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COMMUNICATION

Cerium(IV)-oligoDNA hybrid as highly selective artificial nuclease[1]

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Cerium(IV) ion is attached to the 5′-end of a 19-mer DNA by use of iminodiacetate ligand. At pH 7.2 and 30 °C, the hybrid selectively cuts a 40-mer DNA, which has 19-mer sequence complementary with the DNA in the hybrid, at the target site (the 3′-side of the 19-mer portion of the substrate DNA). Cerium(IV) ion hydrolyzes thymidylyl(3′-5′)thymidine to thymidine without concurrent oxidative cleavage of the ribose, indicating that the selective scission by the hybrid proceeds via the hydrolysis of the phosphodiester linkage.

INTRODUCTION

Selective scission of DNA has been attracting interests, and many elegant systems involving oxidative cleavage of the deoxyribose were reported.[2–5] However, artificial hydrolytic nucleases, which selectively hydrolyze the phosphodiester linkages in DNA as natural nucleases do, have never been prepared yet (except for the one using natural nuclease as the catalytic site).[6] One of the greatest obstacles was the lack of the catalyst for the hydrolysis of enormously stable DNA (half-life of the phosphodiester linkage in DNA at pH 7, 25 °C has been estimated to be 200 million years).[7]

Quite recently, we have shown that lanthanide ions and their complexes efficiently hydrolyze linear DNAs under mild conditions and thus are highly potent as the catalytic sites for the artificial hydrolytic nucleases.[8–11]

Here we report that the Ce(IV) ion bound to a DNA oligomer using iminodiacetate ligand selectively cuts the substrate DNA at the target site. Hydrolytic character of the scission is indicated by the HPLC analysis on the cleavage of thymidylyl(3′-5′)thymidine (TpT) by Ce(IV) ion.

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EXPERIMENTAL SECTION

Iminodiacetate-attached DNA oligomer (DNA-IA) was synthesized according to the well known chemistry, as depicted in Fig 1. A 19-mer DNA having an amino residue at the 5′-end, prepared on a CPG column by use of an automated synthesizer, was reacted with 1,1′-carbonyldiimidazole in 1,4-dioxane (30 mM, 10 ml) at room temperature for 10 min, and was then with diethyl iminodiacetate in dioxane (2 M, 10 ml) at 50 °C for 2 h. The ethyl esters were hydrolyzed by 10 ml of aqueous 0.5 N NaOH solution at room temperature for 1 h, and finally the nuleic bases were deprotected by concentrated ammonia solution in the usual way. Between the reaction steps the CPG column was sufficiently washed by dioxane. After each of the steps a small portion of the product was removed from the column by concentrated ammonia solution and was analyzed by anion-exchange HPLC (TOSOH DEAE-NPR column). The attachment of diethyl iminodiacetate residue to the 5′-end of the DNA oligomer resulted in the longer retention time (corresponding to the conversion of the positively charged amino residue to a urea linkage), and the hydrolysis of the ethyl esters therein to carboxylates further lengthened the retention time due to the formation of two negative charges. A 40-mer DNA, which has 19-mer sequence complementary with the 19-mer DNA in the DNA-IA, was ^{32}P-labelled at the 3′-end by dideoxyadenosine 5′-[α-^{32}P]triphosphate. The sequences of the 19-mer and the 40-mer, chosen rather arbitrarily here, are presented in Fig 3.

Mixtures of the 40-mer DNA and the DNA-IA were heated at 90 °C for 2 min, and then were allowed to stand at 30 °C for 1 h to complete the double helix formation between the two 19-mer sequences.

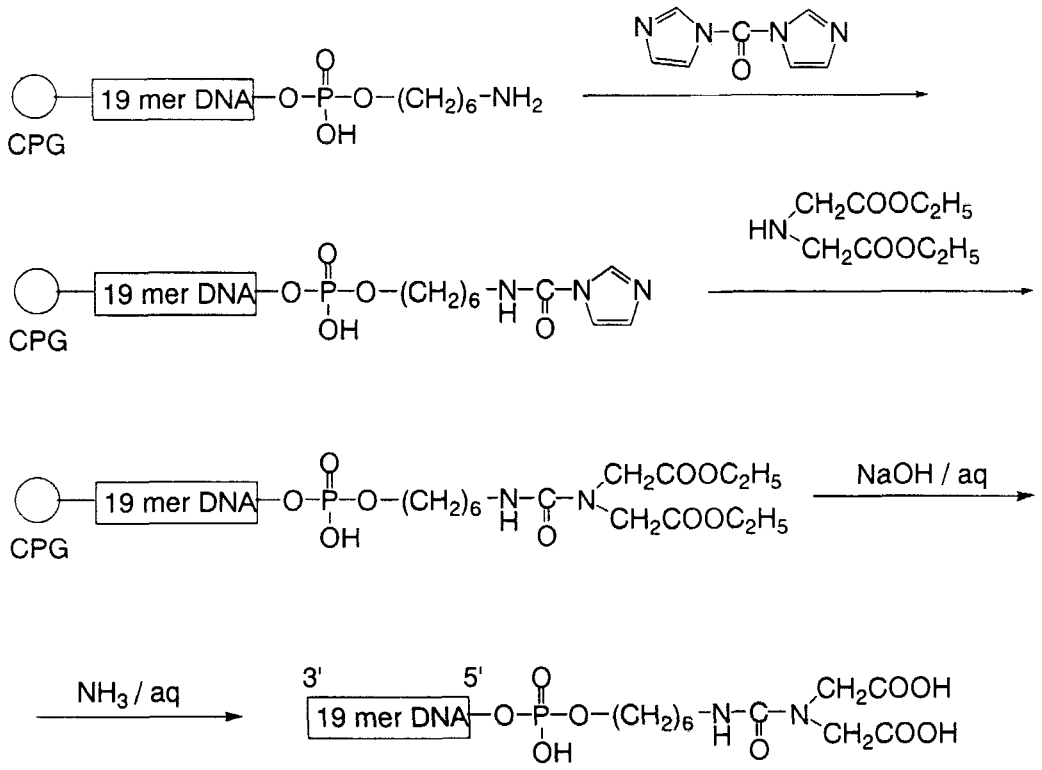

Figure 1 Scheme for the preparation of the iminodiacetate-attached 19-mer DNA (DNA-IA).

Hydrolysis of the 40-mer DNA at pH 7.2 (100 mM Tris buffer) and 30 °C was initiated by the addition of $Ce(NH_4)_2(NO_3)_6$ to the mixture, and was followed by electrophoresis using a denaturing polyacrylamide gel. The scission patterns were quantitatively analyzed by densitometry. Absence of contamination of natural nucleases was substantiated by repeated control experiments.

The hydrolysis of TpT by $Ce(NH_4)_2(NO_3)_6$ was followed by reversed-phase HPLC (Merck Lichrosphere 18(e) column) in the same way as described previously.[9]

RESULTS AND DISCUSSION

Typical electrophoresis pattern for the scission of the 40-mer DNA (3'-labelled) by the Ce(IV) complex of the DNA-IA at pH 7.2, 30 °C for 12 h is presented in lane 1 of Fig 2. Quite significantly, the scission takes place mostly at the linkage between A31 and C32 (see the scission profile in Fig 3). The selectivity is 54% (the total conversion for the scission of the 40-mer DNA is 60 mol%). A highly selective and efficient artificial nuclease has been prepared.

The product migrates more promptly than the Maxam-Gilbert fragment which ranges from A31 to A40 and has a 5'-phosphate terminus (observed at the position of A31 in lane 5), but more slowly than the C32-A40 fragment of 5'-phosphate terminus (at the position of C32 in lane 4). Note that the Maxam-Gilbert reactions provide 5'-phosphate termini[12] and that the sequence scale in the left-hand side in Fig 2 refers to the position of the fragment which has the designated deoxyribonucleotide at the 5'-end and has a 5'-phosphate terminus. Thus the present scission dominantly yields the C32-A40 fragment having a 5'-OH terminus (no alkali-labile species is formed as shown later). Minor cleavages are also perceivable around the main scission site as well as at the 5'-side of G20.

The scission profile is exactly consistent with the molecular design, since the Ce(IV) bound to the DNA-IA is placed near the linkage between A31 and C32 when the complementary 19-mer sequences in the DNA-IA and in the substrate DNA form a double helix. The other minor cleavages are probably associated with the dangling motion of the single-stranded portion in the substrate DNA and/or fluctuation of the rather flexible hexamethylene linker in the DNA-IA (G20 is located almost beneath C32 in the double helix between the two 19-mer sequences and thus can be attacked by the Ce(IV)).

The catalytic activity of Ce(IV) ion for the hydrolysis of phosphodiester linkages in DNA is clearly evidenced by the fact that TpT is hydrolyzed to thymidine (see

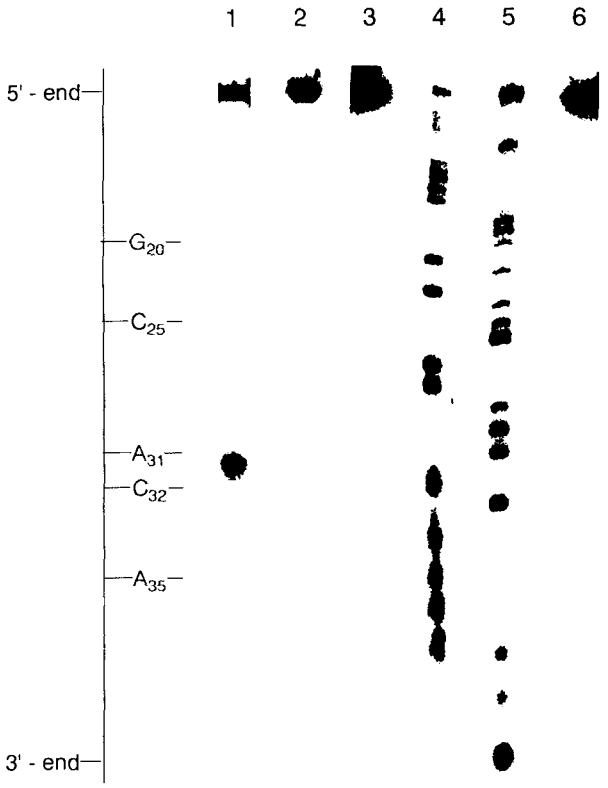

Figure 2 Autoradiographs for the scission of the 40-mer DNA (shown in Fig 3; ^{32}P-labelled at the 3'-end; 1 μM) by the combination of DNA-IA (10 μM) and metal salts (10 μM) at pH 7.2 and 30 °C for 12 h: lane 1, $Ce(NH_4)_2(NO_3)_6$; lane 2, $CeCl_3$; lane 3, $AlCl_3$; lane 4, Maxam-Gilbert A+G sequencing reaction; lane 5, C+T sequencing reaction; lane 6, control. The sequence scale shows the position of the fragment which has the designated deoxynucleotide at the 5'-end and has a 5'-phosphate terminus. These positions have been determined by use of the Maxam-Gilbert reactions in lanes 4 and 5 (the deoxyribonucleotide which is reacted in a specific manner in these reactions is ultimately removed by the subsequent treatments and thus the next deoxyribonucleotide is at the 5'-end of the resultant fragment (ref. 12)).

the HPLC patterns in Fig 4). No thymine is released from the dinucleotide during the hydrolysis (thymine if any should be observed at the retention time 7.9 min in Fig 4). Apparently Ce(IV) ion has no catalytic activity for the oxidative cleavage of the ribose residue. In addition, the post-treatment by 1 M piperidine (at 90 °C for 30 min) of the reaction mixtures for the scission of the 40-mer DNA did not cause any significant changes in the electrophoresis patterns.

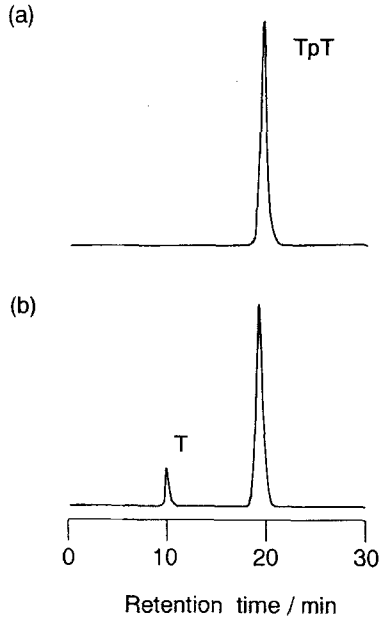

Figure 4 Hydrolysis of TpT by Ce(IV) ion at 30 °C: (a) t = 0 h and (b) t = 12 h: $Ce(NH_4)_2(NO_3)_6$ (10^{-5} mol) was added to 1 ml of 50 mM Hepes buffer containing TpT (10^{-4} M). Thymine, if were formed by the oxidative cleavage of the ribose, should be observed at the retention time 7.9 min.

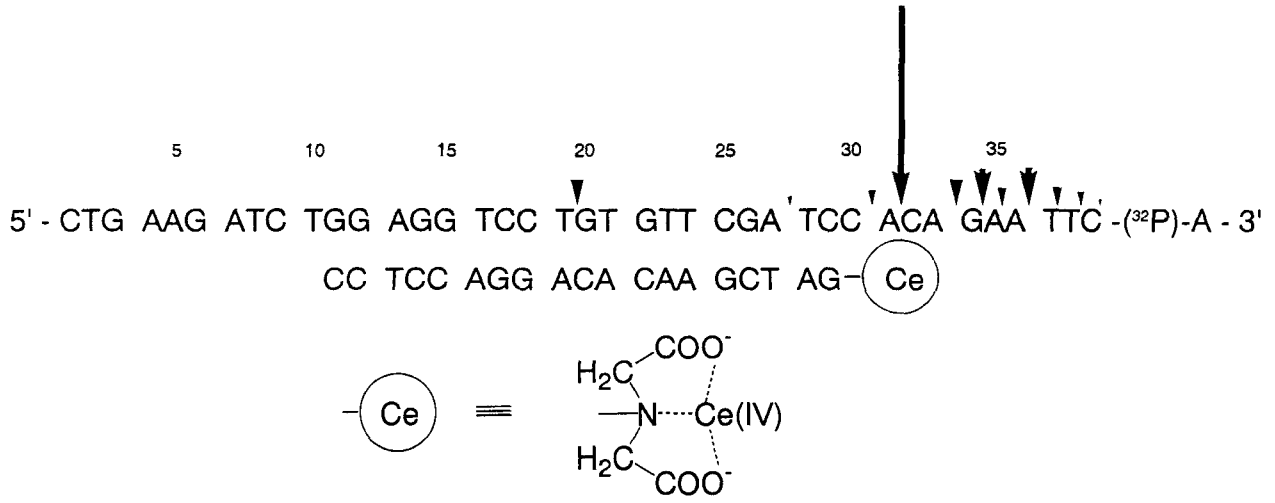

Figure 3 Scission profile for the cleavage of the 40-mer DNA by the Ce(IV)-oligoDNA hybrid: the profile was obtained by densitometry on lane 1 in Fig 2. The length of arrow corresponds to the frequency of scission. Ce denotes the Ce(IV)-iminodiacetate complex bound to the 19-mer DNA via hexamethylene linker.

Alkali-labile sites, which should be formed if the oxidative cleavage were taking place,[4] are not produced. Hydrolytic character of the scission by the Ce(IV)-oligoDNA hybrid is indicated.

The selective DNA scission probably involves intramolecular attack of the Ce(IV)-bound hydroxide ion toward the phosphodiester residue which is coordinating to the Ce(IV) ion.[8-11] The large positive charge of Ce(IV) electrostatically promotes the dissociation of the Ce(IV)-bound water and also stabilizes the negatively charged transition state of the hydrolysis.[13] In addition, the water molecules on the Ce(IV) can function as acid catalysts. Otherwise the reaction hardly proceeds due to electrostatic suppression by the negatively charges of the phosphates of the DNA-IA and the substrate DNA. Consistently the hybrids of Ce(III) and Al(III) ions (other trivalent lanthanide ions also) with DNA-IA are virtually inactive for the DNA hydrolysis under the conditions employed (lanes 2 and 3 in Fig 2). The activities of Ti(IV) and Hf(IV) are also marginal, assumedly because of poor binding to the phosphates of the DNA.

Spectroscopic determination of the formation constant of the iminodiacetate-Ce(IV) complex, which takes advantage of competition between iminodiacetate and a dye 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)phenol for the coordination to Ce(IV),[14] was unsuccessful because of precipitation of the complex between the dye and Ce(IV). However, the value is estimated to be around $10^9\ M^{-1}$, since the formation constant of the iminodiacetate-Ce(III) complex is $10^{6.18}\ M^{-1}$,[15] and the formation constants of the Ce(IV) complexes of both ethylenediaminetetraacetate and citrate are 3 orders of magnitude larger than the values for the corresponding Ce(III) complexes.[16] Thus almost all (>99%) of the Ce(IV) in the reaction mixture should be complexing with the iminodiacetate residue of DNA-IA when $[Ce(IV)]_0 = [DNA\text{-}IA]_0 = 10\,\mu M$ (the molar fraction of the complexing Ce(IV) is 0.90 even when the formation constant of the complex is $10^7\ M^{-1}$).

In conclusion, highly selective DNA scission is successfully achieved by the hybrid of Ce(IV) ion with an oligoDNA as sequence-recognizing moiety. More detailed study on the manner of the scission as well as the preparation of artificial nuclease for the scission of double-stranded DNA is now in progress in our laboratory.

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REFERENCES

1 A part of this work was presented at the 2nd International Symposium on Bioorganic Chemistry, June 6–10, 1993, Fukuoka, Japan (Abstract p. 116).
2 Dervan, P.B.; *Science (Washington D.C.)* **1986**, *232*, 464.
3 Barton, J.K.; *Science (Washington D.C.)* **1986**, *233*, 727.
4 Sigman, D.S.; *Acc. Chem. Res.* **1986**, *19*, 180.
5 Chu, B.C.F.; Oregl, L.E.; *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 963.
6 Zuckermann, R.N.; Schultz, P.G.; *J. Am. Chem. Soc.* **1988**, *110*, 6592.
7 Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X.; *J. Am. Chem. Soc.* **1989**, *111*, 186.
8 Matsumoto, Y.; Komiyama, M.; *Nucleic Acids, Symp. Ser.* **1992**, *27*, 33.
9 Komiyama, M.; Matsumura, K.; Yonezawa, K.; Matsumoto, Y.; *Chem. Express* **1992**, *8*, 85.
10 Shiiba, T.; Yonezawa, K.; Takeda, N.; Matsumoto, Y.; Yashiro, M.; Komiyama, M.; *J. Mol. Catal.* **1993**, *84*, L21.
11 Komiyama, M.; Matsumoto, Y.; Shiiba, T.; Hayashi, N.; Watanabe, K.; *Polym. J.* **1993**, *25*, 1211.
12 Adams, R.L.P.; Knowler, J.T.; Leader, D.P.; *The Biochemistry of the Nucleic Acids*, tenth ed., Chapman and Hall, London, **1986**.
13 Dugas, H., Penney, C.; *Bioorganic Chemistry*, Springer-Verlag, New York, **1981**, pp. 116–118.
14 Ohyoshi, E.; Kohata, S.; *J. Inorg. Biochem.* **1991**, *43*, 45.
15 Thompson, L.C.; *Inorganic Chem.* **1962**, *1*, 490.
16 *Stability Constants of Metal-Ion Complexes* Supplement No. 1, Special Publication No. 25, The Chemical Society, Burlington House, London, **1971**.