Peptide Nucleic Acid for Rapid Gap-selective Hydrolysis of DNA by Ce(IV)/EDTA Complex

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(Received October 20, 2003; CL-030989)

When gap-structures are formed in substrate DNA by using peptide nucleic acid (PNA), these sites are rapidly and selectively hydrolyzed by Ce(IV)/EDTA complex. Scission efficiency at the gap-site is 6-fold higher than that obtained by use of the corresponding DNA additives. Site-selectivity is also higher. The DNA/PNA duplexes must take anti-parallel direction here.

Man-made restriction enzymes are very important for further development of biotechnology. For example, huge genomes of higher animals and plants can be manipulated by them, although it is difficult with naturally occurring enzymes. Previously we reported that the gap-site in DNA substrates is preferentially hydrolyzed by Ce(IV)/EDTA complex.[1] This gap-selective hydrolysis is further promoted by either the addition of oligoamine–acridine conjugates[2] or the attachment of EDTA on the nucleobases near the gap-site.[3] These findings have indicated that PNA (a DNA analogue in which the phosphodiester linkages are replaced with peptide bonds)[4] should be promising additives, since it can show "strand invasion" into double-stranded DNA.[4–6] By forming gap-site or single-stranded region in double-stranded DNA through the strand invasion, the way to site-selective hydrolysis of double-stranded DNA should be opened. Here, we show that PNA is far superior to DNA as the additives for gap-selective hydrolysis of single-stranded DNA by Ce(IV)/EDTA complex. Remarkable effects of the orientation of DNA/PNA duplexes (either anti-parallel or parallel direction) are evidenced.

The sequences of DNA and PNA oligomers are shown in Figure 1. By the addition of $PNA^{(20L)}$ and $PNA^{(20R)}$ (or $DNA^{(20L)}$ and $DNA^{(20R)}$), 10-base gap and 5-base gap are formed in substrates $DNA_{S50}{}^{anti}$ and DNA_{S45}. Here, the DNA/PNA duplexes are in anti-parallel direction. $DNA_{S50}{}^{par}$ forms a 10-base gap structure with $PNA^{(20L)}$ and $PNA^{(20R)}$ in parallel direction. These DNA oligomers were prepared on an automated synthesizer, and purified by the polyacrylamide gel electrophoresis and the reversed-phase HPLC. According to the standard protocol for the solid phase peptide synthesis, PNA oligomers were synthesized using Fmoc-protected PNA monomer and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as a coupling reagent and then purified by the reversed-phase HPLC.

A typical gel electrophoresis pattern for the gap-selective hydrolysis by Ce(IV)/EDTA complex is shown in Figure 2a. In the lanes 1–3, 10-base gap was formed in anti-parallel direction. When $PNA^{(20L)}/PNA^{(20R)}$ was used as the additive, the scission of $DNA_{50}{}^{anti}$ selectively and efficiently occurred at the gap-site (Lane 3). With the use of $DNA^{(20L)}/DNA^{(20R)}$, however, the gap-site scission was much less efficient (Lane 2). The conversion of scission at the gap-site was 9.9% for the $PNA^{(20L)}/PNA^{(20R)}$ combination, whereas the corresponding value with the $DNA^{(20L)}/DNA^{(20R)}$ combination was 1.6% (see the bar graph in the right-hand side). Thus, the scission by the PNA additives is 6 times as fast as that by the DNA additives. For 5-base gap, the scission efficiency and selectivity by the PNAs were also higher than those obtained by use of the DNAs (Lane 6 vs Lane 5). Another advantage of PNA additives is that gap-selective hydrolysis is achievable even at higher temperatures (Figure 2b). The reactions are much faster as expected; the conversions at the gap-site were 7.4% for 2 h at 50 °C and 8.6% for 4 h at 45 °C (Lanes 3 and 5), while the conversion was 9.9% for 17 h at 37 °C as described above. With the use of DNA additives, however, the selectivity was drastically decreased as the reaction temperature became higher (Lanes 2 and 4). The DNA/PNA duplexes are more stable than the DNA/DNA duplexes,[7] and thus can show sufficient "protecting activity" even at the higher temperatures. The selectivity for the gap-selective scission at 50 °C with the PNA additives was still higher than that for the corresponding scission achieved by the DNA additives at 37 °C.

The scission selectivity was enormously decreased when the DNA/PNA duplexes take parallel direction. As shown in Figure 2c, even the double-stranded portions were considerably hydrolyzed by the Ce(IV) complex, resulting in poorer selectivity. In order to accomplish high selectivity, the gap structure must be formed by PNA additives which form anti-parallel duplexes. Probably, parallel duplexes are less stable than anti-parallel ones,[8] and thus the DNA therein can not be sufficiently protected from the Ce(IV) complex.

From the Arrhenius plots in Figure 3, the activation energies for the hydrolysis at the gap-site were determined to be 135 kJ/

Substrate DNA (labeled at 5'-end with ^{32}P)

$DNA_{S50}{}^{anti}$ 5′-CAATTAGAATCAGGAATGGC**TTATGCGCAC**GTGCAGACTGTCGACCTAAG-3′
$DNA_{S50}{}^{par}$ 5′-CGGTAAGGACTAAGATTAAC**TTATGCGCAC**GAATCCAGCTGTCAGACGTG-3′
DNA_{S45} 5′-CAATTAGAATCAGGAATGGC**TTATG**GTGCAGACTGTCGACCTAAG-3′

Complementary strand

$PNA^{(20L)}$ NH_2CO-GTTAATCTTAGTCCTTACCG-NH_2 NH_2CO-CACGTCTGACAGCTGGATTC-NH_2 $PNA^{(20R)}$
$DNA^{(20L)}$ 3′-GTTAATCTTAGTCCTTACCG-5′ 3′-CACGTCTGACAGCTGGATTC-5′ $DNA^{(20R)}$

Figure 1. Sequences of DNA and PNA oligomers used in this study. By the addition of complementary DNAs or PNAs, gap structure is formed at the bases designated in bold type. $DNA_{S50}{}^{anti}$ forms anti-parallel duplex with complementary PNA, whereas $DNA_{S50}{}^{par}$ forms parallel duplex.

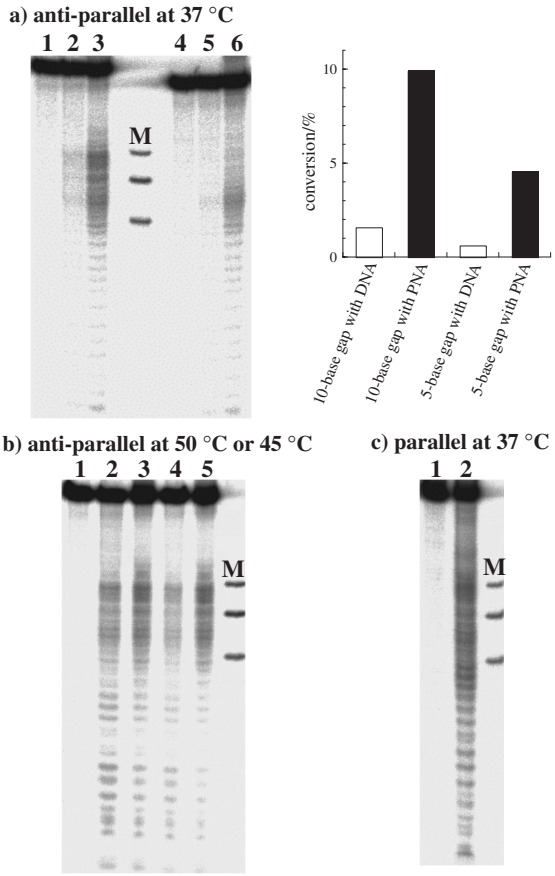

Figure 2. a) Autoradiographs for the Ce(IV)/EDTA-induced hydrolysis of gaps formed with the DNA or PNA additives in anti-parallel direction. Lane 1, control; Lane 2, 10-base gap formed in DNA_{S50}^{anti} with $DNA^{(20L)}/DNA^{(20R)}$; Lane 3, 10-base gap formed with $PNA^{(20L)}/PNA^{(20R)}$; Lane 4, control; Lane 5, 5-base gap formed in DNA_{S45} with $DNA^{(20L)}/DNA^{(20R)}$; Lane 6, 5-base gap formed with $PNA^{(20L)}/PNA^{(20R)}$; M, authentic samples of 20-, 25-, and 30-mer DNA oligomers. The bar graph shows the conversions of hydrolysis at the gap-site formed with the DNA (white bars) or the PNA additives (black bars). b) Hydrolysis of 10-base gap formed in DNA_{S50}^{anti} with the DNA or PNA additives in anti-parallel direction at 50 or 45 °C. Lane 1, control; Lane 2, 50 °C for 2 h with $DNA^{(20L)}/DNA^{(20R)}$; Lane 3, 50 °C for 2 h with $PNA^{(20L)}/PNA^{(20R)}$; Lane 4, 45 °C for 4 h with $DNA^{(20L)}/DNA^{(20R)}$; Lane 5, 45 °C for 4 h with $PNA^{(20L)}/PNA^{(20R)}$; M, authentic samples of 20-, 25-, and 30-mer DNA oligomers. c) Hydrolysis of the gap formed in DNA_{S50}^{par} with $PNA^{(20L)}/PNA^{(20R)}$ in parallel direction. Lane 1, control; Lane 2, 10-base gap formed with the PNAs; M, authentic samples of 20-, 25-, and 30-mer DNA oligomers. Reaction conditions: $[\text{substrate DNA}]_0 = 1\,\mu M$, [each of complementary strands] = 3 μM, [Ce(IV)/EDTA] = 500 μM, [NaCl] = 100 mM, [HEPES] = 5 mM, pH 7.0, 37 °C, and 17 h, unless noted otherwise.

mol for $PNA^{(20L)}/PNA^{(20R)}$ and 195 kJ/mol for $DNA^{(20L)}/DNA^{(20R)}$.[9] The gap-site formed with the PNA additives is more susceptible to hydrolysis than that with the DNAs, primarily because the activation energy is smaller. It is noteworthy that the activation energy for the PNAs is close to the value for the hydrolysis of single-stranded DNA (120 kJ/mol). Probably, molecular flexibility of the target-site is one of the factors governing the reactivity. As shown previously,[1] single-stranded DNA is hydrolyzed by the Ce(IV)/EDTA complex more rapidly than double-stranded DNA, and this notable difference of reactivity is caused by the difference in molecular flexibility of substrate DNA. In PNA/DNA duplexes, the base-pair stacking is less regular than DNA/DNA duplex,[10,11] and this disordered structure might affect the base stacking at the gap-site and provide molecular flexibility for the efficient hydrolysis.

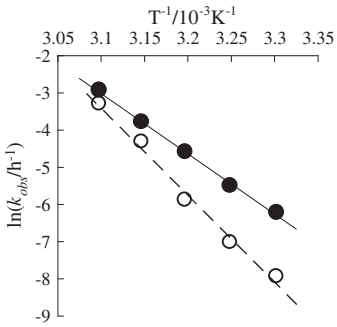

Figure 3. Arrhenius plots on the hydrolysis at the gap-site formed with the PNA additives (closed circles) or the DNA additives (open circles). The solid line (PNA) and dotted line (DNA) are the theoretical ones calculated according to Arrhenius' equation. Reaction conditions: $[DNA_{S50}^{anti}]_0 = 1\,\mu M$, [each of complementary strands] = 1.5 μM, [Ce(IV)/EDTA] = 500 μM, [NaCl] = 100 mM, [HEPES] = 5 mM, pH 7.0.

In conclusion, gap-selective hydrolysis of DNA by Ce(IV)/EDTA complex has been efficiently achieved by using two PNA additives and forming anti-parallel DNA/PNA duplexes. PNA additives are superior to DNA additives, indicating its potential for site-selective scission of double-stranded DNA. These attempts, as well as chemical modification of PNA for further promotion of the gap-selective scission, are under way in our laboratory.

This work was partially supported by PROBRAIN. The support by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan is also acknowledged.

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Published on the web (Advance View) December 20, 2003; DOI 10.1246/cl.2004.76