SHORT COMMUNICATIONS

Hydrolysis of OligoDNAs by Lanthanide Metal(III) Chloride[†]

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Selective scission of nucleic acids has been one of the most challenging topics, and many elegant artificial nucleases were reported.¹ However, most of them take advantage of oxidative cleavage of deoxyribose at the target site, and hydrolytic scission, exhibited by natural nucleases, has never been mimicked. The greatest obstacle is the lack of appropriate catalytic residue for the purpose: non-enzymatic hydrolysis of linear DNAs has not been achieved yet.² Linear DNAs are so stable that the catalyst must show remarkable acceleration (half-life of the phosphodiester linkage at pH 7, 25°C is estimated to be 200 million years).^{3,4} The hydrolytic scission is preferable, at least for some purposes, to oxidative cleavage, since no diffusible species is involved and the resultant DNA fragments can be enzymatically religated when necessary.

Recently the authors found that lanthanide metal ions efficiently cleave plasmid supercoiled DNA.⁵ Here we show that the catalysis by these metal ions is successfully applicable to the hydrolyses of both single-stranded and

double-stranded linear DNAs.

EXPERIMENTAL

Oligo(thymidylic acid) and oligo(deoxyadenylic acid) (oligo(T) and oligo(dA): degrees of polymerization 12—18) were obtained from Pharmacia. Hydrolyses of the oligomers at 50°C, pH 7.2 (Hepes buffer) under nitrogen were followed by ion-exchange HPLC (a Tosoh DEAE-NPR column; a linear gradient from 0 to 0.1 M NaClO₄ at 30 min in 0.02 M pH 7.5 Tris buffer). A 35-mer DNA GGGAATTG-GCCAGTCGACTAGGGGCCGTTAACG-GC, which has a self-complementary 12-base sequence (underlined part), was prepared by an automated synthesizer.⁶ Hydrolysis of the 35-mer, ³²P-labeled at the 5'-end, was achieved at 30°C, pH 7.2, and was analyzed by denaturing polyacrylamide gel. Formation of a double-strand from the 12 base sequence under the reaction conditions was confirmed by the digestion with single-strand specific nuclease S1 in the absence of CeCl₃. The

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greatest caution was paid throughout the experiments to avoid contamination of natural nucleases, which was definitely evidenced by repeated control experiments.

RESULTS AND DISCUSSION

Efficient hydrolysis of the oligo(T) by CeCl_3 has been clearly evidenced by gradual appearance of HPLC peaks of the shorter retention times, which corresponds to formation of the smaller oligomers (Figure 1). No hydrolysis took place in the absence of CeCl_3 . Similarly the oligo(dA) was hydrolyzed to smaller oligomers by CeCl_3 . The oligo(T) and the oligo(dA) were also hydrolyzed by other lanthanide metal(III) chlorides such as LaCl_3 , TmCl_3 , and EuCl_3 , although considerably less efficiently than by CeCl_3 . However, the activities of MgCl_2 , ZnCl_2 , AlCl_3 , and FeCl_3 were virtually none.

The hydrolytic scission of the phosphodiester linkages has been confirmed by the independent reversed-phase HPLC on the monomer and the dimer in the products. TpT and T (thymidine)

are mainly formed from the oligo(T), together with considerably smaller amounts of pTpT, Tp, and pT.⁷ Thymine formation, which should take place if the reaction were involving oxidative cleavage of the ribose residue,⁸ is not perceived at all. Totally hydrolytic nature of the scission is conclusive.

The electrophoresis pattern for the CeCl_3 -catalyzed hydrolysis of duplex of the 35-mer DNA, which has a 12-base self-complementary sequence, at 30°C is depicted in Figure 2. Densitometric analysis indicated that both the double-stranded portion and the single-stranded portion are hydrolyzed at comparable rates, and no specific base-preference is perceived for either of the portions (lanes 1 and 2). The hydrolysis rate is rather independent of the DNA structure. Double-strand formation of the 12 base sequence under the reaction conditions has been substantiated by the digestion with nuclease S1 (lane 7: no scission took place from G_{23} to C_{35}).

The patterns in the lanes 1 and 2 are composed of two types of bands: one of them migrates at the same rate as the DNase I digest

Figure 1. Ion-exchange HPLC patterns for the hydrolysis of the oligo(T) by CeCl_3 (0.01 M) at pH 7.2, 50°C; a, $t = 0$ h; b, $t = 2$ h; c, $t = 4$ h; the initial residual concentration of the oligo(T) is 2.6×10^{-4} M; the monomer and the dimer as well as their phosphates are eluted out as the peak at 0–1 min.

Figure 2. Autoradiograph for the hydrolysis of the duplex of the partially self-complementary 35-mer DNA (shown in (b)); ^{32}P -labeled at the 5'-end; 3.3×10^{-6} M) by CeCl_3 (0.01 M) at pH 7.2, 30°C : lane 1, the reaction for 4 h; lane 2, the reaction for 8 h; lane 3, digested by DNase I; lane 4, Maxam-Gilbert A + G sequencing reaction; lane 5, C + T sequencing reaction; lane 6, control; lane 7, digested by nuclease S1. The DNA sequence in (a) is represented by the residue at the 3'-end. The white triangles indicate a typical example of two types of termini (upper, OH-terminus; lower, phosphate-terminus), both of which are formed by the scission at the 3'-side of T_γ .

does (lane 3), whereas the other has the same mobility as the Maxam-Gilbert sequencing fragments (lanes 4 and 5). Apparently both the OH-terminus and the phosphate-terminus are formed, with a slight preference for the former. These results indicate that the present hydrolysis proceeds by a stepwise scission of the phosphodiester linkage. In the first step, the linkage is cleaved preferentially *via* P-O(5') scission, yielding the phosphate terminus (note

that the DNA oligomer is ^{32}P -labeled at the 5'-end). The terminal phosphomonoester is then hydrolyzed to an OH-terminus. The amounts of the two termini are determined by the ratio of the rate constants for the two steps. The argument is supported by the fact that the authentic sample of Tp is promptly hydrolyzed to T by CeCl_3 at 30°C .⁹

Consistently, the number of the peaks in HPLC for the hydrolysis of the oligo(T) (Figure 1(b) and (c)) is larger than expected simply from the different degrees of polymerization. A number of smaller oligomers having different termini are formed by the stepwise hydrolysis. More favorable formation of the phosphate-terminus in the oligomeric fragments than in the monomeric and dimeric fragments is probably associated with the suppression of the hydrolysis of the phosphate-terminus in the oligomer-Ce(III) complex. The phosphodiester residues in the oligomer coordinate to a Ce(III) ion competitively with the terminal monophosphate and water, making the catalysis less efficient.

Assumedly, the DNA hydrolysis involves nucleophilic attack of the hydroxide ion on a Ce^{+3} ion toward the phosphodiester residue which is coordinating to the same Ce^{3+} .¹⁰ The fission of the resultant penta-coordinated intermediate to the products is also promoted by Ce^{3+} (or water coordinating to the ion) as acid catalyst. The catalysis confers the hydrolytic activity to otherwise highly stable phosphodiester linkages in linear DNAs.

In conclusion, lanthanide metal ions, especially Ce^{3+} , promptly hydrolyze DNA oligomers under mild conditions. The hydrolysis is efficient both for the single-stranded and for the double-stranded DNA, and base-specificity is marginal. The finding strongly indicates high potentialities of the metal ions as the catalytic centers of hydrolytic artificial nucleases.

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