Efficient and Oxygen-independent Hydrolysis of Single-stranded DNA by Cerium(IV) Ion

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Cerium(IV) ion efficiently hydrolyses the phosphodiester linkages in DNAs, even in the absence of molecular oxygen. The pseudo first-order rate constant for the hydrolysis of thymidylyl-(3',5')thymidine (TpT) by $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) at pH 7 and 50 °C is 1.9×10^{-1} h^{-1} (the half-life is 3.6 h), either with or without molecular oxygen. DNA hydrolysis by $CeCl_3$ requires molecular oxygen to convert the Ce^{III} ion to Ce^{IV}. Addition of hydrogen peroxide causes various side-reactions rather than accelerating the hydrolysis. The hydrolysis by Ce^{IV} proceeds *via* P–O scission, as confirmed by the absence of ^{18}O incorporation into thymidine (T) during the reaction in an $H_2{}^{18}O$–$H_2{}^{16}O$ mixture. There exists no specific base-preference in the scission, and concurrent oxidative cleavage of the deoxyribose is nil. The activity of Ce^{IV} is more than 200 fold greater than those of trivalent lanthanide ions and of other tetravalent ions. A significant D_2O solvent isotope effect and the pH independence of the hydrolysis rate indicate that the hydrolysis proceeds *via* an intramolecular attack by the Ce^{IV}-bound hydroxide ion and that the reaction is further assisted by the general acid catalysis of another water bound to the Ce^{IV} ion.

Non-enzymatic hydrolysis of DNA is increasingly attracting interest. A supercoiled plasmid DNA has been cleaved *via* the hydrolytic pathway either by the combination of a ruthenium complex and a divalent metal ion or by a diiron complex.[1] Under physiological conditions, linear DNAs are too stable to be non-enzymatically hydrolysed (the half-life of the phosphodiester linkage at pH 7 and 25 °C is estimated to be 200 million years).[2]

In previous papers,[3] we succeeded in demonstrating the first non-enzymatic hydrolysis of linear DNAs by use of lanthanide metal(III) salts and their complexes: $CeCl_3$ is the most active.†,‡ Then, we[4] and Chin *et al.*[5] independently found that the Ce^{IV} ion formed *in situ* is responsible for catalysis. The argument was supported by the effective catalysis of $Ce^{IV}(NH_4)_2(NO_3)_6$ for DNA hydrolysis.[4,6] Furthermore, selective scission of DNA at specific target sites has been successfully achieved by artificial nucleases, prepared from Ce^{IV} ion and DNA oligomers as sequence-recognizing moieties.§,[6,7]

We proposed that the hydroxide ion bound to Ce^{IV} intramolecularly attacks the DNA to effect hydrolysis.[3,4] According to Takasaki and Chin,[5] however, hydrogen peroxide is first formed (together with Ce^{IV}) from $CeCl_3$ and molecular oxygen and DNA hydrolysis takes place owing to Ce^{IV}-promoted nucleophilic attack by the hydrogen peroxide toward the phosphate. A systematic study is required to clarify the reaction mechanism.

We report here a full account of the DNA hydrolysis by Ce^{IV} ion, obtained directly from $Ce(NH_4)_2(NO_3)_6$ or prepared *in situ* from $CeCl_3$. No requirement of molecular oxygen and hydrogen peroxide for the catalytic process is evidenced in either case. The hydrolytic character of the scission is substantiated by product analysis and by isotope-exchange experiments. The base-preference for the scission reaction is also investigated. Furthermore, a reaction mechanism is proposed on the basis of kinetic and spectroscopic evidence.

Table 1 Rate constants (in 10^{-1} h^{-1}) for the hydrolysis of deoxyribodinucleotides by $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) at pH 7 and 50 °C, as well as the values for the corresponding ribodinucleotides

Dimer of deoxyribonucleotide	Rate constant	Dimer of ribonucleotide	Rate constant/10^{-1} h^{-1}
TpT	1.9	UpU	52
d(ApA)	1.6	ApA	50
d(TpA)	5.0	UpA	160
d(GpG)	2.0	GpG	43

Results

DNA Hydrolysis by Ce^{IV} *Ion.*—$Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) promptly and stoichiometrically hydrolysed thymidylyl-(3',5')thymidine (TpT) to thymidine (T) as depicted in Fig. 1(*a*). The rate constant at pH 7 and 50 °C is 1.9×10^{-1} h^{-1} (the half-life is 3.6 h), and the activation energy (estimated at 20–60 °C) is 23 ± 2 kcal mol^{-1}. The catalysis is also effective for the hydrolysis of other dinucleotides (Table 1). The rate of hydrolysis is not remarkably dependent on the kind of nucleic acid base on either the 3'-side or the 5'-side of the phosphodiester linkage.

† A marginal activity of La^{III} for DNA hydrolysis at pH 8.6 (5.3% conversion for 30 day reaction) was claimed 40 years ago: E. Bamann, H. Trapmann and F. Fischler, *Biochem. Z.*, 1954, **326**, 89. According to another paper, however, La^{III} is totally inactive for DNA hydrolysis: M. Shimomura and F. Egami, *Bull. Chem. Soc. Jpn.*, 1953, **20**, 263.

‡ $CeCl_3$ is the most active also for the hydrolysis of adenosine 3',5'-cyclic monophosphate: J. Sumaoka, M. Yashiro and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, 1992, 1707.

§ A number of artificial nucleases, which cleave the deoxyribose at the target site *via* oxidative process, have been reported: (*a*) P. B. Dervan, *Science*, 1986, **232**, 464; (*b*) J. K. Barton, *Science*, 1986, **233**, 727; (*c*) D. S. Sigman, *Acc. Chem. Res.*, 1986, **19**, 180; (*d*) B. C. F. Chu and L. E. Orgel, *Proc. Natl. Acad. Sci., U.S.A.*, 1985, **82**, 963. A photo-induced artificial nuclease was also reported: L. Perrouault, U. Asseline, C. Rivalle, N. T. Thuong, E. Bisagni, C. Giovannangeli, T. L. Doan and H. Hélène, *Nature*, 1990, **344**, 358.

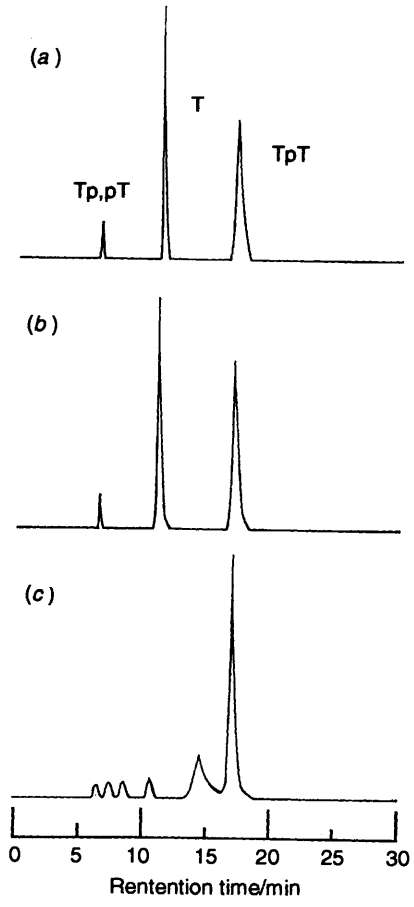

Fig. 1 Reversed-phase HPLC patterns for the hydrolysis of TpT by $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) at pH 7 (0.05 mol dm^{-3} HEPES buffer) and 50 °C: (*a*) for 3 h under air; (*b*) for 3 h in the absence of molecular oxygen; (*c*) for 1 h in the presence of hydrogen peroxide (0.01 mol dm^{-3})

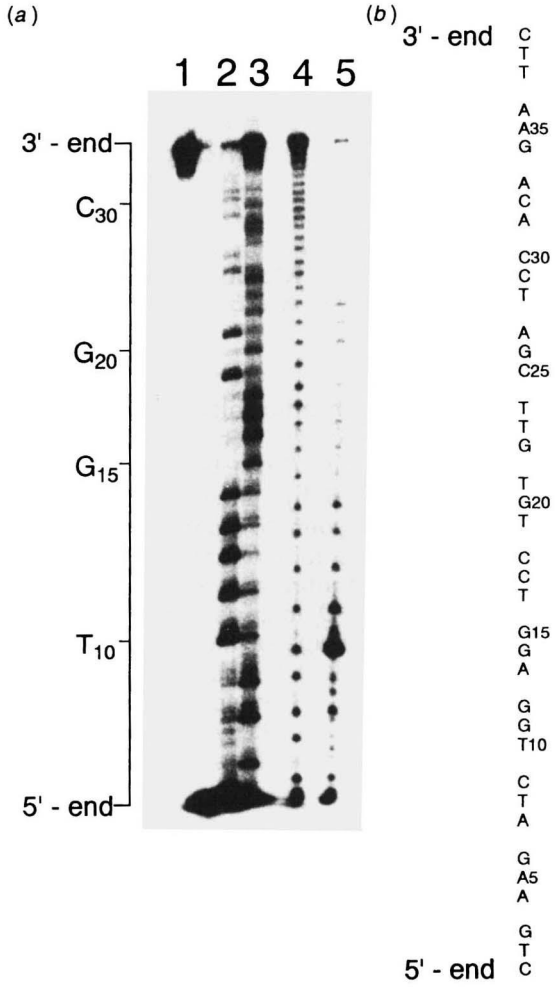

Fig. 2 Denaturing polyacrylamide gel (20%) electrophoresis patterns for the hydrolysis of the 39-mer DNA (10^{-6} mol dm^{-3}) by $Ce(NH_4)_2(NO_3)_6$ (10^{-2} mol dm^{-3}) at pH 7 (0.05 mol dm^{-3} Tris buffer) and 50 °C for 4 h; the oligomer in (*b*) was ^{32}P-labelled at the 5'-end. Lane 1, control; lane 2, Maxam-Gilbert A + G sequencing reaction; lane 3, C + T sequencing reaction; lane 4, scission by Ce^{IV}; lane 5, digested by DNase I. The DNA scale represents the residue at the 3'-end of the corresponding fragment.

The hydrolysis proceeds stepwise *via* the nucleoside monophosphates as intermediates, although they are promptly hydrolysed to the final product nucleosides and do not significantly accumulate in the mixtures. The time-courses for the dinucleotide hydrolyses are consistent with the theoretical ones calculated by use of the hydrolysis rates of authentic samples of the monophosphates. Thus the rate constants for the hydrolysis of Tp and pT under the conditions are 2.7 and 4.6 h^{-1}, respectively, which are 14 and 24 times as large as the value for the hydrolysis of TpT. No other by-products are formed. An independent reversed-phase HPLC system using different conditions showed that the Tp:pT ratio in the early stage of the reaction is around 1:1. Both the P–O(5') and the P–O(3') linkages are cleaved at comparable rates.

Phosphodiester linkages in a DNA oligomer (39-mer) are also effectively hydrolysed by $Ce(NH_4)_2(NO_3)_6$ (Fig. 2). The hydrolysis occurs almost uniformly throughout the DNA chain without significant base-preference (lane 4). According to a densitometric analysis of the lane, the scission efficiencies of the linkages differ from each other by less than two fold. The fragments (^{32}P-labelled at the 5'-end) comigrate with the digests by DNase I (lane 5), but not with the Maxam-Gilbert sequencing fragments (lanes 2 and 3). Apparently, 3'-OH termini are formed here. These results are consistent with the HPLC analysis on the dinucleotide hydrolysis described above.

Ribonucleotide dimers are also hydrolysed. The rates are 20–40 fold larger than those for the corresponding dimers of deoxyribonucleotides (Table 1). The only products are hydrolytic ones. Accumulation of small amounts of the 2',3'-cyclic monophosphates of ribonucleosides strongly indicates that the 2'-OH residues intramolecularly attack the phosphorus atoms as is usually the case in the enzymatic and non-enzymatic hydrolysis of RNA.

Product Analysis and Isotope-Exchange Experiments.—The final product T from the hydrolysis of TpT was concretely characterized by both ^1H NMR and HPLC–mass spectroscopy ($MH^+ = 243$). Release of thymine, expected for the oxidative cleavage of the deoxyribose residues, was not observed at all, confirming the totally hydrolytic character of the scission.

When TpT was hydrolysed by $Ce(NH_4)_2(NO_3)_6$ in a 20:80 $H_2{}^{18}O$–$H_2{}^{16}O$ mixture, no ^{18}O was incorporated into the product T. The intensity of the weak signal at $MH^+ = 245$, which corresponds to the incorporation of one ^{18}O atom into the T, was exactly the same as the value estimated in terms of natural abundance of the isotopes for the C, H, N and O atoms (Fig. 3). Consistently the intensity was identical with that at $MH^+ = 245$ for an authentic sample of T (the signal at 244 is also ascribed to the presence of the isotopes of natural abundance in the T). It is concluded that the present hydrolysis proceeds *via* the direct scission of the P–O bond in the

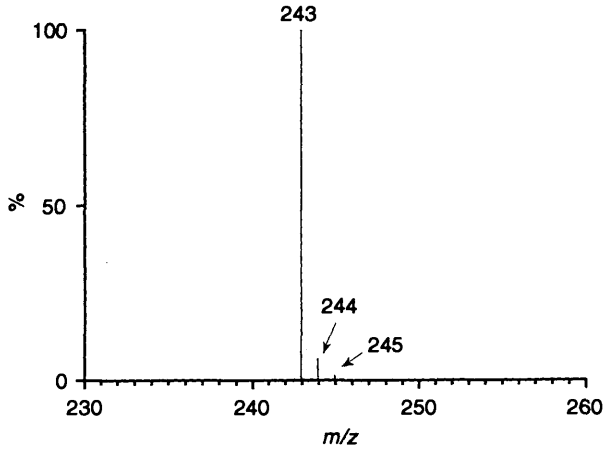

Fig. 3 HPLC–Mass pattern for the product of the TpT hydrolysis by $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) in a 20:80 $H_2{}^{18}O$–$H_2{}^{16}O$ mixture at pH 7 and 50 °C: only the MH^+ 230–260 region is presented

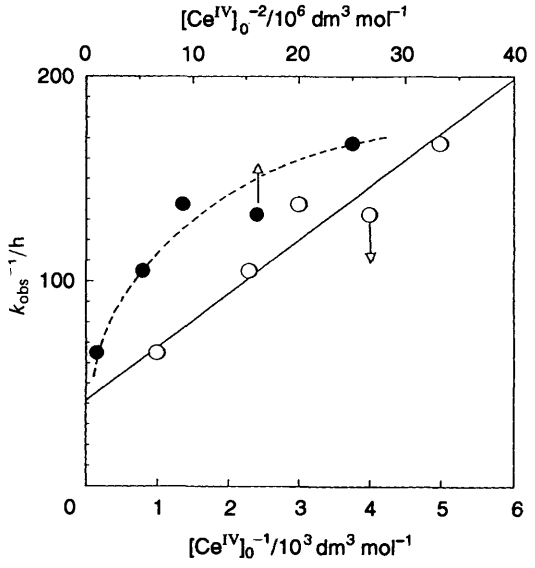

Fig. 4 Plot of $1/k_{obs}$ *vs.* $1/[Ce^{IV}]_0$ for TpT hydrolysis by $Ce(NH_4)_2(NO_3)_6$ at pH 2: $[TpT]_0 = 10^{-5}$ mol dm^{-3}. The closed circles show the plot of $1/k_{obs}$ *vs.* $1/[Ce^{IV}]_0{}^2$.

phosphodiester linkage, without any contribution from C–O scission.[8]

Furthermore, ^{1}H-NMR spectroscopy has shown that the hydrolysis in D_2O does not give rise to any H–D exchange in either TpT or T. No proton is abstracted from the deoxyribose during the hydrolysis.

Effects of Molecular Oxygen and Hydrogen Peroxide on the Ce^{IV}*-Induced DNA Hydrolysis.*—The rate constant for the $Ce(NH_4)_2(NO_3)_6$-induced hydrolysis of TpT in the absence of molecular oxygen was identical with that under air within experimental error [compare Fig. 1(*b*) with (*a*)]. Here molecular oxygen was thoroughly removed from the mixtures by repeated freeze-thaw cycles. Apparently molecular oxygen is not necessary for DNA hydrolysis by Ce^{IV}.

Hydrogen peroxide (0.01 mol dm^{-3}), when added to the reaction mixtures (either with or without molecular oxygen), showed no measurable acceleration of the hydrolysis, and rather gave varieties of by-products [see the complicated HPLC pattern in Fig. 1(*c*)]. One of them at the retention time 6.4 min was assigned to thymine by coinjection of an authentic sample, but others were not assignable. In addition, the total peak area in the HPLC gradually decreased with increasing reaction time, indicating that some destructive reaction was taking place. These results contrast sharply with the selective formation of the hydrolytic product T (as well as the hydrolysis intermediates pT and Tp) in the absence of hydrogen peroxide [Fig. 1(*a*) and (*b*)].

When a pH 7 solution of $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) without any buffer agent was incubated at 50 °C in the absence of molecular oxygen, no significant pH change was observed. This result indicates that formation of hydrogen peroxide from water and Ce^{IV} is virtually nil, since it should be accompanied by the release of protons.

Kinetic and Spectroscopic Analysis of the Ce^{IV}*-induced Hydrolysis of TpT.*—The mixtures for the DNA hydrolysis by $Ce(NH_4)_2(NO_3)_6$ at pH 7 involve some precipitation, presumably because of formation of the metal hydroxide. However, the DNA hydrolysis occurs over a wide pH range (from 1 to 9), and thus a detailed kinetic analysis has been carried out below pH 3.3 where the mixtures are homogeneous. No oxidative cleavage of the deoxyribose occurs here, as shown by the HPLC.¶ The titration study (see the Experimental section) has confirmed that no Ce^{IV} is reduced to Ce^{III} during the DNA hydrolysis.

The hydrolysis rate is virtually constant from pH 1.3 to 3.3. This indicates an essential role of the Ce^{IV}-bound hydroxide ion for the DNA hydrolysis: its concentration (pK_a *ca.* 0)[9] remains almost constant in the pH region. The pK_a of the phosphodiester linkage of TpT has been independently determined to be 0.4 ± 0.3 by titration using ^{31}P NMR chemical shifts, and thus the TpT mostly exists as a monoanion throughout the pH region.‖

A plot of the hydrolysis rate *vs.* $[Ce^{IV}]_0$ under conditions where $[Ce^{IV}]_0 \gg [TpT]_0$ shows saturation at large values of $[Ce^{IV}]_0$, corresponding to complex formation between Ce^{IV} and TpT. Thus the data have been analysed in terms of eqn. (1) which is based on the assumption of 1:1 complex formation.

$$1/k_{obs} = 1/k_c + 1/(k_cK) \times 1/[Ce^{IV}]_0 \quad (1)$$

Here k_c refers to the rate constant for the hydrolysis of the TpT in the complex. As shown by the open circles in Fig. 4, the plot gives a fairly good straight line, substantiating that the TpT hydrolysis proceeds *via* the 1:1 complex between Ce^{IV} and TpT. The formation constant K of the complex is determined to be 1700 ± 200 dm^3 mol^{-1}. In contrast, the plot of $1/k_{obs}$ *vs.* $1/[Ce^{IV}]_0{}^2$ shows a considerable deviation from linearity with a notable curvature (the closed circles in Fig. 4). Thus, the participation of two (or more) Ce^{IV} ions in the catalysis is inconsistent with the results.

The ^{31}P NMR signal of TpT shifted towards lower magnetic field on complex formation (2.3 ppm when $[TpT]_0 = 0.01$ and $[Ce^{IV}]_0 = 0.03$ mol dm^{-3}), showing that the phosphodiester linkage of TpT, rather than the nucleic acid bases, coordinates to Ce^{IV}. Consistently, the ^{1}H NMR chemical shifts of the thymine residues in TpT were hardly affected by 0.01 mol dm^{-3} of $Ce(NH_4)_2(NO_3)_6$. Thus the DNA scission proceeds without remarkable base-preference.

It is noteworthy that a significant D_2O solvent isotope effect (2.0 ± 0.2) has been observed for the DNA hydrolysis. The

¶ Release of the nucleic acid bases due to cleavage of the ribose was detected only when the pH was 1.0 or smaller.

‖ The rate of DNA hydrolysis gradually decreased with decreasing pH below pH 1.3, due to the protonation of TpT. However, a detailed analysis of the pH-rate constant profile in the highly acidic region was unsuccessful, since the oxidative cleavage of TpT yielded complicated products (see note ¶).

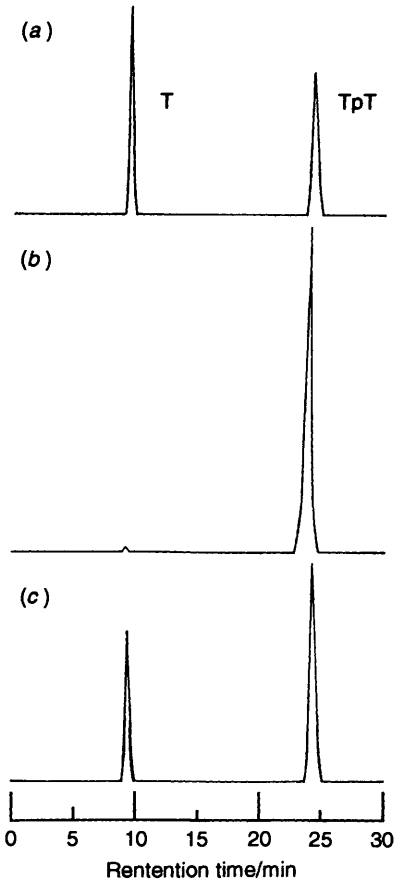

Fig. 5 HPLC patterns for the hydrolysis of TpT by $CeCl_3$ (0.01 mol dm^{-3}) at pH 7 (0.05 mol dm^{-3} HEPES buffer) and 50 °C for 3 h: (*a*) under air; (*b*) in the absence of oxygen; (*c*) $CeCl_3$ was preincubated at pH 7 and 50 °C for 1 h under air and then the DNA hydrolysis was achieved in the absence of molecular oxygen (see text for detail)

magnitude of the isotope effect was constant irrespective of pH. Apparently the catalysis involves a rate-limiting proton-transfer.

Comparison of the Catalytic Activity of Ce^{IV} *with that of other Trivalent and Tetravalent Metal Ions.*—Ce^{IV} ion greatly (by more than 200-fold) exceeds lanthanide metal^{III} ions and other tetravalent ions in its activity. When $CeCl_3$ (0.01 mol dm^{-3}) is used in the absence of molecular oxygen, the conversion of the TpT hydrolysis at 3 h is only 0.2 mol% [Fig. 5(*b*): compare this chart with Fig. 1(*a*) and (*b*)]. The complete removal of molecular oxygen (by freeze-thaw cycles) is essential here, since otherwise the Ce^{III} ion is promptly oxidized to Ce^{IV} during the reaction (*vide infra*: the absence of the Ce^{IV} formation in these experiments has been confirmed by titration).

The activities of La^{III}, Pr^{III}, Nd^{III}, Sm^{III}, Eu^{III}, Gd^{III}, Tb^{III}, Dy^{III}, Ho^{III}, Er^{III}, Tm^{III}, Yb^{III} and Lu^{III} (used as the chlorides) are almost identical to that of Ce^{III} and are not affected by molecular oxygen. $HfCl_4$ and $ZrCl_4$ show only marginal catalysis (the conversions at 4 h are less than 0.2 mol%), whereas $TiCl_4$ is inactive. The superiority of Ce^{IV} is conclusive.

DNA Hydrolysis by Ce^{IV} *Prepared in situ from* $CeCl_3$.—Instead of the use of $Ce(NH_4)_2(NO_3)_6$, catalytically active Ce^{IV} can be obtained *in situ* by carrying out the DNA hydrolysis with $CeCl_3$ under air [Fig. 5(*a*)]. The titration study has shown that about 60% of the Ce^{III} is oxidized to Ce^{IV} at 50 °C and pH 7 for 3 h. The conversion for the TpT hydrolysis at 3 h is 43 mol%, which is in strong contrast with the marginal DNA hydrolysis in the absence of molecular oxygen [Fig. 5(*b*)]. This result is consistent with the previous findings[3,5] that $CeCl_3$ is overwhelmingly more active than other lanthanide(III) chlorides. Of all the lanthanide ions, only cerium ion has a stable tetravalent state in aqueous solutions.[10]

The catalytic activity of the Ce^{IV} ion, prepared *in situ* from $CeCl_3$, is not affected by molecular oxygen to a considerable extent, as shown by the following result. An aqueous solution of $CeCl_3$ was first incubated at 50 °C under air for 1 h, and then molecular oxygen was removed by freeze-thaw cycles. About 40% of the Ce^{III} ion was oxidized to Ce^{IV} during this procedure, as determined by a titration study. As depicted in Fig. 5(*c*), the DNA hydrolysis is efficient under these conditions. Apparently molecular oxygen is used only for the formation of Ce^{IV} from Ce^{III} and not for the catalysis by the resultant Ce^{IV}. Addition of hydrogen peroxide to these solutions provided complicated HPLC patterns which are similar to that [Fig. 1(*c*)] for the DNA hydrolysis by the combination of $Ce(NH_4)_2(NO_3)_6$ and hydrogen peroxide.

Discussion

DNA Hydrolysis by Ce^{IV}.—Phosphodiester linkages in DNA, which are remarkably stable and highly resistant to hydrolysis, have been hydrolysed by Ce^{IV} ion under mild conditions.[3–7] The Ce^{IV} ions are either obtained from Ce^{IV} salts or prepared *in situ* from Ce^{III} salts. The deoxyribose residues remain intact during the DNA scission. It is noteworthy that Ce^{IV}, which is usually used as an oxidation agent, promotes the hydrolysis of the phosphodiester linkage of DNA around neutral pH. Concurrent oxidative cleavage of the deoxyribose is detectable only under highly acidic conditions,** which are far away from the physiological ones.

Neither the $Ce(NH_4)_2(NO_3)_6$-induced DNA hydrolysis nor the $CeCl_3$-induced one (under air) employs molecular oxygen as a cofactor in the catalytic process. Only when the Ce^{III} salts such as $CeCl_3$ are additives for the hydrolysis, molecular oxygen is required to convert the Ce^{III} ions to highly active Ce^{IV} ions. Thus hydrolysis by the Ce^{IV} salt is successful even in the total absence of molecular oxygen throughout the reaction. Hydrogen peroxide induces quite complicated side-reactions rather than accelerates the hydrolysis [Fig. 1(*c*)]. Presumably, the combination of Ce^{IV} and hydrogen peroxide produces some radical species, which oxidatively cleave the deoxyribose residues. The concurrent release of the nucleic acid base (thymine) is consistent with this interpretation. Any essential role of hydrogen peroxide or its relevant species, formed *in situ* if any, in the $CeCl_3$-induced DNA hydrolysis[5,11] seems to be unlikely.

Ce^{IV} ion exhibits no remarkable base-preference in DNA scission (Table 1 and Fig. 2), and thus is highly potent as the catalytic centre of artificial nucleases for site-selective DNA scission. In fact, the Ce^{IV}-iminodiacetate complex, attached to a DNA oligomer as sequence-recognizing moiety, selectively cleaved the substrate DNA at the target site.[6,7] The DNA scission does not need molecular oxygen, and can be achieved even under anaerobic conditions. This is quite important from a practical standpoint, since the interior of cells, for example, is considerably anaerobic (the concentration of molecular oxygen in cells is 0.1–10 μmol dm^{-3}, whereas the value in water under air is 250 μmol dm^{-3}).[12] The scission by Ce^{IV} prepared *in situ* from $CeCl_3$ is effective for the hydrolysis of both single-stranded and double-stranded DNA, as reported in a preliminary communication.[3d]

Proposed Mechanism.—The proposed mechanism of the DNA hydrolysis by Ce^{IV} ion is schematically depicted in Fig. 6.

** See note ¶ on p. 271.

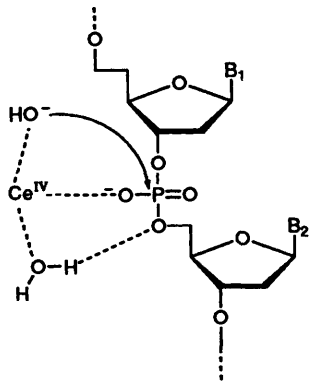

Fig. 6 Proposed mechanism for the DNA hydrolysis by Ce^{IV} ion: P–O(3′) scission is depicted here, although P–O(5′) scission is also plausible

Metal-bound hydroxide ion intramolecularly attacks the phosphate of DNA, which is bound to the same Ce^{IV} ion. Similar intracomplex reactions have been proposed for the hydrolysis of activated phosphate esters by lanthanide metal ions and complexes.††[1,13] The intramolecular reaction is further promoted by the general acid catalysis of another water on the Ce^{IV} ion, as confirmed by the significant D_2O solvent isotope effect (2.0). The general acid catalysis can take place concertedly with intramolecular attack by the metal-bound hydroxide. As the P–O bond is formed, the effective positive charge on the cerium ion is gradually increased and thus the dissociation of the metal-bound water is facilitated. The lanthanide ion has many (8–9) coordination water molecules[14] so that one (or some) of them should be located in quite an appropriate position for the general acid catalysis. Furthermore, the large positive charge of the Ce^{IV} ion electrostatically stabilizes the negatively charged transition state of the DNA hydrolysis. A similar electrostatic catalysis is achieved by the lysine-41 residue of ribonuclease A in RNA hydrolysis.[15] The cooperation of the acid–base catalysis and the electrostatic catalysis should be responsible for the efficient hydrolysis of the stable DNA under mild conditions.

The overwhelming importance of the Ce^{IV}-bound hydroxide as an intramolecular nucleophile is clearly evidenced by the fact that the hydrolysis rates of deoxyribodinucleotides are only 20–40 fold smaller than those for ribodinucleotides (Table 1). DNAs are intrinsically much (several orders of magnitude) less reactive than RNAs[2,13b] because of the absence of the 2′-OH for the intramolecular attack. In the Ce^{IV} catalysis, the metal-bound hydroxide serves as a highly eminent substitute of the 2′-OH residue, giving rise to the efficient hydrolysis of DNA. The possibility that the TpT in the Ce^{IV}–TpT complex is intermolecularly attacked by an uncomplexed water molecule is unlikely. The activation of TpT by complex formation with Ce^{IV} is not remarkable: the ^{31}P-chemical shift change (2.3 ppm) is considerably smaller than those (10–20 ppm) observed for the co-ordination of phosphoesters to the Co^{III} complexes.†† The bimolecular reaction between the Ce^{IV}–TpT complex and the Ce^{IV}–bound hydroxide is ruled out by the results in Fig. 4.

Trivalent lanthanide metal ions show much smaller activities, partially because their co-ordinated water is less acidic (the pK_a is 8–9).[9] In addition, the smaller positive charge on the metal ions is less favourable for the electrostatic catalysis. An estimation using the formation constant of the Ce^{III}–TpT complex (30 dm^3 mol^{-1})[7] indicates that about one quarter of TpT is complexed with Ce^{III} under the conditions employed in Fig. 5(*b*). The DNA hydrolysis is virtually nil there, and thus the great difference in the activity between Ce^{IV} and Ce^{III} cannot be ascribed to the difference in the concentration of the complexed DNA.

†† Co^{III} complexes also hydrolyse activated aryl phosphates by intracomplex reactions: (*a*) D. R. Jones, L. F. Lindoy and A. M. Sargeson, *J. Am. Chem. Soc.*, 1983, **105**, 7327; (*b*) R. M. Milburn, M. Gautem-Basek, R. Tribolet and H. Siegel, *J. Am. Chem. Soc.*, 1985, **107**, 3315; (*c*) ref. 2.

Conclusions

Non-enzymatic hydrolysis of the phosphodiester linkages in linear DNAs has been successfully achieved by Ce^{IV} ions under physiological conditions. The activity is far greater than that of any other metal ions. The scission is totally hydrolytic, and shows no specific base-preference. The high potential of Ce^{IV} as the catalytic centre of artificial hydrolytic nucleases is evidenced.[6,7] Molecular oxygen is not necessary at all for the catalysis by Ce^{IV}. The addition of hydrogen peroxide induces various side-reactions, probably due to oxidative cleavage of the deoxyribose, rather than accelerates the hydrolysis.

The reaction involves a complex between the DNA and Ce^{IV}. All the kinetic and spectroscopic evidence indicates that the hydroxide ion bound to the Ce^{IV} ion intramolecularly attacks the phosphate of the DNA, which coordinates to the Ce^{IV} ion. The reaction is assisted both by the general acid catalysis of the water bound to the Ce^{IV} ion and by the electrostatic catalysis of the Ce^{IV} ion. The large number of the co-ordinated water molecules on Ce^{IV} and their high acidities are mainly responsible for the superb activity of Ce^{IV}. The present DNA hydrolysis efficiently proceeds even under anaerobic conditions and thus has a number of potential applications.

Experimental

Materials.—Lanthanide metal salts from Soekawa (except for $LaCl_3$ from Nacalai) were used without further purification. Dinucleotides were purchased from Sigma. $H_2{}^{18}O$ (80.3 atom% ^{18}O) and D_2O (99.9 atom% D) were obtained from Commissariat a L'Energie Atomique and Aldrich, respectively. All the buffers [I = 0.1 mol dm^{-3} (KCl)] were sterilized immediately before use, and great care was taken to avoid contamination by natural nucleases throughout the experiments. A 39-mer DNA oligomer was prepared by an automated synthesizer and was ^{32}P-labelled at the 5′-end by the action of T4 polynucleotide kinase and adenosine 5′-[γ-^{32}P]triphosphate. The choice of the sequence was rather arbitrary.

Hydrolysis of Dinucleotides.—The dinucleotide hydrolysis was analysed by a reversed-phase HPLC [Merck LiChrosphere RP-18(e) ODS column; water–acetonitrile = 92/8 or 94/6 (v/v)]. The reaction mixture was prepared by the addition of $Ce(NH_4)_2(NO_3)_6$ to a buffer solution, followed by the pH adjustment with a small amount of NaOH. The initial concentration of the dinucleotide was 10^{-4} mol dm^{-3} unless otherwise noted.

At an appropriate interval, a 30 mm^3 portion of the reaction mixture was sampled and was treated with 3 mm^3 of 10% phosphoric acid to decompose the complex between the dinucleotide and Ce^{IV}. The specimen was injected to the HPLC after being passed through a disposable pretreatment filter (Tosoh; W-3-2). Unequivocal assignment of the HPLC signals was made by coinjection of authentic samples.

All the reactions satisfactorily showed pseudo-first-order kinetics. The rate constants reported in the present paper are the averages of the results of at least duplicate runs which coincide with each other within ± 5%.

For the experiments in the absence of molecular oxygen, the reaction mixtures were first subjected to repeated (3 times or

more) freeze–thaw cycles, and then the DNA hydrolysis was carried out under nitrogen of the purity >99.99%.

Determination of Ce^{IV} *in the Reaction Mixture.*—The concentration of Ce^{IV} in the reaction mixture was determined by titration. A given amount of $FeSO_4$ was added to the mixture and the resultant solution was back-titrated with $Ce(NH_4)_4(SO_4)_4$ using *o*-phenanthroline as an indicator. At least duplicate runs were achieved for each of the determinations. The solutions of $FeSO_4$ and $Ce(NH_4)_4(SO_4)_4$ were prepared immediately before use.

Hydrolysis of DNA Oligomer.—Hydrolysis of the 39-mer DNA oligomer (^{32}P-labelled at the 5′-end; 10^{-6} mol dm^{-3}) was carried out in 50 mmol dm^{-3} Tris buffer and was followed by electrophoresis using a denaturing polyacrylamide gel. The scission patterns were analysed by densitometry.

Spectroscopy.—The ^1H NMR spectra of the reaction products were taken in D_2O on a JNM-GX 400 FT NMR spectrometer. The reaction mixture, in which TpT was partially or almost completely hydrolysed in D_2O by $Ce(NH_4)_2(NO_3)_6$, was treated with a disposable pretreatment filter (Kurabo; CHROMATODISK 13A) after being concentrated by evaporation.

The HPLC–mass spectra were measured on a PLATFORM (Fisons Instruments Organic Analysis Biotech MS), in which the ionization was made by an electronic spray method. Before the injection, the specimens were passed through a cation-exchange column (Tosoh; TOYOPAK IC-SP) to remove the Ce^{IV} ion.

The ^{31}P NMR spectroscopy was run on a JNM-GX 400 FT NMR spectrometer with 85% D_3PO_4 as an external standard. The pK_a of the phosphate of TpT was determined in D_2O by the titration using the chemical shift changes.

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