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# Hydrolysis of DNA by cerium(IV)/EDTA complex

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**Abstract**—Homogeneous solutions are formed from equimolar amounts of $Ce(NH_4)_2(NO_3)_6$ and EDTA. These solutions hydrolyze single-stranded DNA far more efficiently than double-stranded DNA. In contrast, Ce(IV) hydroxide gel, obtained in the absence of EDTA, hydrolyzes both single-stranded and double-stranded DNAs at almost the same rates. In order to achieve this substrate-specificity sufficiently, the mole ratio of EDTA to $Ce(NH_4)_2(NO_3)_6$ must be 1.0 or greater. By using this remarkable specificity of Ce(IV)/EDTA complex, gap-site in substrate DNA is selectively hydrolyzed. The scission-site is modulated by use of appropriate combination of oligonucleotide additives. The reactions have been kinetically analyzed.


## 1. Introduction

Non-enzymatic hydrolysis of DNA and RNA has been attracting interests, mainly because it is essential for further developments of biotechnology, molecular biology, therapy, etc.[1] The present biotechnology is based on site-selective scission of DNA by naturally-occurring restriction enzymes. However, the sequence-specificity of these enzymes is too low to cut the DNAs of higher animals and higher plants at desired site. Most of them recognize a specific sequence composed of 4 or 6 DNA-bases and thus too many scission-sites exist in large DNAs.[2] Thus, artificial enzymes, which selectively hydrolyze DNA at the target position with a desired specificity, are crucially important. They are also valuable as the tools for therapy, regulation of cell-growth, and others. However, the phosphodiester linkages in DNA are enormously stable, and could not be hydrolyzed until recently without using naturally occurring enzymes.[3] Non-enzymatic hydrolysis of RNA was also difficult.

About 10 years ago, the remarkable catalyses by lanthanide ions for the hydrolysis of DNA[4,5] and RNA[6] were discovered, and both of them were for the first time hydrolyzed at reasonable rates under physiological conditions. For DNA hydrolysis Ce(IV) ion is the most active,[7,8] whereas Tm(III), Yb(III), and Lu(III) are quite effective for RNA hydrolysis.[9] The acceleration by these metal ions is as large as $10^8-10^{12}$ fold. Interestingly and importantly, these enormous catalytic activities are completely specific to the lanthanide ions, and none of non-lanthanide ions shows a notable activity. Furthermore, artificial enzymes for site-selective scission of DNA[10] and RNA[11] were prepared by conjugating lanthanide complexes to sequence-recognizing oligonucleotides. The DNA fragments, obtained by the scission, could be transformed to desired forms by using natural enzymes so that the matching between the lanthanide catalysis and the current biotechnology is straightforward.[12]

As described above, Ce(IV) is virtually the sole catalyst for DNA hydrolysis. Its electron-withdrawing activity is overwhelmingly greater than those of other metal ions, and thus the electrophilicity of the phosphodiester linkage in DNA is enormously promoted.[13] However, this metal ion rapidly forms metal hydroxide gel at around pH 7, and this feature is unfavorable for its practical application. Recently,[14] we found that the complex of Ce(IV) with ethylenediamine-N,N,N′,N′-tetraacetate (EDTA) is homogeneous at pH 7 and active for DNA hydrolysis. The DNA scission by this complex was further promoted by oligoamines such as ethylenediamine and spermine.[15] Here we report that the activity of Ce(IV)/EDTA complex for DNA hydrolysis is strongly dependent on the structure of substrate. Single-stranded DNA is efficiently hydrolyzed but double-stranded DNA is hardly hydrolyzed. The effect of mole ratio of Ce(IV) to EDTA on the catalytic activity is investigated. The structure of the Ce(IV)/EDTA complex is also analyzed by light-scattering method. Furthermore, the results of kinetic study on the DNA hydrolysis by Ce(IV)/EDTA complex are reported.

*Keywords*: cerium(IV); EDTA; DNA; hydrolysis; structure specificity.
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## 2. Results and discussion

### 2.1. Hydrolysis of single-stranded DNA and double-stranded DNA by homogeneous solution of Ce(IV)/EDTA complex

The sequences of substrate DNA and oligonucleotide additives are presented in Figure 1. Figure 2 shows the gel electrophoresis patterns for the DNA hydrolysis by homogeneous solution of Ce(IV)/EDTA complex at pH 7.0 and 37°C. This homogeneous solution was prepared by mixing $Ce(NH_4)_2(NO_3)_6$ and EDTA (4Na salt) in 1:1 mole ratio (20 mM each). Hydrolytic character of the scission has been definitely confirmed by the previous HPLC analysis.[14] In the absence of oligonucleotide additive, the substrate $DNA^{(S)}$ (50-mer) was cut almost randomly without any specific base-preference (lane 2). In the presence of $DNA^{(F)}$ which is complementary with the whole part of $DNA^{(S)}$, however, the $DNA^{(S)}$ was hardly cleaved (lane 3). When either $DNA^{(G)}$-L1 or $DNA^{(G)}$-R1 was added so that a predetermined portion of the substrate DNA formed a duplex with the additive (lanes 4 and 5), the single-stranded portion in the substrate was hydrolyzed far more efficiently than was the double-stranded portion. Thus, there exists remarkable substrate-specificity in the DNA hydrolysis by these homogeneous solutions of Ce(IV)/EDTA complex. In the scission of single-stranded portion, no specific base-preference or sequence-preference was observed. These facts indicate the possibility of new strategy for site-selective DNA scission, which requires no covalent fixation of molecular scissors (Fig. 3).

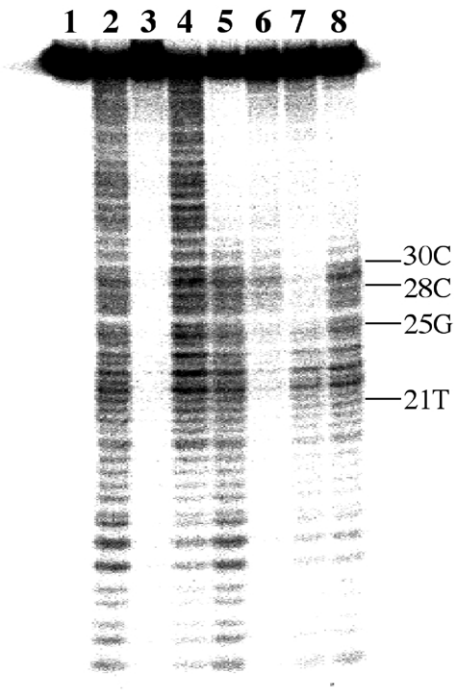


**Figure 2.** Autoradiographs for the hydrolysis of DNA having gap-structure by homogeneous solution of Ce(IV)/EDTA complex. Lane 1, control; lane 2, $DNA^{(S)}$ only; lane 3, with $DNA^{(F)}$; lane 4, with $DNA^{(G)}$-L1; lane 5, with $DNA^{(G)}$-R1; lane 6, 3-base gap ($DNA^{(G)}$-L2+$DNA^{(G)}$-R1); lane 7, 5-base gap ($DNA^{(G)}$-L1+$DNA^{(G)}$-R2); lane 8, 10-base gap ($DNA^{(G)}$-L1+$DNA^{(G)}$-R1). Reaction conditions: $[Ce(NH_4)_2(NO_3)_6]_0$=$[EDTA]_0$=500 μM, $[DNA^{(S)}]_0$=1.0 μM, [each of oligonucleotide additives]$_0$=1.5 μM, [NaCl]=100 mM, [spermine]=100 μM, and [HEPES]=2.5 mM at pH 7.0 and 37°C for 4 days.

### 2.2. Gap-selective DNA hydrolysis by homogeneous solution of Ce(IV)/EDTA complex

One of the most successful site-selective DNA scissions, based on the strategy in Figure 3, is presented in lanes 6–8 in Figure 2.[16] Here, only the target scission-site is kept single-stranded, and the other portions are protected from the scission by forming a duplex with DNA additives. When gap-structures were formed in the substrate DNA by using two oligonucleotide additives, the scission was strictly restricted to the corresponding gap-site. In lane 6, the nucleotides C28, A29 and C30 in $DNA^{(S)}$ were unpaired, and all others formed Watson–Crick base pairs with $DNA^{(G)}$-L2 and $DNA^{(G)}$-R1. The scission by homogeneous solution of the Ce(IV) complex selectively occurred at these three unpaired nucleotides. The scission at A29 was the most efficient. In the presence of both $DNA^{(G)}$-L1 and $DNA^{(G)}$-R2, five nucleobases from T21 to G25 in the substrate DNA formed a gap-structure and site-selective hydrolysis occurred at these sites (lane 7). The scissions at T22 and A23 were dominant, and minor bands were observed at T21, T24, and G25. For the 10-base gap, the scission took place also within the gap (lane 8). The total conversion of scission in this region was 12% under the conditions employed. The double-stranded region was not hydrolyzed to a measurable extent, except for the minor scission near the gap-edges. It is noteworthy that the scission-site moves concurrently with the shift of gap-site. Apparently, the site of selective scission by the Ce(IV) complex, which is not covalently bound anywhere, can be modulated by using appropriate combination of oligonucleotide additives. Site-selective scission by using gap-strategy was also successful for all other DNA substrates investigated. Both the site-selectivity and the scission-efficiency were not much dependent on the substrate-sequence.

Substrate DNA

$DNA^{(S)}$ 5'-CAATTAGAATCAGGAATGGCTTATGCGCACGTGCAGACTGTCGACCTAAG-3'

Additive DNAs

$DNA^{(F)}$ 3'-GTTAATCTTAGTCCTTACCGAATACGCGTGCACGTCTGACAGCTGGATTC-5'

$DNA^{(G)}$-L1 3'-GTTAATCTTAGTCCTTACCG-5'

$DNA^{(G)}$-L2 3'-GTTAATCTTAGTCCTTACCGAATACGC-5'

$DNA^{(G)}$-R1 3'-CACGTCTGACAGCTGGATTC-5'

$DNA^{(G)}$-R2 3'-GCGTGCACGTCTGACAGCTGGATTC-5'

**Figure 1.** The substrate DNA and oligonucleotide additives used in this study.

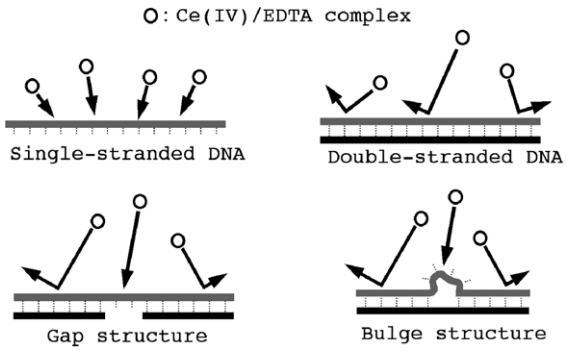


**Figure 3.** New strategy for site-selective DNA hydrolysis which requires no covalent fixation of molecular scissors.

The fragments of all these scissions showed the same mobilities as authentic samples which were obtained by use of a synthesizer, confirming that the present DNA scission is hydrolytic. The phosphates at the 3′-termini of these fragments were removed by the phosphomonoesterase activity of the homogeneous solution of Ce(IV)/EDTA complex.[17] The weak bands between the strong ones in the electrophoresis gels are probably associated with the fragments bearing the 3′-terminal phosphate.

In addition to these gap-strategies, site-selective DNA hydrolysis by the Ce(IV)/EDTA complex was successfully accomplished by forming a bulge-structure at the target site.[16] Only the single-stranded site in the bulges was selectively hydrolyzed. The scission efficiency increased with increasing bulge-length (5-, 6-, 8-, and 10-base bulges) and, in all the cases, was the largest at around the center of bulge.

### 2.3. DNA hydrolysis by Ce(IV) hydroxide gel

When $Ce(NH_4)_2(NO_3)_6$ was dissolved in HEPES buffer in the absence of EDTA and the pH was raised to 7, gel of Ce(IV) hydroxide was formed. Significantly, this hydroxide gel hydrolyzed both single-stranded DNA and double-stranded DNA at almost the same rates. This result is highly in contrast with the preferential scission of single-stranded DNA by homogeneous Ce(IV)/EDTA complex. Even when gap-structures were formed in the substrate DNA, the scission occurred randomly throughout the DNA chain. This hydroxide gel hydrolyzed both the single-stranded portion and the double-stranded portion even when there existed a gap-structure in the DNA.

### 2.4. Effect of the mole ratio of Ce(IV) to EDTA on the substrate-specificity in DNA hydrolysis

In Figure 4, the ratio of $Ce(NH_4)_2(NO_3)_6$ to EDTA was varied, and the substrate-specificity of these solutions was investigated. All of these solutions were homogeneous, and efficiently hydrolyzed single-stranded DNA, irrespective of the Ce(IV)/EDTA ratio (lanes 2–5). In contrast, double-stranded DNA was hardly hydrolyzed, when the Ce(IV)/EDTA ratio was 0.5 (lane 6) and 1.0 (lane 7). Quite interestingly, however, even double-stranded DNA was hydrolyzed, when the Ce(IV)/EDTA ratio was 2.0 (lane 8) or 4.0 (lane 9) and there existed excess amount of Ce(IV) ion in the mixtures. The hydrolysis of double-stranded DNA

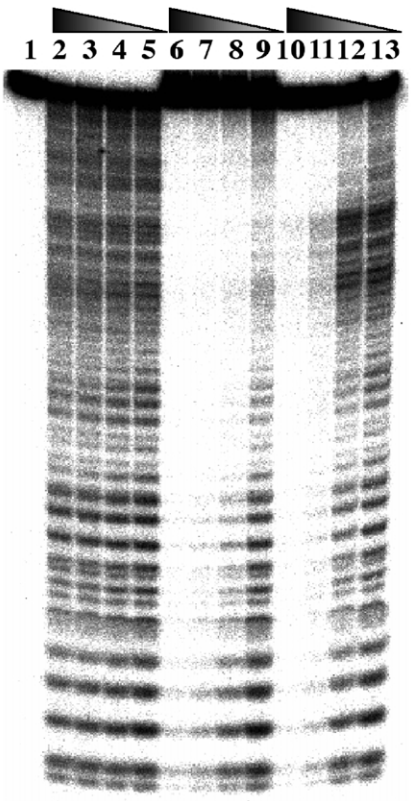


**Figure 4.** Effect of the mole ratio of Ce(IV) to EDTA on the DNA hydrolysis. Lane 1, no Ce(IV) complex; lanes 2, 6 and 10, Ce(IV):EDTA=1:2; lanes 3, 7 and 11, Ce(IV):EDTA=1:1; lanes 4, 8 and 12, Ce(IV):EDTA=1:1/2; lanes 5, 9 and 13, Ce(IV):EDTA=1:1/4. Lanes 1-5, $DNA^{(S)}$ only; lanes 6-9, with $DNA^{(F)}$; lanes 10-13, 10-base gap ($DNA^{(G)}$-L1+$DNA^{(G)}$-R1). The initial concentration of $Ce(NH_4)_2(NO_3)_6$ was kept constant at 500 μM and the concentration of EDTA was varied. $[DNA^{(S)}]_0$=1.0 μM, [each of oligonucleotide additives]$_0$=1.1 μM, [NaCl]=100 mM, [spermine]=100 μM, and [HEPES]=2.5 mM at pH 7.0 and 37°C for 43 h.

at the Ce(IV)/EDTA ratio 4.0 was faster than that at the ratio 2.0. Thus, the preference of single-stranded DNA to double-stranded one decreases with decreasing amount of EDTA. Consistently, site-selective scission at gap-site was successful when the Ce(IV)/EDTA ratio was 0.5 (lane 10) and 1.0 (lane 11), but was unsuccessful at the ratio 2.0 (lane 12) or 4.0 (lane 13).

### 2.5. Light-scattering experiments on homogenous solution of Ce(IV)/EDTA complex

As shown in Figure 5, the homogeneous solution contains some colloidal particles (averaged diameter=7 nm), in addition to the Ce(IV) complex which is small and could not be detected by the present light-scattering experiments.[18] The instrument used here can detect particles only when they are 1.4 nm or larger. These colloidal particles should be formed during the preparation of the homogeneous solution of Ce(IV)/EDTA complex. Consistently, the size distribution, evaluated by the light-scattering method, was hardly changed even when the solutions were incubated at room temperature for a week.

In order to remove the colloidal particles, the homogeneous solutions of Ce(IV)/EDTA complex, prepared by mixing

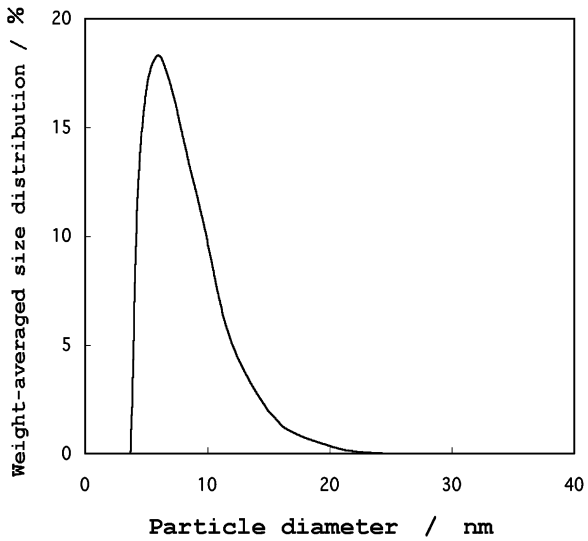


**Figure 5.** Light-scattering measurement on the homogeneous solution of Ce(IV)/EDTA complex; $[Ce(NH_4)_2(NO_3)_6]_0$=$[EDTA]_0$=1 mM.

$Ce(NH_4)_2(NO_3)_6$ and EDTA, were treated with a membrane filter (Nuclepore Polycarbonate Track-Etch Membrane: see Experimental section for details). As expected, the filtrate showed no light-scattering signal, confirming that there exist no particles greater than 1.4 nm.

### 2.6. Kinetic analysis on the DNA hydrolysis by the Ce(IV)/EDTA complex

The concentration of Ce(IV) ion in the filtrate was determined by ICP-MS analysis, and the catalytic activity of this filtrate for the hydrolysis of single-stranded DNA was investigated. For the purpose of comparison, the activities of the homogeneous solutions prior to the filtration were also examined. In Figure 6, the rate of DNA hydrolysis ($V_0$) was plotted as the function of concentration of Ce(IV) ion. The plots for both of the solutions showed saturation phenomena at large value of $[Ce(IV)]_0$. Apparently, the DNA forms a

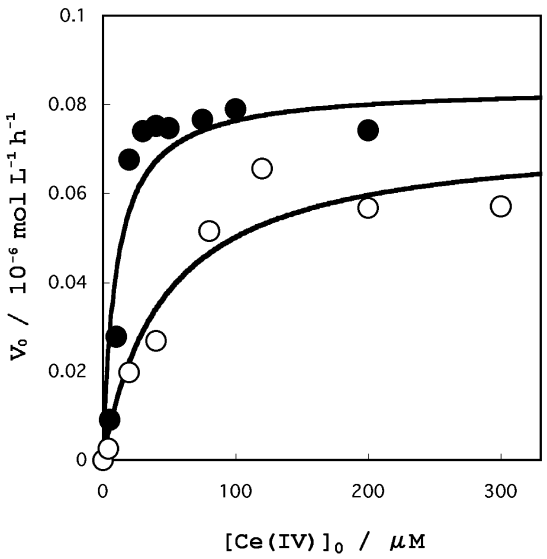


**Figure 6.** Dependence of the rate of hydrolysis of single-stranded DNA ($DNA^{(S)}$) on the concentration of Ce(IV) in the homogeneous solutions of Ce(IV)/EDTA complex before the filtration (●) and the filtrate (○).

complex with the Ce(IV)/EDTA complex prior to the chemical transformation.

Accordingly, these plots were analyzed in terms of Michaelis–Menten kinetics. The kinetic parameters obtained (the maximal rate constant $k_{cat}$ and the Michaelis–Menten constant $K_m$) are listed in Table 1. The $k_{cat}$ value of the Ce(IV) complex in the filtrate is similar to that of the colloidal particle. In contrast, the $K_m$ for the complex (47 μM) is notably greater than the value (14 μM) of the large particle. Thus, the large particles bind the phosphodiester linkage of DNA more strongly. Electrostatic attraction by many positive charges in the particles is probably responsible for this difference.

**Table 1**. The values of $k_{cat}$ and $K_m$ for the homogeneous solutions of Ce(IV)/EDTA complex before the filtration and the filtrate at pH 7.0 and 37°C

| Ce(IV)/EDTA complex | $k_{cat}$ ($h^{-1}$) | $K_m$ (μM) |
|---|---|---|
| Before the filtration | $7.8\times10^{-2}$ | 14 |
| Filtrate | $7.4\times10^{-2}$ | 47 |

## 3. Conclusion

Homogeneous solutions of $Ce(NH_4)_2(NO_3)_6$ and EDTA (Ce(IV)/EDTA ratio=1.0) preferentially hydrolyze single-stranded DNA, while double-stranded DNA is hardly hydrolyzed by these solutions. This highly contrasts with the fact that Ce(IV) hydroxide gel hydrolyzes both single-stranded and double-stranded DNAs. Accordingly, gap-site in substrate DNA is selectively hydrolyzed by homogeneous solution of Ce(IV)/EDTA complex. It has been found that the homogeneous solutions, prepared by mixing $Ce(NH_4)_2(NO_3)_6$ and EDTA, contain some colloidal particles in addition to the Ce(IV) complex. Thus, these particles are removed by filtration, and the catalytic activity of the filtrate has been analyzed. Kinetic study on the hydrolysis of single-stranded DNA indicates that the Ce(IV)/EDTA complex is as active as the large particles, although its DNA-binding activity is smaller. Double-stranded portion in DNA is not much hydrolyzed by either of these particles. The attempts to improve the site-selectivity and the scission-efficiency are currently under way in our laboratory.

## 4. Experimental

### 4.1. Materials

The substrate DNA and oligonucleotide additives were synthesized on an automated synthesizer. They were purified by denaturing 20% polyacrylamide gel electrophoresis and further by reversed-phase HPLC. Commercially obtainable $Ce(NH_4)_2(NO_3)_6$ (from Nacalai Tesque), EDTA 4Na salt (from Tokyo Kasei), HEPES [(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid: from Nacalai Tesque), Tris [tris(hydroxymethyl)aminomethane: from Aldrich] were used without further purification. Water was ion-exchanged by Millipore purification system (MilliQX; specific resistance of the water >18.3 MΩ $cm^{-1}$) and sterilized in an autoclave immediately before use. The substrate $DNA^{(S)}$ (50-mer) was $^{32}P$-labeled at its 5′-end by

[$\gamma$-$^{32}$P]-ATP (from Amersham) with T4 kinase at pH 7.6 and 37°C for 3 h. The Tris–HCl buffer (50 mM, pH 8.0) for this enzymatic reaction contained $MgCl_2$ (10 mM) and dithiothreitol (5 mM). The labeled DNA was purified by polyacrylamide gel electrophoresis. Polycarbonate Track-Etch Membrane filter (NUCLEPORE®, catalogue code: 111101) was purchased from Whattman.

### 4.2. Preparation of homogeneous solution of Ce(IV)/EDTA complex

Homogeneous Ce(IV)/EDTA complexes were prepared immediately before use by mixing $Ce(NH_4)_2(NO_3)_6$ (20 mM) and appropriate amount of EDTA (4Na salt) in 25 mM HEPES buffer. In order to remove large particles, these homogeneous solutions were filtrated by Polycarbonate Track-Etch Membrane described above.

### 4.3. DNA scission by Ce(IV)/EDTA complex

The substrate $DNA^{(S)}$ ($^{32}$P-labeled at its 5′-end; the initial concentration=1.0 μM) was treated with homogeneous solutions of Ce(IV)/EDTA complex at pH 7.0 and 37°C. Gap structures were formed by adding appropriate oligonucleotides to the solutions (the initial concentration of each was 1.1 μM). These mixtures of oligonucleotides in aqueous NaCl solution (9 μL) were heated to 90°C for 1 min and then slowly cooled down to room temperature. Finally, 1 μL of the solution of Ce(IV)/EDTA complex in pH 7.0 HEPES buffer was added to the mixture.

The DNA scission was stopped by adding 5 μL of Tris/borate buffer (90 mM) containing EDTA (10 mM), inorganic phosphate (70 mM), urea (7 M), Xylene Cyanol FF (0.02%), and Bromophenol Blue (0.02%). Then the solution (5 μL) was subjected to denaturing 20% polyacrylamide gel electrophoresis. The scission fragments were quantified with a Fuji Film FLA-3000G imaging analyzer.

### 4.4. Dynamic light-scattering (DLS) measurement

Homogeneous solutions of Ce(IV)/EDTA complex were analyzed by an Otsuka Electronics FDLS-3000 spectrometer at 25°C. The light source was a diode pumped solid state laser (100 mW) with wavelength of 532 nm. This analyzing system can detect the particles which are greater than 1.4 nm in diameter, and provide the number- and weight-averaged size distribution of the particles.

### 4.5. Kinetic analysis of the hydrolysis of single-stranded DNA by Ce(IV)/EDTA complex

Homogeneous solutions of the complex before the filtration (with Nuclepore Polycarbonate Track-Etch Membrane: vide ante) and after that were used as the catalysts. The concentration of Ce(IV) ion in the reaction mixture was determined by ICP-MS analysis (HEWLETT PACKERD HP-4500). The hydrolysis of single-stranded DNA was analyzed in terms of Eq. (1), which is based on the Michaelis–Menten kinetics.

$$V_0 = k_{cat}[DNA^{(S)}]_0[Ce(IV)]_0/(K_m + [Ce(IV)]_0) \quad (1)$$

Here, $k_{cat}$ is the rate constant for the hydrolysis of substrate DNA which is coordinated to the Ce(IV)/EDTA complex, and $K_m$ is the apparent equilibrium constant for the dissociation of the complex between Ce(IV)/EDTA complex and substrate DNA. The initial rate ($V_0$) of DNA hydrolysis was determined from the time-conversion curve under the conditions that the conversion was below 20 mol%.

## Acknowledgements

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## References

1. Reviews; (a) Komiyama, M.; Sumaoka, J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 751–757. (b) Oivanen, M.; Kuusela, S.; Lönnberg, H. *Chem. Rev.* **1998**, *98*, 961–990. (c) Trawick, B. N.; Daniher, A. T.; Bashkin, J. K. *Chem. Rev.* **1998**, *98*, 939–960. (d) Pratviel, G.; Bernadou, J.; Meunier, B. *Advances in Inorganic Chemistry*; Sykes, A. G., Ed.; Academic: San Diego, 1998; Vol. 45, pp 251–312. (e) Perreault, D. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 432–450. (f) Chin, J. *Curr. Opin. Chem. Biol.* **1997**, *1*, 514–521. (g) Kimura, E.; Koike, T. *Advances in Inorganic Chemistry*; Sykes, A. G., Ed.; Academic: San Diego, 1997; Vol. 44, pp 229–261 and references cited therein.
2. The scission site of a restriction enzyme, which recognizes a sequence of 6 DNA-bases, appears at every $4^6$ DNA-base sequence. If the DNA of human beings is treated with this restriction enzyme, the scission occurs at more than $10^5$ sites.
3. The half-life of the phosphodiester linkage in DNA at pH 7 and 30°C is estimated to be 200 million years.
4. (a) Matsumoto, Y.; Komiyama, M. *Nucleic Acids, Symp. Ser.* **1992**, *27*, 33–34. (b) Komiyama, M.; Matsumura, K.; Yonezawa, K.; Matsumoto, Y. *Chem. Express* **1993**, *8*, 85–88. (c) Shiiba, T.; Yonezawa, K.; Takeda, N.; Matsumoto, Y.; Yashiro, M.; Komiyama, M. *J. Mol. Catal.* **1993**, *84*, L21–L25.
5. Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443–1451.
6. (a) Matsumura, K.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1992**, 640–641. (b) Morrow, J. R.; Buttrey, L. A.; Shelton, V. M.; Berback, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 1903–1905.
7. (a) Komiyama, M.; Shiiba, T.; Kodama, T.; Takeda, N.; Sumaoka, J.; Yashiro, M. *Chem. Lett.* **1994**, 1025–1028. (b) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1994**, *116*, 1121–1122.
8. Komiyama, M.; Takeda, N.; Takahashi, Y.; Uchida, H.; Shiiba, T.; Kodama, T.; Yashiro, M. *J. Chem. Soc., Perkin Trans. 2* **1995**, 269–274.
9. Matsumura, K.; Komiyama, M. *J. Biochem.* **1997**, *122*, 387–394.
10. (a) Komiyama, M.; Shiiba, T.; Takahashi, Y.; Takeda, N.;

Matsumura, K.; Kodama, T. *Supramol. Chem.* **1994**, *4*, 31–34. (b) Komiyama, M. *J. Biochem.* **1995**, *118*, 665–670.
11. (a) Matsumura, K.; Endo, M.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1994**, 2019–2020. (b) Hall, J.; Hüsken, D.; Häner, R. *Nucleosides Nucleotides* **1997**, *16*, 1357–1368. (c) Magda, D.; Wright, M.; Crofts, S.; Lin, A.; Sessler, J. L. *J. Am. Chem. Soc.* **1997**, *119*, 6947–6948. (d) Huang, L.; Chappell, L. L.; Iranzo, O.; Baker, B. F.; Morrow, J. R. *J. Biol. Inorg. Chem.* **2000**, *5*, 85–92.
12. Sumaoka, J.; Azuma, Y.; Komiyama, M. *Chem. Eur. J.* **1998**, *4*, 205–209.
13. Shigekawa, H.; Ikawa, H.; Yoshizaki, R.; Iijima, Y.; Sumaoka, J.; Komiyama, M. *Appl. Phys. Lett.* **1996**, *68*, 1433–1435.
14. Igawa, T.; Sumaoka, J.; Komiyama, M. *Chem. Lett.* **2000**, 356–357.
15. Sumaoka, J.; Igawa, T.; Yagi, T.; Komiyama, M. *Chem. Lett.* **2001**, 20–21.
16. Kitamura, Y.; Komiyama, M. *Nucleic Acids Res.* **2002**, *30*, e102.
17. Monophosphates are also hydrolyzed by Ce(IV) ion: Miyama, S.; Asanuma, H.; Komiyama, M. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1685–1688.
18. The kinetic study on the DNA hydrolysis by $Ce(NH_4)_2(NO_3)_6$ in homogeneous acidic solutions showed that dinuclear Ce(IV) cluster is the active species (Ref. 5). This result indicates that the Ce(IV)/EDTA complex involving two Ce(IV) is the active species, although more detailed analysis is certainly required to obtain a concrete conclusion.