Site-selective DNA hydrolysis by the combination of Ce(IV) and oligonucleotide bearing EDTA groups

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ABSTRACT

By using appropriately modified oligonucleotides, a gap-structure is formed in substrate DNA, and 2-6 ethylenediamine-N,N,N'-triacetate residues are placed near this gap. When these composites are treated with homogenous Ce(IV)/EDTA complex, the phosphodiester linkages in the gap-site are selectively hydrolyzed at much greater rates than achieved previously by using unmodified oligonucleotides.

INTRODUCTION

Man-made restriction enzymes are essential for precise manipulation of huge DNA of higher animals and higher plants. Site-selective DNA hydrolysis was achieved by conjugating Ce(IV) ion (catalyst for DNA hydrolysis) with oligonucleotides (Figure 1a). In the preparation of these artificial enzymes, however, hydroxide gel of Ce(IV) ion is concurrently formed, making the system complicated. For further developments of the relevant fields, new strategy for site-selective DNA scission is desirable. Recently, we found that homogenous Ce(IV)/EDTA complex is active for DNA hydrolysis. Quite interestingly, this complex hydrolyzes single-stranded DNA far more effectively than double-stranded DNA. Accordingly, phosphodiester linkages at the target site were selectively hydrolyzed by placing these linkages at gap- or bulge-sites and keeping the other linkages in double-strands (Figure 1b). Here, we show that this gap-selective hydrolysis by Ce(IV)/EDTA complex is greatly promoted when ethylenediamine-N,N,N'-triacetate groups as eminent metal-binding ligands are immobilized near the gap-sites (Figure 1c). Unprecedentedly fast site-selective DNA hydrolysis has been accomplished.

MATERIALS AND METHODS

The substrate DNA and oligonucleotide additives are presented in Figure 2. All of them were prepared on an automated synthesizer, and purified by the usual method. The X residues bearing an ethylenediamine-N,N,N'-triacetate on thymidine were introduced to these oligonucleotides by using the phosphoramidite monomer purchased from GLEN research. Homogeneous Ce(IV)/EDTA complex was prepared immediately before use from equimolar amounts of Ce(NH₄)₂(NO₃)₆ and EDTA (4Na salt). The hydrolysis of DNA (32P-labelled at the 5' end) was initiated by adding the solution of Ce(IV)/EDTA complex to the reaction mixtures, and carried out at pH 7.0 (2.5 mM Hepes buffer) and 37°C; [substrate DNA]₀ = 1.0 µM, [each of oligonucleotide additives]₀ = 1.5 µM, and [NaCl] = 100 mM. After a predetermined time, the reaction mixtures were analyzed by denaturing 20% polyacrylamide gel electrophoresis, and the scission fragments were quantified with a Fuji Film FLA-3000G imaging analyzer.

RESULTS

By using appropriate oligonucleotide additives, gap-structures of various sizes were formed in the substrate DNA. When two oligonucleotides bearing an ethylenediamine-N,N,N'-triacetate group at the terminus were used as the additives and two of these groups were immobilized near the gap-site (1-, 5-, or 10-base gap), the DNA was selectively and efficiently hydrolyzed at the gap-site by Ce(IV)/EDTA complex. Typical polyacrylamide gel electrophoresis patterns are presented in Figure 3a. It is noteworthy that these DNA scissions with the combinations of two oligonucleotides bearing

![Figure 1. Three types of artificial enzymes for site-selective DNA hydrolysis](https://academic.oup.com/nass/article-abstract/3/1/137/1050173/37760173)
ethylenediamine-N,N,N'-triacetate group (on the X residue) are far faster than those with unmodified oligonucleotides. The remarkable promotion effects by the two ethylenediamine-N,N,N'-triacetate groups for the selective hydrolysis at the gap-site are evident. With increasing gap-length, the scission efficiency monotonously increases (lane 1 > lane 2 > lane 3).

The gap-selective DNA hydrolysis was still faster, when two ethylenediamine-N,N,N'-triacetate groups were bound to the terminus of each of the two oligonucleotide additives and four groups were immobilized near the gap-site (compare lane 2 with lane 1 in Figure 3b). The scission sites were satisfactorily restricted to the gap-site. Similarly, efficient gap-selective hydrolysis by Ce(IV)/EDTA complex was successful with six ethylenediamine-N,N,N'-triacetate groups at the gap-site (lane 3). Significantly, these scissions are successfully achieved even at 1-base gap-site. This is highly in contrast with the fact that virtually no scission occurs at 1-base gap when there exist no ethylenediamine-N,N,N'-triacetate groups there. The promotion by these groups is further substantiated. With longer gaps, the scission efficiency is still higher.

In conclusion, highly selective and efficient site-selective scission has been accomplished by immobilizing ethylenediamine-N,N,N'-triacetate groups at the gap-site and treating these systems with Ce(IV)/EDTA complex. Further studies to improve the site-selectivity and scission-efficiency are currently under way in our laboratory.

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REFERENCES


