Cooperation of the Ce(IV)/EDTA Complex and Oligoamine for Prompt Scission of DNA

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DNA hydrolysis by the Ce(IV)/EDTA complex is enormously accelerated by the cooperation with selected oligoamines. The reaction in the presence of ethylenediamine (100 mmol dm^{-3}) is 50 times as fast as that in its absence.

Non-enzymatic hydrolysis of DNA has been attracting much interest, mainly because of potential applications to molecular biology, therapy, and others.[1] Notable catalysis by Ce(IV) ion has been already documented.[2,3] However, this metal ion forms hydroxide gel at physiological pH, which has been imposing limitations to practical applications. Recently, it has been reported that homogeneous Ce(IV)/EDTA complex hydrolyzes DNA and is promising for various applications (EDTA = *N*,*N*,*N*',*N*'-ethylenediaminetetraacetate).[4,5] Here, we show that the activity of this complex is significantly increased by the cooperation with selected oligoamines.

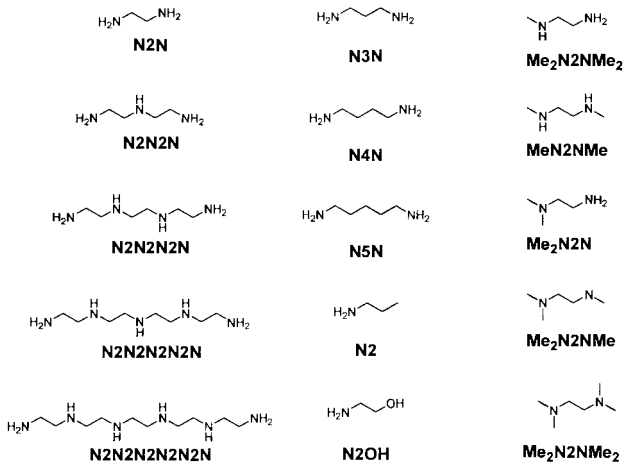

Figure 1. Amines used in the present study.

The oligoamines used in this study are presented in Figure 1. The Ce(IV)/EDTA complex was prepared from equimolar amounts of $Ce(NH_4)_2(NO_3)_6$ and EDTA (2Na salt), as described previously.[4] When this complex was combined with the oligoamines, the substrate DNA (30-mer of thymidine: ^{32}P-labeled at its 5'-end) was rapidly cleaved at pH 7.0 and 37 °C (see lanes 3–10 in Figure 2).[6] The scission occurred almost randomly throughout the DNA chain (In lanes 4–6, secondary scission is also perceived).[7] The scission products comigrated with the fragments obtained by the digestion with naturally occurring enzymes.[8] Apparently, the present DNA scission proceeds through hydrolysis of the phosphodiester linkages, as does the DNA hydrolysis by the enzymes and also by the Ce(IV)/EDTA complex.[4]

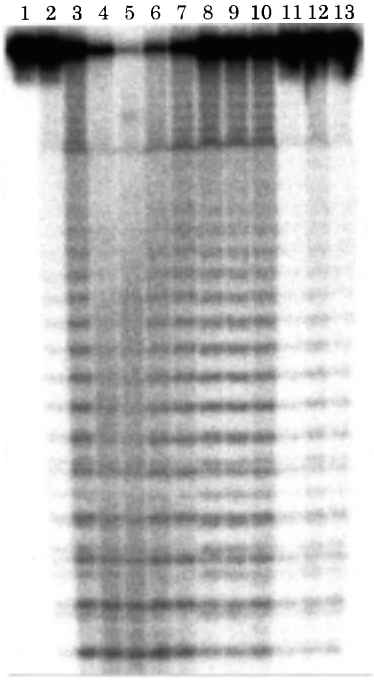

Figure 2. Polyacrylamide-gel electrophoresis patterns for the hydrolysis of single-stranded 30-mer of thymidine (^{32}P-labelled at the 5'-end) by the combinations of the Ce(IV)/EDTA complex (5 μmol dm^{-3}) and amines (10 mmol dm^{-3}) at pH 7.0 (20 mmol dm^{-3} Hepes buffer) and 37°C for 15 h. Lane 1, control; lane 2, the Ce(IV)/EDTA complex only; lane 3, Ce(IV)/EDTA + N2N; lane 4, + N2N2N; lane 5, + N2N2N2N; lane 6, + N2N2N2N2N; lane 7, + N2N2N2N2N2N; lane 8, + N3N; lane 9, + N4N; lane 10, + N5N; lane 11, + Me_2N2NMe_2; lane 12, + N2; lane 13, + N2OH.

It is noteworthy that the DNA hydrolysis by these Ce(IV)/EDTA/oligoamine systems is far faster than that by the Ce(IV)/EDTA complex. The concentration of the Ce(IV)/EDTA complex employed here (5 μmol dm^{-3}) is rather low so that the DNA hydrolysis is quite slow in the absence of amines (lane 2 in Figure 2).[9] Only when the oligoamines coexist, the scission is efficient (lanes 3–10). Significant synergism between the Ce(IV)/EDTA complex and the oligoamines is evident (note that, in the absence of the Ce(IV) complex, all the oligoamines are inactive for DNA scission). The hydrolysis rate monotonically increases with increasing concentration of the oligoamine, although it attains a plateau at quite high concentrations (see Figure 3). Thus, the acceleration by 100 mmol dm^{-3} ethylenediamine (N2N) is more than 50 fold.

The efficiency of cooperation with the Ce(IV)/EDTA complex is in the following order: triethylenetetramine (N2N2N2N) > tetraethylenepentamine (N2N2N2N2N) > pentaethylenehexa-

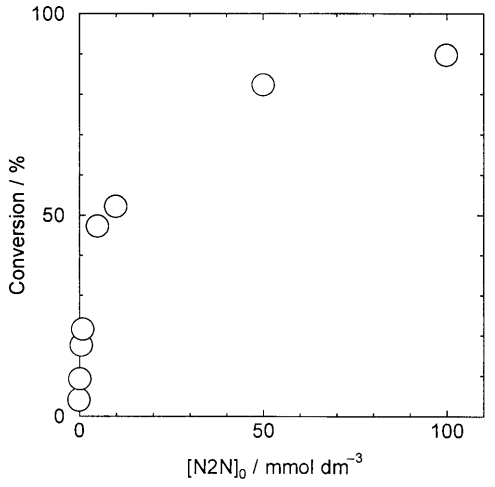

Figure 3. Dependence of the catalytic activity of the Ce(IV)/EDTA/N2N system on $[N2N]_0$ at pH 7.0 and 37 °C for 10 h: the $[Ce(IV)/EDTA]_0$ was kept constant at 5 μmol dm^{-3}. The conversion refers to the disappearance of the substrate DNA.

mine (N2N2N2N2N2N) > diethylenetriamine (N2N2N) > ethylenediamine (N2N). Here, the concentration of amine is kept constant. Trimethylenediamine (N3N), tetramethylenediamine (N4N), and pentamethylenediamine (N5N) are also effective (lanes 8–10 in Figure 2). In contrast with these remarkable cooperations by the oligoamines, monoamines (e.g., ethylamine and hydroxyethylamine) are inactive for the purpose (lanes 12 and 13). Two or more amino residues in the amines are essential for this cooperation. When the nitrogen atoms are methylated, the cooperation-effect is suppressed. The activities of N2N derivatives decrease as follows: N2N > MeN2N > MeN2NMe = Me_2N2N > Me_2N2NMe >> Me_2N2NMe_2. The fully *N*-methylated N2N derivative (Me_2N2NMe_2) is virtually inactive for the cooperation (lane 11).

The pH-rate profile for the DNA hydrolysis by the Ce(IV)/EDTA/N2N system showed the maximum at around pH 7 (data not presented). The dominant species of N2N here is a monocation, in which one of the amino groups is protonated and the other is in its neutral form.[10] The other oligoamines also involve both protonated and neutral amino residues under the reaction conditions. In a plausible mechanism, a water molecule is efficiently activated by the acid/base cooperative catalysis between the protonated amine and the neutral amine, and accordingly an eminent nucleophile is provided. Alternatively, the negatively-charged transition state for the DNA hydrolysis is stabilized by the positive charge(s) of the oligoamines.

In conclusion, the DNA hydrolysis by the Ce(IV)/EDTA complex is notably accelerated by the synergism with the oligoamines. Applications of the present findings to the preparation of artificial nucleases are now in progress in our laboratory.

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Reference and Notes

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6 The concentration of the DNA substrate was around 0.1 μmol dm^{-3}.

7 When a single-stranded 40 mer DNA (5'-GCAGTCGAGC-CTCCGCACCCGGCAGCGCAGCCACGTGACG-3') was used as a substrate, there was no specific base-preference or sequence-preference in the scission.

8 According to detailed gel-electrophoresis study, the present scission provides two kinds of DNA fragments. One of them comigrates with the digests by micrococcal nuclease, and the other with the digests by S1 nuclease. The former has 3'-phosphate termini, and the latter has 3'-OH termini.

9 The rate of DNA hydrolysis monotonically increases with the concentration of the complex (ref 4).

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