Site-selective Scission of Double-stranded DNA by Combining Peptide Nucleic Acids and Ce(IV)/EDTA

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By using two pseudo-complementary peptide nucleic acids (pcPNAs), invasion-structures were formed in double-stranded DNA so that the nucleotides adjacent to the DNA/PNA duplexes were kept unpaired. When these DNA/PNA conjugates were treated with Ce(IV)/EDTA complex, these gap-like sites in both DNA strands were preferentially hydrolyzed.

Site-selective scission of DNA has been attracting much interest, mainly because it is essential for advanced biotechnology and molecular biology.[1] To date, several artificial enzymes for site-selective scission of single-stranded DNA have been prepared.[2] However, few reports have been ever made on site-selective scission of double-stranded DNAs which are widely spread in nature.[3]

It was recently reported that Ce(IV)/EDTA complex selectively hydrolyzes gap-sites in DNA substrates.[4] Although the complex is not fixed to any sequence-recognizing moieties, the phosphodiester linkages in gap-sites are preferentially hydrolyzed over the other linkages. Moreover, PNA additives are much more effective than DNAs for this gap-selective hydrolysis.[5] These findings have prompted us to use pcPNAs for site-selective hydrolysis of double-stranded DNA, because they feasibly invade into double-stranded DNA.[6]

Substrate DNAs and pcPNAs used are shown in Figure 1.[7,8] The DNA^{S60} and DNA^{C60} are complementary with each other. When pcPNA2/pcPNA4 combination invades into DNA^{S60}/DNA^{C60} duplex, five nucleotides in both strands, designated by the underlines, are unpaired (T21–T25 in DNA^{S60} and T21–G25 in DNA^{C60}; the structure is schematically shown in lanes 5 and 6 in the right-hand side of Figure 3). These gap-like portions are the target sites for site-selective scission by Ce(IV)/EDTA. On the other hand, no single-stranded portions as reactive sites are formed in substrate DNAs when pcPNA1/pcPNA3 combination is used (see the structure in lanes 3 and 4 in the right-hand side of Figure 3).

The results of gel-shift assay for the invasion complex formation are shown in Figure 2. In Figure 2A, 1:1 mixture of DNA^{S60} and DNA^{C60} was mixed with different amount of 1:1 mixture of pcPNA1 and pcPNA3. Upon adding 1:1 pcPNA1/pcPNA3 mixture, a new band of smaller mobility appeared and its intensity gradually increased with increasing amount of the pcPNA mixture. Apparently, invasion-complex DNA^{S60}/DNA^{C60}/pcPNA1/pcPNA3 is formed under these conditions. Formation of this complex is almost complete when the mole ratio DNA^{S60}/DNA^{C60}/pcPNA1/pcPNA3 = 1/1/2/2 (lane 3

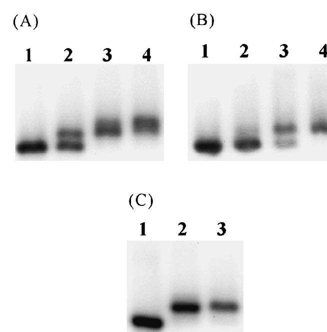

Figure 2. Agarose gel-shift assay for the invasion of (A) 1:1 pcPNA1/pcPNA3 combination or (B) 1:1 pcPNA2/pcPNA4 combination into DNA^{S60}/DNA^{C60} duplex ($[DNA^{S60}] = [DNA^{C60}] = 1\,\mu M$). For both (A) and (B): lane 1, without pcPNAs; lane 2, [each pcPNA] = 1 μM; lane 3, [each pcPNA] = 2 μM; lane 4, [each pcPNA] = 3 μM. The mixtures were incubated at 37 °C and pH 7.0 for 6 h ([NaCl] = 10 mM), and subjected to 2% agarose gel electrophoresis using 0.5 × TBE buffer as running buffer. The bands were stained with GelStar. In (C), the stabilities of these invasion-complexes under the conditions for the site-selective DNA scission (in Figure 3) were investigated. The complexes were first formed by incubating mixtures of DNA and pcPNAs at [NaCl] = 10 mM and 37 °C for 6 h, and then NaCl was added to a final concentration 100 mM. The gel-shift assay was made after the resultant solutions were incubated at 37 °C and pH 7.0 for 24 h. Lane 1, without pcPNAs; lane 2, [pcPNA1] = [pcPNA3] = 3 μM; lane 3, [pcPNA2] = [pcPNA4] = 3 μM.

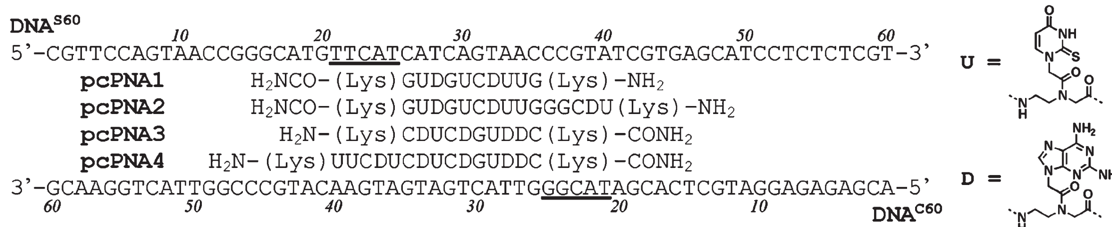

Figure 1. DNA substrates and pcPNA additives used. Invasion-complexes were prepared by mixing DNA^{S60}/DNA^{C60} mixture with pcPNA1/pcPNA3 mixture (or pcPNA2/pcPNA4 mixture). In the invasion-complex DNA^{S60}/DNA^{C60}/pcPNA2/pcPNA4, the underlined nucleotides in DNA^{S60} and DNA^{C60} remain unpaired.

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in Figure 2A). The results of gel-shift assay for the DNA^{S60}/DNA^{C60}/pcPNA2/pcPNA4 system are shown in Figure 2B. A new band for the invasion-complex also appeared at smaller mobility, and its formation is almost complete at the mole ratio DNA^{S60}/DNA^{C60}/pcPNA2/pcPNA4 = 1/1/3/3 (lane 4).

In Figure 3, these systems were treated with Ce(IV)/EDTA and analyzed by polyacrylamide gel electrophoresis.[9] In both lanes 5 and 6, the pcPNA2/pcPNA4 combination was used (see the structure of invasion complex in the right side). In lane 5, DNA^{S60} was ^{32}P-labeled at the 5′-end and thus the scission of this strand by Ce(IV)/EDTA was analyzed. The main products were 22-mer, 23-mer, 24-mer, and 25-mer fragments. Apparently, the scission mainly occurred in the single-stranded portion of DNA^{S60} (the 20-mer and 25-mer markers in lane M show the position of the single-stranded portion). In lane 6, DNA^{C60} was ^{32}P-labeled. The main products for the scission of this strand were 22-mer and 23-mer fragments. The scission of DNA^{C60} occurred also in the single-stranded region. These bands were not observed without Ce(IV)/EDTA complex (data not shown). Apparently, both DNA^{S60} and DNA^{C60} in the invasion-complex were selectively hydrolyzed in the single-stranded region. It is concluded that double-stranded DNA is site-selectively hydrolyzed by Ce(IV)/EDTA complex when invasion-structure is formed using two pcPNAs and the target sites are kept single-stranded.[10] This is the first site-selective hydrolysis of double-stranded DNA by artificial systems, although the scission efficiency is not very high at present.

All these arguments are strongly supported by the fact that no site-selective scission is observed in either of the DNA strands in lanes 3 and 4. Here, the pcPNA1/pcPNA3 combination invades into DNA^{S60}/DNA^{C60} duplex. The two strands in DNA^{S60} and DNA^{C60}, which are bound by pcPNA1 and pcPNA3, are complementary with each other, and thus no single-stranded portions are formed in substrate DNAs.

In all the reactions described above, the invasion-complexes were first prepared by incubating the mixture of double-stranded DNA and pcPNAs at [NaCl] = 10 mM, since low salt concentrations are more favorable for the formation of invasion-complexes.[11] Then, NaCl was added to the mixture to a final concentration of 100 mM. The subsequent addition of NaCl was necessary to stabilize the DNA/DNA duplexes in both ends of the DNA^{S60}/DNA^{C60}/pcPNA2/pcPNA4 complex and achieve sufficiently high site-selectivity.[10] As shown in Figure 2C, the invasion complexes are evidently formed under the reaction conditions for the site-selective scission.

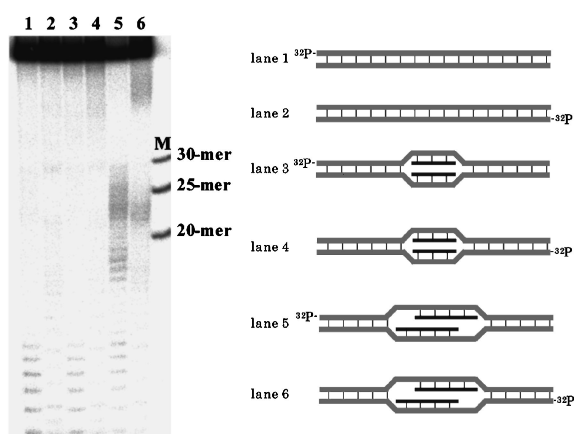

Figure 3. Ce(IV)/EDTA complex-induced site-selective hydrolysis of DNA^{S60}/DNA^{C60} duplex using two pcPNA additives. Lanes 1 and 2, Ce(IV)/EDTA alone; lanes 3 and 4, pcPNA1/pcPNA3 + Ce(IV)/EDTA; lanes 5 and 6, pcPNA2/pcPNA4 + Ce(IV)/EDTA; M, authentic samples of 20-, 25-, and 30-mer DNA oligomers. In lanes 1, 3, and 5, DNA^{S60} is ^{32}P-labeled at the 5′-end, whereas DNA^{C60} is ^{32}P-labeled at the 5′-end in lanes 2, 4, and 6. [^{32}P-labeled DNA] = 1 μM, [unlabeled DNA] = 1.2 μM, [each of pcPNAs] = 5 μM, [Ce(IV)/EDTA] = 500 μM, [NaCl] = 100 mM, [HEPES] = 4.75 mM, pH 7.0, 37 °C, and 60 h.

In conclusion, double-stranded DNA was selectively hydrolyzed by Ce(IV)/EDTA at the target sites by using two pseudo-complementary PNAs as additives. Appropriate modification of these PNAs (e.g., introduction of metal-binding sites) should be useful for further promotion of the scission with respect to both scission-efficiency and site-selectivity. These attempts are currently under way in our laboratory.

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References and Notes

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8 The melting temperatures are as follows: pcPNA1/pcPNA3, 41.0 °C; pcPNA2/pcPNA4, 49.7 °C; pcPNA1/DNA^{S20}, 77.3 °C; pcPNA3/DNA^{C20}, 85.5 °C; pcPNA2/DNA^{S20} and pcPNA4/DNA^{C20}, >85 °C. DNA^{S20} and DNA^{C20} are T21–A40 of DNA^{S60} and T21–A40 of DNA^{C60}, respectively. Measurement conditions: [pcPNA] = [DNA] = 3 μM, [NaCl] = 10 mM, [HEPES] = 1 mM, pH 7.0.

9 In pH 7.0 HEPES buffer solution containing 10 mM NaCl, the invasion-complex composed of DNA^{S60}/DNA^{C60} (either of these two DNAs was labeled by ^{32}P at 5′-end) and pcPNAs was prepared by incubating the mixtures for 6 h at 37 °C. Then, NaCl was added to a final concentration of 100 mM. The DNA hydrolysis was started by adding aqueous solution of Ce(IV)/EDTA, and the mixture was incubated for 60 h at 37 °C. After the reaction, the mixture was subjected to 20% denaturing polyacrylamide gel electrophoresis.

10 The DNA scission at the sites other than the target ones is tentatively ascribed to breathing motion of the DNA/DNA double-strands in both ends of the DNA^{S60}/DNA^{C60}/pcPNA2/pcPNA4 complex. Consistently, preliminary experiments on the scission of longer double-stranded DNAs provided still clearer site-selectivity. Detailed study is now being made.

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