Hydrolysis of Oligonucleotides by Homogeneous Ce(IV)/EDTA Complex

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Homogeneous Ce(IV) complex of EDTA promptly hydrolyzes oligonucleotides under physiological conditions. In contrast, dinucleotides are not hydrolyzed to measurable extents. The degree of polymerization of DNA substrate is crucial for the present catalysis.

Non-enzymatic hydrolysis of DNA has been one of the most significant targets for chemists.[1] Several years ago, the remarkable catalysis by the lanthanide ions was found, and highly stable phosphodiester linkages in DNA were hydrolyzed at pH 7.[2,3] The Ce(IV) ion is especially active.[4-6] However, the Ce(IV) easily forms a gel of metal hydroxide, and this feature imposes limitations to the scope of its application. Homogeneous and catalytically active Ce(IV) complexes are required for more versatile applications.

Previously,[7] the authors found that homogeneous complexes of Ce(IV) with ethylenediamine-*N,N,N',N'*-tetraacetate (EDTA), poly(vinylpyrrolidone), and dextran hydrolyze plasmid DNA. Interestingly, Ce(IV) hydroxide gel is virtually inactive for the purpose, although it is highly active for the hydrolysis of dinucleotides and oligonucleotides. This finding prompted us to study the effect of the structure of DNA substrates on the catalysis by these homogeneous complexes. Here we show that homogeneous Ce(IV)/EDTA complex hydrolyzes oligonucleotides, although it is inactive for the hydrolysis of dinucleotides. Significant role of the degree of polymerization of DNA substrate is clearly evidenced.

Homogeneous solutions of the Ce(IV)/EDTA complex were prepared by adding equimolar amounts of $Ce(NH_4)_2(NO_3)_6$ and EDTA (2Na salt) to Hepes buffer (20 mmol dm^{-3}). The pH was adjusted to 7.0 by NaOH. Under these conditions, the complex is almost quantitatively formed (the formation constant of the 1:1 complex is $10^{24.2}$ at 25 °C).[8] Consistently, the solutions were kept completely homogeneous throughout the reactions. The reversed-phase HPLC pattern for the scission of T_{12} is presented in Figure 1 (A).[9] The signal for T_{12} gradually weakened, and concurrently several peaks (corresponding to smaller fragments) appeared at shorter retention-times. In contrast, thymidylyl(3' → 5')thymidine (T_2) as well as other dinucleotides was not hydrolyzed at all under the same conditions (B). The degree of polymerization (n) of DNA substrate shows a drastic effect on the present catalysis.

As depicted in Figure 2, three oligo(thymidine)s (n = 4, 8, and 12: the 5'-ends were ^{32}P-labeled) were notably hydrolyzed by the complex. When $[\text{the complex}]_0$ = 0.5 mmol dm^{-3} at pH 7.0 and 37 °C, about 25% of pT_8 were converted to smaller fragments in 15 h. The catalytic activity of this complex was comparable to that of Ce(IV) hydroxide gel. The other substrates were also hydrolyzed effectively. The present homogeneous catalyst is sufficiently active. Oligonucleotides of vari-

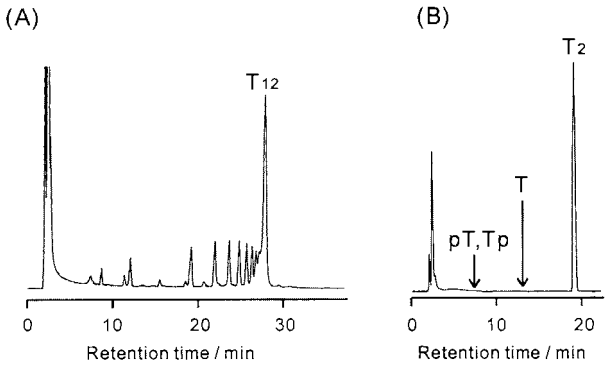

Figure 1. HPLC patterns for the hydrolysis of T_{12} (A) and T_2 (B) by the Ce(IV)/EDTA complex at pH 7.0 and 50 °C for 2 days; $[\text{phosphodiester linkage}]_0$ = 0.1, $[Ce(NH_4)_2(NO_3)_6]_0 = [EDTA]_0$ = 0.5, and [NaCl] = 100 mmol dm^{-3}. The peaks at around 2 min are associated with the buffer agents and others, which hardly interact with the column.

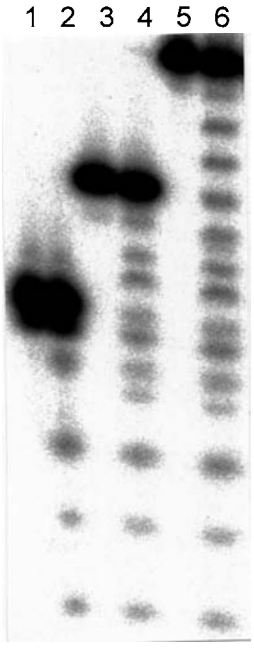

Figure 2. Polyacrylamide-gel electrophoresis patterns for the hydrolysis of oligo(thymidine)s of different degrees of polymerization at pH 7.0 and 37 °C for 15 h. Lanes 1 and 2, pT_4; lanes 3 and 4, pT_8; lanes 5 and 6, pT_{12}. Lanes 2, 4, and 6; with the Ce(IV)/EDTA complex. Lanes 1, 3, and 5; without the complex. The substrates were ^{32}P-labelled at the 5'-ends. $[\text{phosphodiester linkage}]_0$ = 1.0, $[Ce(NH_4)_2(NO_3)_6]_0 = [EDTA]_0$ = 0.5, and [NaCl] = 100 mmol dm^{-3}.

ous sequences (involving all of A, G, C, and T) were hydrolyzed without specific base-preference (data not presented). The scission took place almost randomly throughout the DNA chain.

The scission of the internal phosphodiester linkages in pT_4 (also in T_4) was confirmed by the reversed-phase HPLC (Figure 3).[10] All the products were firmly assigned to the hydrolytic ones by the coinjection with authentic samples (see the assign-

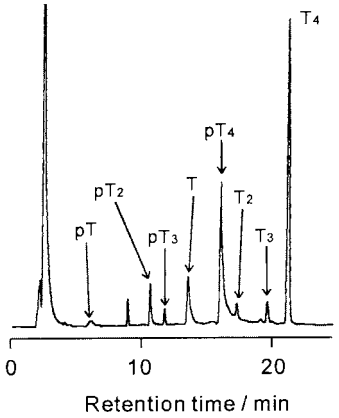

Figure 3. Assignments of the HPLC signals for the hydrolysis of pT_4 by the Ce(IV)/EDTA complex at pH 7.0 and 37 °C for 3 days; [phosphodiester linkage]$_0$ = 0.1, [$Ce(NH_4)_2(NO_3)_6$]$_0$ = [EDTA]$_0$ = 1.0, and [NaCl] = 100 mmol dm^{-3}.

ments in the Figure).[11] The scission occurs via hydrolytic pathway.[12] Similarly, the hydrolysis of the internal linkages in pT_3 was evidenced by the HPLC. However, the phosphodiester linkages in T_3 and pT_2 were hardly hydrolyzed by the complex.[12] These results are consistent with the fact that T_2 was kept intact even in the presence of the complex (see Figure 1 (B)). Thus, the number of phosphates in the substrate must be greater than 3, in order for the Ce(IV)/EDTA complex to show a notable catalysis. Assumedly, several phosphates must be simultaneously coordinated to Ce(IV) for the Ce(IV)/EDTA complex to form the catalyst/substrate complex, since the net positive-charge on the Ce(IV) in this complex is diminished due to the negative-charges of the EDTA.[13]

In conclusion, it has been found that the Ce(IV)/EDTA complex efficiently hydrolyzes oligonucleotides under physiological conditions. This complex is homogeneous and stable, and thus a promising candidate for the catalytic sites of sequence-selective artificial nucleases and the relevant artificial enzymes.

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10 Although the terminal phosphates in pT_2, pT_3, and pT_4 were also hydrolyzed rather efficiently, all the products were well characterized by the HPLC and thus the efficiency for the scission of the internal linkages could be precisely evaluated.

11 When the internal linkages in pT_4 are hydrolyzed, plausible products are pT, pT_2, pT_3, T, T_2, T_3, pTp, pT_2p, and pT_3p. The first six are clearly observed in Figure 3.

12 When the reaction mixtures were treated with hot-piperidine (90 °C for 30 min), the polyacrylamide-gel electrophoresis patterns were virtually unchanged. No alkaline-labile sites were formed during the reactions, providing a further evidence for the hydrolytic nature of the scission.

13 Similar chelate-structures were previously proposed in the metal-mediated hydrolysis of DNA (Ref. 4d) and RNA (S. Kuusela, A. Guzaev, and H. Lönnberg, *J. Chem. Soc., Perkin Trans. 2*, **1996**, 1895).