dependent on the kind of the adjacent nucleic acid bases. Thus, a 22-mer DNA oligomer (GCATCACCAGCGGTCCTAGCAT) was hydrolyzed almost randomly throughout the DNA chain, as shown by the polyacrylamide gel electrophoresis.

A similar synergism is evident, when the Ce^{IV} ion is combined with intrinsically inactive Nd^{III} ion (see Table 1). The magnitude of the synergetic acceleration is 7-8 fold. These results are reminiscent of the bimetallic cooperation in some of natural phosphoesterases.[2b] In contrast, other lanthanide ions, Y^{III}, Sc^{III}, and non-lanthanide ions (Mg^{II}, Al^{III}, Mn^{II}, Ni^{II}, Co^{II}, and Fe^{II}) suppressed the activity of the Ce^{IV} ion.

The Ce^{IV}-Pr^{III} mixtures in pH 7 buffers were centrifuged,

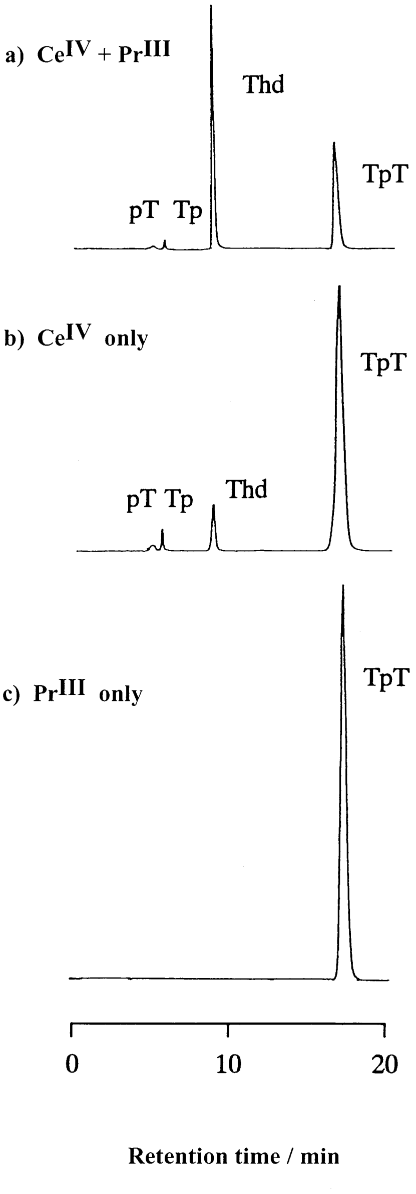

Figure 1. Reversed-phase HPLC profiles for the hydrolysis of TpT at pH 7.0 and 50 °C for 30 min: (a) by the 2:1 combination of Ce^{IV} and Pr^{III}; (b) by Ce^{IV} only; (c) by Pr^{III} only. To 1 cm^3 of 50 mmol dm^{-3} Hepes buffer, $Ce(NH_4)_2(NO_3)_6$ (10 μmol) and/or $PrCl_3$ (5 μmol) were added, and then the pH was adjusted to 7 by use of a small amount of NaOH. $[TpT]_0$ = 0.1 mmol dm^{-3}.

Table 1. The pseudo first-order rate constants for the hydrolysis of TpT at pH 7.0 by the 2:1 Ce^{IV}-Pr^{III} and Ce^{IV}-Nd^{III} combinations [a]

Metal ions	Rate constant / h^{-1}	
	at 50 °C	at 30 °C
$Ce^{IV} + Pr^{III}$	1.7 (0.4)	0.11 (6.3)
Ce^{IV} only	0.16 (4.3)	0.013 (53)[b]
Pr^{III} only	0.0	0.0
$Ce^{IV} + Nd^{III}$	1.1 (0.6)	0.11 (6.3)
Nd^{III} only	0.0	0.0

[a] The numbers in parentheses show the half-lives of TpT in h.

[b] Evaluated by the extrapolation using the activation energy (97 kJ mol^{-1}).

and both the liquid phase and the gel were analyzed separately by UV-visible absorption spectroscopy, ion chemical plasma spectroscopy, and elemental analysis. Only the gel was active for the DNA hydrolysis. When the $[Pr^{III}]_0/[Ce^{IV}]_0$ ratio was smaller than 0.5, all the Pr^{III} and Ce^{IV} ions were present in the gel. At the larger feed ratio, however, the Pr^{III}/Ce^{IV} ratio in the gel was kept constant at 0.5, and the excess Pr^{III} ions remained in the liquid phase (all the Ce^{IV} ions were in the gel). The gel was free from ammonium, chloride, and nitrate ions. All these results clearly show that 2:1 mixed hydroxide cluster, formed from the two kinds of metal ions, is responsible for the remarkable DNA hydrolysis. Consistently, k_{obsd} for the TpT hydrolysis increased linearly with $[Pr^{III}]_0$ up to the $[Pr^{III}]_0/[Ce^{IV}]_0$ ratio 0.5, but gradually decreased thereafter due to the competitive and non-productive binding of TpT by the free Pr^{III} ion in the mixture ($[Ce^{IV}]_0$ was kept constant here). The pH-rate constant profile indicated that the DNA hydrolysis involves the catalysis by a basic residue of pK_a 6.2. The reaction does not require molecular oxygen, since its removal by repeated freeze-thaw cycles did not affect either the hydrolysis rate or the product distribution.

According to the core level photoelectron spectroscopy, the binding energy (134.7 eV) of the 2p level of the P atom in the TpT, which is complexing with the 2:1 Ce^{IV}-Pr^{III} combination, is greater by 0.3 eV than that (134.4 eV) for the TpT complexing with the Ce^{IV} ion. The electron density at the P atom in the former TpT is considerably smaller than that in the latter TpT. Apparently, the electrophilicity of the P atom in TpT is increased by the bimetallic combination in a greater extent than is by the Ce^{IV} ion alone.[8] It is strongly indicated that the phosphate residue of the dinucleotide simultaneously interacts with two Ce^{IV} ions in the 2:1 mixed hydroxide cluster. Assumedly, the phosphate thus activated is attacked by the hydroxide ion bound to the Pr^{III} ion in the mixed hydroxide cluster. The kinetically determined pK_a (6.2) is slightly smaller than the pK_a value (7.1) [9] of the water bound to the Pr^{III} ion, reflecting the electron withdrawal from the Pr^{III} ion by the adjacent Ce^{IV} ions. The large positive charges of the cluster stabilize the negatively charged transition state of the reaction. Although the detailed mechanism is not yet clear, the present bimetallic combinations can hydrolyze DNA at reasonable rates under the physiological conditions: the TpT hydrolysis by the 2:1 Ce^{IV}-Pr^{III} combination at pH 7 and 30 °C is nearly completed within 1 day, although the conversion by the Ce^{IV} alone is less than 30%.

This work was supported by Grants-in-Aid for Scientific Research on Priority Areas "New Development of Rare Earth Complexes" from The Ministry of Education, Science, Sports and Culture, Japan and by JSPS Research Fellowships for Young Scientists (for N. T. and J. S.).

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1 Part of this work was presented at the NATO Workshop on "DNA Cleavers and Chemotherapy of Cancer and Viral Diseases," Toulouse, Sept. 11-14, 1995.

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6 Of the bimetallic combinations, only the La^{III}-Fe^{III} and the La^{III}-Sn^{IV} systems hydrolyze linear DNA.[5a] Their activities were more than three orders of magnitude smaller than those of the Ce^{IV}-Pr^{III} and the Ce^{IV}-Nd^{III} combinations.

7 Authentic samples of pT and Tp were hydrolyzed by the combination about 10 fold faster than TpT was.

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