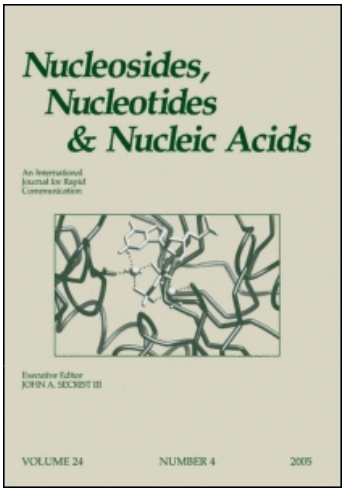

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Kinetic Studies on CE(IV)-Induced Hydrolysis of Single-Stranded and Double-Stranded Oligonucleotides

Tomoyuki Igawa[a]; Jun Sumaoka[a]; Makoto Komiyama[a]
[a] Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Tokyo, JAPAN

To cite this Article Igawa, Tomoyuki , Sumaoka, Jun and Komiyama, Makoto(2000) 'Kinetic Studies on CE(IV)-Induced Hydrolysis of Single-Stranded and Double-Stranded Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 19: 5, 891 — 902
To link to this Article: DOI: 10.1080/15257770008033030
URL: http://dx.doi.org/10.1080/15257770008033030

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(5&6), 891–902 (2000)

KINETIC STUDIES ON CE(IV)-INDUCED HYDROLYSIS OF SINGLE-STRANDED AND DOUBLE-STRANDED OLIGONUCLEOTIDES

Tomoyuki Igawa, Jun Sumaoka, and Makoto Komiyama*
Department of Chemistry and Biotechnology, Graduate School of Engineering,
The University of Tokyo, Hongo, Tokyo 113-8656, JAPAN

ABSTRACT: The Ce(IV)-induced hydrolyses of DNA are kinetically investigated. The formation constants of the Ce(IV)-DNA complexes are in the following order: the single-stranded DNA > the double-stranded DNA >> the dinucleotide. On the other hand, the catalytic rate constants for the single-stranded DNA and the double-stranded DNA are comparable with each other, but both of them are much smaller than the value for the dinucleotide hydrolysis.

Scission of DNA is one of the most fundamental techniques in biotechnology and molecular biology, and thus the preparation of catalysts for DNA hydrolysis has been widely attempted by many chemists.[1,2] However, non-enzymatic hydrolysis of DNA is quite difficult, because of the enormous stability of the phosphodiester linkages therein. Several years ago, remarkable catalysis of the lanthanide ions was discovered, and DNA was for the first time hydrolysed at reasonable rates under physiological conditions (the Ce(IV) ion is especially active).[3-11] Furthermore, artificial restriction-enzymes, which hydrolyse DNA at the target site, were prepared by attaching the Ce(IV) complex to DNA oligomers.[12,13]

The Ce(IV)-induced hydrolysis of dinucleotides was already investigated in detail, and the reaction mechanism was proposed.[2] Oligonucleotides were also hydrolysed by the Ce(IV) ion, but mechanistic and kinetic information on these reactions has been scarce. In some of the physicochemical properties, which could make notable effects on the catalysis, oligonucleotides significantly differ from dinucleotides. For example,

* Corresponding author: Tel. +81-3-5841-7314; Fax. +81-3-5841-7314; E-mail: mkomi@chembi.t.u-tokyo.ac.jp

oligonucleotides have a number of phosphodiester linkages as potential coordination-sites to the Ce(IV), whereas the coordination-site of dinucleotides is only the internal phosphodiester linkage. Furthermore, the conformations of oligonucleotides (especially of their duplexes) are much more rigid than those of dinucleotides. These factors should greatly affect the structures and stabilities of the Ce(IV)-substrate complexes (and also of the transition states for the hydrolysis). Thus, oligonucleotides and dinucleotides might be hydrolysed by different mechanisms. The information on these points is crucially important to design still more useful tools for practical applications.

In the present paper, double-stranded DNA and single-stranded DNA are hydrolysed by the Ce(IV) ion. The rates of these reactions are quantitatively compared with each other, and also with the values for the hydrolysis of dinucleotides. All of the reactions are kinetically analysed, and the Michalis-Menten parameters are determined. The difference in the reactivities is discussed in terms of the molecular structures of the substrates.

EXPERIMENTAL SECTION

Materials. All the oligonucleotides were prepared on an automated synthesizer, and purified in the usual manner. The substrate oligonucleotide was ^{32}P-labeled at the 5'-end by using adenosine γ-^{32}P-triphosphate and T4 polynucleotide kinase. Thymidylyl(3'-5')thymidine (TpT) and $Ce(NH_4)_2(NO_3)_6$ were commercially obtained. Water was purified by a Milli-Q Lab purification system (the specific resistance > 18.3 $M\Omega\,cm^{-1}$), and furthermore sterilized in an autoclave immediately before use.

DNA Hydrolysis. The DNAs (either single-stranded or double-stranded) were hydrolysed at pH 7.0 (20 *mM* Hepes buffer) and 37 °C in the presence of 100 *mM* NaCl. Throughout the present study, the initial concentration of the substrate DNA (with respect to the total amount of phosphodiester linkages) was kept constant at 1 *mM*. For the hydrolysis of the single-stranded DNA, the required amount of unlabeled DNA, which has the same sequence as the ^{32}P-labeled target DNA, was added to the reaction mixtures. In the scission of the double-stranded DNA, the initial concentrations of both the target DNA (^{32}P-labeled one + unlabeled one) and its complementary

counterpart were 0.5 *mM*. After a predetermined time, the reaction mixture was subjected to 20% denaturing polyacrylamide gel electrophoresis. The electrophoresis patterns were quantified by using a Fujix BAS-1000 II system. The pH change during the reactions was smaller than 0.2 units.

The hydrolysis of TpT was achieved under the same conditions as the oligonucleotide hydrolysis; pH 7.0 (20 *mM* Hepes buffer) and 37°C in the presence of NaCl (100 *mM*). The initial concentration of TpT was also 1 *mM*. With appropriate intervals, a small portion of the reaction mixture was subjected to the reversed-phase HPLC (a Merck LiChrospher RP-18(e) ODS column; phosphate buffer/acetonitrile = 92/8 (v/v)).

Kinetic Analysis. The Ce(IV)-induced DNA hydrolysis was analysed in terms of Equation 1, which was based on the Michaelis-Menten kinetics. Here, k_{cat} is the rate constant for the hydrolysis of the phosphodiester linkage which is coordinated to the Ce(IV) ion, and K_m is the apparent equilibrium constant for the dissociation of the complex between the Ce(IV) and the DNA. The initial rate (V_0) for the DNA hydrolysis, determined at the conversion below 20 mole%, was used for the present analysis. The values of k_{cat} and K_m were evaluated by the best-fitting method. When some of the reactions were followed up to higher conversions, fair pseudo-first-order kinetics was observed.

$$V_0 = k_{cat}\,[DNA]_0 \cdot [Ce(IV)]_0 / (K_m + [Ce(IV)]_0) \qquad (1).$$

The scission of all the DNAs took place almost randomly throughout the DNA chain (see FIG. 2). Thus, the rate of hydrolysis (with respect to the phosphodiester linkage) was determined under the assumption that all the linkages are hydrolysed at the same rate.

Competitive Inhibition Experiments. In addition to the Michaelis-Menten analyses describe above, the relative stabilities of the complexes between the Ce(IV) ion and the substrate DNA were evaluated as follows. To the reaction mixture for the hydrolysis of ^{32}P-labeled DNA (ca. 5 μM with respect to the phosphodiester linkage),

Substrate DNAs
Single-stranded 40-mer
5'-^{32}P-GCAGTCGAGCCTCCGCACCCGGCAGCGCAGCCACGTGACG-3'

Double-stranded 40-mer
5'-^{32}P-GCAGTCGAGCCTCCGCACCCGGCAGCGCAGCCACGTGACG-3'
3'-CGTCAGCTCGGAGGCGTGGGCCGTCGCGTCGGTGCACTGC-5'

Competitive Inhibitors
Single-stranded 25-mer DNA
5'-ATGCTAGGACCGCTGGTGATGCCGC-3'

Double-stranded 25-mer DNA
5'-ATGCTAGGACCGCTGGTGATGCCGC-3'
3'-TACGATCCTGGCGACCACTACGGCG-5'

FIG. 1. The sequences of substrate DNAs and competitive inhibitors.

various types of unlabeled DNAs (1 *mM*) were added as competitive inhibitors. The inhibition should be more explicit, when the Ce(IV)-binding activity of the inhibitor is greater. The rate of hydrolysis of the ^{32}P-labeled DNA as the substrate was determined by the gel electrophoresis. The relative stability of the Ce(IV)-inhibitor complex was estimated from the magnitude of the inhibition.

RESULTS AND DISCUSSION

Hydrolysis of Single-Stranded and Double-Stranded Oligonucleotides by the Ce(IV). The sequences of the substrate DNAs (either single-stranded or double-stranded), as well as those of the DNAs used as the competitive inhibitors, are presented in FIG. 1. The substrate DNAs do not take any specific intra-strand structures, and are not complementary with any part (of tetranucleotides or longer sequences) in the competitive inhibitors. As shown by the polyacrylamide gel electrophoresis patterns in FIG. 2, both the single-stranded DNA and the double-stranded DNA are hydrolysed by $Ce(NH_4)_2(NO_3)_6$ almost randomly throughout the DNA chain (see the lanes 3-6). There is no specific base-preference or sequence-preference in the scission. This is totally consistent with the previous results that all the dinucleotides investigated are hydrolysed by the Ce(IV) at virtually the same rates.[4c] The nucleic acid bases do not take any important roles in the Ce(IV)-induced DNA hydrolysis.

FIG. 2. Autoradiographs for the hydrolysis of single-stranded and double-stranded DNAs by $Ce(NH_4)_2(NO_3)_6$ at pH 7.0 and 37 °C. Lane 1, no treatment; lane 2, control (15 h); lane 3, the hydrolysis of the single-stranded 40-mer DNA by $[Ce(IV)]_0$ = 1 *mM* (15 h); lane 4, the double-stranded 40-mer DNA by $[Ce(IV)]_0$ = 1 *mM* (15 h); lane 5, the single-stranded DNA by $[Ce(IV)]_0$ = 10 *mM* (3 h); lane 6, the double-stranded DNA by $[Ce(IV)]_0$ = 10 *mM* (3 h). [DNA (with respect to the phosphodiester residue)]$_0$ = 1 and $[NaCl]_0$ = 100 *mM*.

When the concentration of the Ce(IV) is 1 *mM*, the phosphodiester linkages in the single-stranded DNA are hydrolysed much more promptly than are those in the double-stranded one (compare the lanes 3 and 4). At a greater $[Ce(IV)]_0$ value (e.g., 10 *mM*), however, the difference in the rates is much smaller (the lanes 5 and 6). Apparently, the rate of hydrolysis of the double-stranded DNA depends on the $[Ce(IV)]_0$ much more drastically than does the rate of hydrolysis of the single-stranded DNA. It is indicated

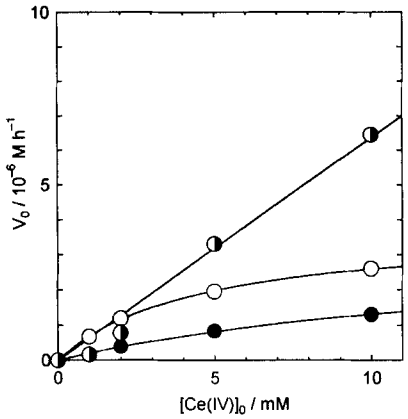

FIG. 3. The $[Ce(IV)]_0$ dependencies of the rates of hydrolysis of the single-stranded 40-mer (○), the double-stranded 40-mer (●), and TpT (◑) at pH 7.0 and 37 °C.

that the stabilities of the corresponding Ce(IV)-DNA complexes are significantly different from each other. The reaction rates are kept virtually constant when the pH is varied between 6 and 8.

Dependencies of the Hydrolysis Rates on $[Ce(IV)]_0$. In FIG. 3, the initial rates of the hydrolysis of the phosphodiester linkages in the three types of DNAs (double-stranded, single-stranded, and dinucleotide) are plotted as the functions of the initial concentration of Ce(IV). For both of the single-stranded DNA and the double-stranded DNA (the open and the closed circles, respectively), the rates of hydrolysis gradually saturate at high concentrations of the Ce(IV). These reactions involve the formation of rather stable Ce(IV)-DNA complexes prior to the chemical transformation. In contrast, the rate of TpT hydrolysis monotonously increases with increasing $[Ce(IV)]_0$, up to 10 *mM*. The curvature is very small. Thus, the phosphodiester linkage in the dinucleotide is hydrolysed far more promptly than those in the oligonucleotides, when the $[Ce(IV)]_0$ is large. At low concentrations of Ce(IV), however, both of them are hydrolysed at rather comparable rates.

TABLE 1. The values of k_{cat} and K_m for the Ce(IV)-induced hydrolysis of DNA at pH 7.0 and 37 °C [a]

Substrate DNA	k_{cat} (10^3 h^{-1})	K_m (*mM*)
Single-stranded 40-mer	3.7	4.4
Double-stranded 40-mer	3.3	15
TpT	>35	>41

a. The sequences of the DNAs are presented in FIG. 1.

Michaelis-Menten Analysis. The k_{cat} value for the single-stranded DNA, determined by using Equation 1, is almost the same as the value for the double-stranded one (see TABLE 1). On the other hand, the K_m value for the former is 3.5 times as small as that for the latter. The single-stranded DNA binds the Ce(IV) more strongly than does the double-stranded one. In the hydrolysis of TpT, both the K_m and the k_{cat} values are quite large (they could not be precisely determined by the present analysis). Similar results are obtained for the hydrolysis of various dinucleotides (the hydrolysis rates increases almost linearly with $[Ce(IV)]_0$).[4c] The catalyst-substrate complex in the dinucleotide hydrolysis is unstable, but is highly active.

From these results, the followings are concluded. The oligonucleotides, either single-stranded or double-stranded, exceed the dinucleotides in the Ce(IV)-binding activity. However, the chemical transformation for the phosphodiester hydrolysis is notably suppressed in the oligonucleotides, compared with that in the dinucleotides.

Competitive Inhibition Experiments. In order to confirm the validity of these kinetic analyses, the following competitive inhibition experiments have been also achieved (TABLE 2). Here, the concentration of the ^{32}P-labeled single-stranded 40-mer DNA (the substrate) is kept constant, and various types of DNAs are added as competitive inhibitors. When unlabeled single-stranded 40-mer DNA (having the same sequence as the substrate DNA) is added to the reaction mixture, for example, the Ce(IV) ions in the solution are competitively bound by both the ^{32}P-labeled DNA and

TABLE 2. Competitive inhibition of the Ce(IV)-induced hydrolysis of the single-stranded 40-mer DNA by various types of DNAs at pH 7.0 and 37 °C [a]

Competitive inhibitor	Conversion at 16 h (mole%)	Inhibition Efficiency (%) [b]
None	42	---
40-mer DNA (of the same sequence as the substrate)	23	45
Single-stranded 25-mer DNA	19	55
Double-stranded 25-mer DNA	38	10
TpT	37	12

a. [^{32}P-labeled 40-mer DNA (with respect to the phosphate residue)]$_0$ = 5 μM; [competitive inhibitor]$_0$ = 1, [Ce(IV)]$_0$ = 1, and [NaCl]$_0$ = 100 mM. Any part of the competitive inhibitor is not complementary with the substrate DNA.
b. The inhibition efficiency is defined as the magnitude of suppression of hydrolysis by the inhibitor.

the unlabeled DNA. Because of this competition, the rate of hydrolysis of the labeled-DNA is decreased to almost a half. The single-stranded 25-mer DNA also notably (55%) decelerates the hydrolysis of the substrate single-stranded 40-mer DNA (^{32}P-labeled).[14] This 25-mer DNA binds the Ce(IV) still more strongly. However, the double-stranded DNA (25-mer) causes only a small effect on the rate of hydrolysis of the substrate DNA. Apparently, the single-stranded DNAs bind the Ce(IV) more strongly than do the double-stranded ones (note that the inhibitors are not complementary with the substrate DNA, and thus cannot suppress the reaction due to the duplex formation with the substrate).

On the other hand, TpT only slightly decreases the rate of hydrolysis. This dinucleotide binds the Ce(IV) so weakly that it removes the Ce(IV) ion from the substrate DNA only in a small extent. From these analyses, the Ce(IV)-binding

activities are in the following order: the single-stranded DNA > the double-stranded DNA >> the dinucleotide. Virtually the same results are obtained, when the competitive-inhibition experiments are achieved by using a ^{32}P-labeled single-stranded 25-mer DNA, in place of the ^{32}P-labeled single-stranded 40-mer (data not presented).[15] The validity of the results of kinetic analysis in TABLE 1 has been concretely substantiated.

Origins of the Difference in the Hydrolysis Rate. The stronger Ce(IV)-binding by oligonucleotides (both single-stranded and double-stranded) than those of dinucleotides is ascribed to the following two reasons. First, a number of negatively-charged phosphates are accumulated there, and efficiently attract the positively-charged Ce(IV) by electrostatic interactions. Second, several phosphodiester linkages bound to the backbone of the same DNA can be simultaneously coordinated to the Ce(IV).[16] Dinucleotides cannot fulfill either of these requirements. The single-stranded DNA binds the Ce(IV) more strongly than does the double-stranded DNA (TABLE 1), probably because its structure is more flexible than that of the double-stranded one. As the result, the phosphodiester linkages in the single-stranded DNA can be feasibly placed at the appropriate positions for the simultaneous coordination to one Ce(IV) ion.

It is noteworthy that the k_{cat} values for the oligonucleotides are significantly smaller than those for the dinucleotides (TABLE 1). The difference exceeds 10 fold. Oligonucleotides (especially double-stranded ones) are less flexible than dinucleotides. Thus, the formation of the transition state, which accompanies some conformational change of the substrate, is energetically unfavorable. Furthermore, the transition state is destabilized by the interactions with the negative charges of the phosphates in the oligonucleotides (the transition state is negatively charged in a greater magnitude than is the initial state, and thus interacts more strongly with the adjacent charges).

In conclusion, the phosphodiester linkages in oligonucleotides are hydrolysed by the Ce(IV) at the comparable rates as those in dinucleotides, since both of k_{cat} and K_m are smaller than the values for the dinucleotides. These two factors are compensated with each other. When $[Ce(IV)]_0$ is small, single-stranded oligonucleotides, which have smaller K_m, are hydrolysed preferentially to double-stranded DNA. At greater $[Ce(IV)]_0$, however, the difference becomes less significant. The information obtained

here should be useful for the design of active catalysts for DNA hydrolysis and of artificial restriction-enzymes.

ACKNOWLEDGMENTS

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

REFERENCES AND NOTES

1. Reviews: (a) Komiyama M.; Sumaoka, J. *Curr. Opin. Chem. Biol.*, **1998**, ***2***, 751-757; (b) Pratviel, G.; Bernadou J.; Meunier, B. in *Advances in Inorganic Chemistry*, ed. A.G. Sykes, Academic Press, New York, 1997, vol.45, p.251-312.
2. The mechanism of lanthanide ion-induced hydrolysis of DNA and RNA was recently discussed; Komiyama, M.; Takeda, N.; Shigekawa, H. *J. Chem. Soc., Chem. Commun*, **1999**, 1443-1451.
3. (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.
4. (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. (c) Komiyama, M.; Takeda, N.; Takahashi, Y.; Uchida, H.; Shiiba, T.; Kodama, T.; Yashiro, M. *J. Chem. Soc. Perkin Trans. 2*, **1995**, 269-274. (d) Shigekawa, H.; Ikawa, H.; Yoshizaki, R.; Iijima, Y.; Sumaoka, J.; Komiyama, M. *Apply. Phys. Lett.* **1996**, ***68***, 1433-1435. (e) Sumaoka, J.; Azuma, Y.; Komiyama,M. *Chem. Eur. J.*, **1998**, ***4***, 205-209.
5. Hydrolysis of dinucleotides by non-lanthanide ions was reported: (a) Ihara, T.; Shimura, H.; Ohmori, K.; Tsuji, H.; Takeuchi, J.; Takagi, M. *Chem. Lett*, **1996**, 687-688. (b) Ott, R.; Krämer, R. *Angew. Chem. Int. Ed. Engl*, **1998**, ***37***, 1957-1960.
6. Supercoiled DNA, which is activated by internal strain, was cleaved via the hydrolytic pathway by metal complexes: (a) Basile, L. A.; Raphael, A. L.; Barton, J. K. *J. Am. Chem. Soc.*, **1987**, ***109***, 7550-7551. (b) Schnaith, L. M. T.; Hanson, R.

S.; Que, L., Jr. *Proc. Natl. Acad. Sci. USA.*, **1994**, ***91***, 569-573. (c) Rammo, J.; Hettich, R.; Roigk, A.; Schneider, H.-J. *J. Chem. Soc. Chem. Commun.*, **1996**, 105-107. (d) Hashimoto, S.; Y. Nakamura, *J. Chem. Soc. Perkin Trans. 1*, **1996**, 2623-2628. (e) Ragunathan, K. G.; Schneider, H.-J. *Angew. Chem. Int. Ed. Engl.*, **1996**, ***35***, 1219-1221. (f) Itoh, T.; Hisada, H.; Sumiya, T.; Hosono, M.; Usui, Y.; Fujii, Y. *J. Chem. Soc., Chem. Commun.*, **1997**, 677-678. (g) Dixon, N. E.; Geue, R. J.; Lambert, J. N.; Moghaddas, S.; Pearce D. A.; Sargeson,A. M. *J. Chem. Soc., Chem. Commun.*, **1996**, 1287-1288. (h) Roigk, A.; Hettich R.; Schneider, H.-J. *Inorg. Chem.* **1998**, ***37***, 751-756. (i) Kimizuka, N.; Watanabe, E.; Kunitake, T. *Chem. Lett.*, **1999**, 29-30.

7. RNA hydrolysis by lanthanide ions: (a) George, A.; Draganac P.; Farkas, W. R. *Inorg. Chem.*, **1985**, ***24***, 3627-3631. (b) Ciesiolka, J.; Marciniec T.; Krzyzosiak, W.J. *Eur. J. Biochem.*, **1989**, ***182***, 445-450. (c) Breslow R.; Huang, D.-L. *Proc. Natl. Acad. Sci. USA*, **1991**, ***88***, 4080-4083. (d) Komiyama, M.; Matsumura, K.; Matsumoto, Y. *J. Chem. Soc. Chem. Commun.*, **1992**, 640-641. (e) Morrow, J. R.; Buttrey, L. A.; Shelton V. M.; Berback, K. A. *J. Am. Chem. Soc.*, **1992**, ***114***, 1903-1905. (f) Chin, K. O. A.; Morrow, J. R. *Inorg. Chem.*, **1994**, ***33***, 5036-5041. (g) Morrow J. R.; Shelton, V. M. *New, J. Chem.*, **1994**, ***18***, 371-375. (h) Matsumura, K.; Komiyama, M. *J. Biochem.*, **1997**, ***122***, 387-394. (i) Kamitani, J.; Sumaoka, J.; Asanuma, H.; Komiyama, M. *J. Chem. Soc., Perkin Trans. 2*, **1998**, 523-527.

8. Hydrolysis of other phosphodiesters by lanthanide ions: (a) Sumaoka, J.; Yashiro M.; Komiyama, M. *J. Chem. Soc., Chem. Commun.*, **1992**, 1707-1708. (b) Sumaoka, J.; Miyama S.; Komiyama, M. *J. Chem. Soc., Chem. Commun.*, **1994**, 1755-1756. (c) Matsumura, K.; Komiyama, M. *J. Inorg. Biochem.*, **1994**, ***55***, 153-156. (d) Morrow, J. R.; Buttrey, L. A.; Berback, K. A. *Inorg. Chem.* **1992**, ***31***, 16-20. (e) Kuusela, S.; Lönnberg, H. *J. Phys. Org. Chem.*, **1992**, ***5***, 803-811. (f) Schneider, H.-J.; Rammo, J.; Hettich, R. *Angew. Chem. Int. Ed. Engl.*, **1993**, ***32***, 1716-1719. (g) Tsubouchi, A.; Bruice, T. C. *J. Am. Chem. Soc.*, **1994**, ***116***, 11614-11615. (h) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.*, **1995**, ***117***, 8582-8585. (i) Moss, R. A.; Park, B. D.; Scrimin, P.; Ghirlanda, G. *J. Chem. Soc., Chem. Commum.*, **1995**, 1627-1628. (j) Breslow R.; Zhang, B. *J. Am. Chem. Soc.*, **1994**,

116, 7893-7894. (k) Zhu, B.; Wang, Z.-Q.; Li, X.-M.; Zhao, D.-Q.; Ni, J.-Z. *J. Mol. Cat. A*, **1997**, ***118***, L5-L7. (l) Moss, R. A.; Ragunathan,K. G. *Langmuir*; **1999**, *15*, 107-115. (m) ref. 1.

9. Hydrolysis of phosphomonoesters: (a) Millich, F.; Jayes, E.L., Jr. *J. Am. Chem. Soc.*, **1964**, ***86***, 2914-2918. (b) Oh, S. J.; Song K. H.; Park, J. W. *J. Chem. Soc., Chem. Commun.*, **1995**, 575-576.
10. Hydrolysis of phosphotriesters by lanthanide ions: (a) Hay R. W.; Govan, N. *J. Chem. Soc. Chem. Commun.*, **1990**, 714-715. (b) Oh, S. J.; Yoon C. W.; Park, J. W. *J. Chem. Soc., Perkin Trans 2*, **1996**, 329-331.
11. Hydrolysis of ATP: Yohannes, P. G.; Bowman-James, K. *Inorg. Chim. Acta*, **1993**, ***209***, 115-117.
12. (a) Komiyama, M.; Shiiba, T.; Takahashi, Y.; Takeda,N.; Matsumura K.; Kodama, T. *Supramol. Chem.*, **1994**, ***4***, 31-34; (b) M. Komiyama, N. Takeda, T. Shiiba, Y. Takahashi, Y. Matsumoto and M. Yashiro, *Nucleosides Nucleotides*, **1994**, ***13***, 1297-1309. (c) Komiyama, M. *J. Biochem.*, **1995**, ***118***, 665-670.
13. Artificial enzymes for the sequence-selective scission of RNA were also prepared by conjugating the lanthanide ions with DNA oligomers: (a) Matsumura, K.; Endo, M.; Komiyama, M. *J. Chem. Soc. Chem. Commun.*, **1994**, 2019-2020. (b) Magda, D.; Miller, R. A.; Sessler, J. L.; Iverson, B. L. *J. Am. Chem. Soc.*, **1994**, ***116***, 7439-7440. (c) Hall, J.; Hüsken D.; Häner, R. *Nucleic Acid Res.*, **1996**, ***24***, 3522-5326.
14. The inhibition efficiencies are somewhat affected by the base sequences of the inhibitor DNAs. Detailed study on the relationship between the base sequence and the inhibition efficiency is in progress.
15. The single-stranded competitive inhibitor in FIG. 1 is used as the substrate here.
16. Simultaneous coordination of two or more phosphates of DNA or RNA to metal ions was proposed previously: (a) Miyama, S.; Asanuma, H.; Komiyama, M. *J. Chem. Soc., Perkin Trans. 2*, **1997**, 1685-1688. (b) ref. 4e; (c) Kuusela S.; Guzaev, A.; Lönnberg, H. *J. Chem. Soc., Perkin Trans. 2*, **1996**, 1895-1899. (d) Kuusela, S.; Azhayev, A.; Guzaev, A.; Lönnberg, H. *J. Chem. Soc., Perkin Trans. 2*, **1995**, 1197-1202.

Received 11/18/99
Accepted 2/8/00