Homogeneous Ce(IV) Complexes for Efficient Hydrolysis of Plasmid DNA

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Homogeneous Ce(IV) solutions, prepared by using appropriate additives, promptly hydrolyzed a plasmid DNA. The inactivity of Ce(IV)-hydroxide gel for this reaction, which is in contrast with its remarkable catalysis for the hydrolysis of linear DNA, has been ascribed to the steric hindrance between the supercoiled DNA and the polymeric catalyst.

Non-enzymatic hydrolysis of DNA is quite important for further developments of biotechnology and molecular biology.[1] However, DNA is so strongly resistant to hydrolysis that the challenges of chemists were unsuccessful for a long time. Only several years ago, the remarkable catalyses by the lanthanide ions were found, and a new era has been opened.[2-4] For the hydrolysis of dinucleotides and oligonucleotides, the Ce(IV) ion is overwhelmingly more active than the lanthanide(III) ions.[5] In the hydrolysis of plasmid DNA, however, the Ce(IV) is virtually inactive, and far inferior to the lanthanide(III) ions.[6] The order in the activity of the Ce(IV) is completely reversed between the linear DNA and the supercoiled DNA. This drastic substrate-specificity has been ascribed to either of the following two possibilities: (1) some of the phosphodiester linkages in plasmid DNA are notably activated so that they are more susceptible to the catalysis by the lanthanide(III) ions, and (2) the hydrolysis of plasmid DNA is suppressed by steric repulsion between the Ce(IV)-hydroxide gel, formed in the reaction mixtures, and the polymeric substrate. However, any definite conclusion has not yet been obtained. This point must be sufficiently clarified, since plasmid DNA is often used as the substrate for non-enzymatic cleavage of DNA, due to experimental convenience (high reactivity and easy detection of the products).[6,7]

The present paper reports that homogeneous solutions of Ce(IV), prepared by using poly(vinylpyrrolidone) (PVP), dextran, and ethylenediamine-N,N,N',N'-tetraacetate (EDTA) as the additives, are sufficiently active for the hydrolysis of a plasmid DNA. Predominant significance of steric hindrance in the hydrolysis of plasmid DNA is evidenced, providing a concrete answer to the above long-standing question.

Into a Hepes buffer (20 mmol dm^{-3}), were added $Ce(NH_4)_2(NO_3)_6$ and PVP (the averaged molecular weight = 24500: from Nacalai). The pH of the solution was adjusted to 7.0 by a small amount of NaOH. Homogeneous Ce(IV) solutions with dextran (the averaged molecular weight = 50000-70000; from Nacalai) and EDTA were prepared in similar manners. The resultant solutions were stable, and no turbidity was observed even after 1 week. In the absence of the additives, however, the gel of Ce(IV) hydroxide precipitated immediately on the addition of NaOH. The DNA hydrolysis was initiated by adding pBR322 plasmid DNA (from Toyobo) to the mixture and was carried out at 37 °C.

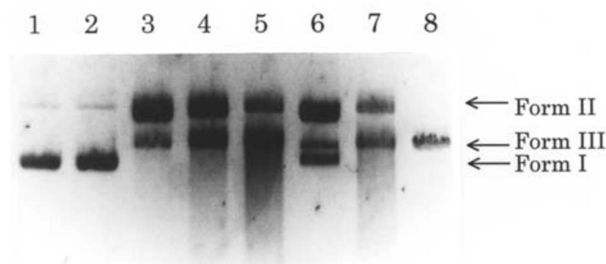

Figure 1. Agarose-gel electrophoresis patterns for the hydrolysis of pBR322 DNA at pH 7.0 and 37°C. Lane 1, control (2 h); lane 2, Ce(IV) alone (2 h); lane 3, Ce(IV)/PVP mixture (30 min); lane 4, the Ce(IV)/PVP mixture (1 h); lane 5, the Ce(IV)/PVP mixture (2 h); lane 6, Ce(IV)/EDTA mixture (2 h); lane 7, Ce(IV)/dextran mixture (2 h); lane 8, Form III DNA (*Eco*RI digest of pBR322). [DNA (monomeric residue)]$_0$ = 0.2, [Ce(IV)]$_0$ = 0.5, [PVP (monomeric residue)]$_0$ = 250, [EDTA]$_0$ = 0.5, and [dextran (monomeric residue)]$_0$ = 1 mmol dm^{-3}.

The typical agarose-gel electrophoresis patterns for the reactions by the homogeneous Ce(IV) solutions are presented in Figure 1. The concentration of the Ce(IV) was kept constant at 0.5 mmol dm^{-3}. As shown in lane 3, the form I DNA (supercoiled structure) was rapidly converted to the relaxed form II and then to the linearized form III. Within 30 min, more than 95% of the form I DNA was cleaved. This activity is far greater (>10 fold) than those of the corresponding solutions of the lanthanide(III) ions.[8] The superiority of the Ce(IV) is evident, as is the case in the hydrolysis of linear DNA. The homogeneous Ce(IV)/dextran solutions are also active.[9] When [Ce(IV)]$_0$ = 0.5 and [dextran (monomeric residue)]$_0$ = 1.0 mmol dm^{-3}, the form I DNA almost completely disappeared within 2 h (lane 7). The homogeneous 1:1 Ce(IV)/EDTA mixture also efficiently hydrolyzed the plasmid DNA (lane 6). More than 50 % of form I DNA was hydrolyzed to form II and form III DNA.[10] In contrast, the Ce(IV) ion itself (the gel of Ce(IV) hydroxide) showed virtually no catalysis (lane 2), as was reported previously.[6] The Ce(IV) ion is inactive for the hydrolysis of plasmid DNA, only when it forms the hydroxide gel and the reaction mixture is heterogeneous. Significance of steric effect is strongly indicated.[11]

At a longer reaction time, the bands for the form II and the form III DNA gradually weakened, and each of them was converted to consecutive bands having higher mobilities (see the lanes 4 and 5 of Figure 1). Apparently, the phosphodiester linkages in the form II and the form III DNA were hydrolyzed by these homogeneous Ce(IV) solutions, and smaller fragments were formed. With the lanthanide(III) ions, however, these post-reactions never occurred. These ions are rather poor for

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Figure 2. Polyacrylamide-gel electrophoresis patterns for the hydrolysis of the oligonucleotide (single-stranded 40 mer; ^{32}P-labelled at the 5'-end) at pH 7.0 and 37 °C for 15 h. Lane 1, control (PVP alone); lane 2, Ce(IV) alone; lane 3, the Ce(IV)/PVP mixture. $[DNA]_0$ = 0.1, $[Ce(IV)]_0$ = 2.0, and $[PVP]_0$ = 200 mmol dm^{-3}. The sequence of the substrate oligonucleotide: 5'-GCAGTCGAGCCTCCGCACCCGGCAG-CGCAGCCACGTGACG-3'.

the hydrolysis of linear DNA.[5]

Consistently, the Ce(IV)/PVP solutions hydrolyzed a single-stranded 40 mer DNA almost randomly throughout the DNA chain (the lane 3 in Figure 2). It is noteworthy that this homogeneous solution is 3 times as active as the gel of Ce(IV) hydroxide (compare the lanes 2 and 3 in Figure 2). Homogeneous catalysts are advantageous (as in the hydrolysis of plasmid DNA), since the steric hindrance between the Ce(IV)-hydroxide gel and the oligonucleotide substrate notably suppresses the catalysis.[12] This Ce(IV)/PVP polymer complex is the first homogeneous catalyst, which exceeds the gel of Ce(IV) hydroxide in the activity for the hydrolysis of linear DNA. The homogeneous Ce(IV)/EDTA complex also promptly hydrolyzed an oligonucleotide (T_{12}) to small fragments.[13]

The hydrolytic character of the present DNA scission is definitely confirmed by the HPLC analysis on the hydrolysis of thymidylyl(3' → 5')thymidine (TpT) by the Ce(IV)/PVP system. The product was only thymidine (its 3'- and 5'-monophosphates were rapidly hydrolyzed to thymidine, and not much accumulated). No by-products, assignable to oxidative cleavage, were observed. In conclusion, the inactivity of Ce(IV)-hydroxide gel for the hydrolysis of plasmid DNA mostly comes from the steric hindrance between the supercoiled substrate and the polymeric catalyst. Detailed mechanisms are currently being investigated.

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References and Notes

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10. The activity of Ce(IV)/EDTA is lower than those of the other two homogeneous system probably because of the negative charges of the EDTA. The positive charge on Ce(IV) is mostly compensated by EDTA. The positive charge electrostatically stabilizes the negatively charged transition state for DNA hydrolysis.
11. Assumedly, the Ce(IV)-hydroxide gel cannot get close to the phosphodiester linkages in the plasmid DNA, which are exceptionally reactive and more easily hydrolyzed than the others.
12. The argument is supported by the fact that the homogeneous Ce(IV)/PVP solution ($[Ce(IV)]_0$ = 10 mmol dm^{-3}) is 3 fold less active for the hydrolysis of TpT than is the corresponding Ce(IV)-hydroxide gel. In this small substrate, the steric hindrance is not so significant as in the hydrolysis of plasmid DNA and oligonucleotides.
13. Interestingly, the Ce(IV)/EDTA complex is virtually inactive for the hydrolysis of dinucleotides, although it efficiently hydrolyzes the plasmid DNA and the oligonucleotides. Detailed study on these points is in progress.