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Origin of High Fidelity in Target-Sequence Recognition by PNA−Ce(IV)/EDTA Combinations as Site-Selective DNA Cutters

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Abstract: Double-duplex invasion of pseudocomplementary peptide nucleic acid (pcPNA) is one of the most important strategies for recognizing a specific site in double-stranded DNA (*Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11804−11808). This strategy has recently been used to develop artificial restriction DNA cutters (ARCUTs) for site-selective scission of double-stranded DNA, in which a hot spot formed by double-duplex invasion of PNA was hydrolyzed by Ce(IV)/EDTA (*Nat. Protoc.* **2008**, *3*, 655−662). The present paper shows how and where the target sequence in double-stranded DNA is recognized by the PNA−Ce(IV)/EDTA combinations for site-selective scission. The mismatch-recognizing activities in both the invasion process and the whole scission process are evaluated. When both pcPNA additives are completely complementary to each strand of the DNA, site-selective scission is the most efficient, as expected. Upon exchange of one DNA base pair at the invasion site with another base pair, which introduces mismatches between the pcPNAs and the DNA, the site-selective scission by the ARCUT is notably diminished. Mismatches in (or near) the central double-invasion region are especially fatal, showing that Watson−Crick pairings of the DNA bases in this region with the pcPNA strands are essential for precise recognition of the target sequence. Both gel-shift assays and melting temperature measurements on the double-duplex invasion process have confirmed that the fidelity in this process primarily governs the fidelity of the DNA scission. According to these systematic analyses, the typical ARCUT involving two 15-mer pcPNAs precisely recognizes 14−16 base pairs in substrate DNA. This remarkable fidelity is accomplished at rather high salt concentrations that are similar to the values in cells.

Introduction

Chemical conversion of DNA at a predetermined site by artificial agents is one of the major challenges from the point of view of molecular biology, nanotechnology, and many other applications. Artificial enzymes for site-selective scission of DNA serve as a typical example.[1−7] The sequences that naturally occurring restriction enzymes recognize are too short to manipulate genomic DNA, and thus, new scission tools that recognize longer sequences are required. To date, site-selective scission of single-stranded DNA has been achieved by attaching catalysts for DNA hydrolysis to sequence-recognizing oligonucleotides (or their equivalents).[8−10] However, in regard to site-selective scission of double-stranded DNAs, which are widespread in nature, few reports have been published.[11] Recently, artificial restriction DNA cutters (ARCUTs) have been developed by combining Ce(IV)/EDTA (which acts as molecular scissors for the hydrolysis of phosphodiester linkages) with two pseudocomplementary peptide nucleic acid (pcPNA) additives (which recognize the target scission site in the double-stranded DNA).[12−20] Site-selective scission by an ARCUT is based on double-duplex invasion of the two pcPNA strands, which was

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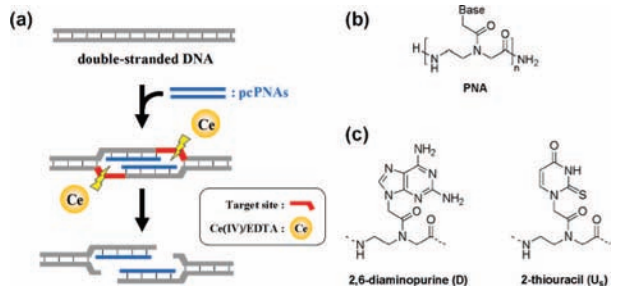

Figure 1. (a) Scheme of site-selective DNA scission by an ARCUT. Single-stranded portions (red lines) formed by the invasion of two pcPNA strands (blue lines) on the DNA substrate (gray lines) are hydrolyzed by Ce(IV)/EDTA. (b) Generic structure of PNA. In pcPNA, 2,6-diaminopurine (D) and 2-thiouracil (U_s) [shown in (c)] are used together with G and C.

elegantly developed by Nielsen et al.[21−23] to recognize a site in DNA and alter the physicochemical properties there. The binding sites of the two pcPNA strands to the two strands of the substrate DNA are laterally shifted relative to one another (see Figure 1a). Thus, in the double-duplex invasion complexes, single-stranded portions are formed at predetermined sites in the two DNA strands.[21−23] The other component of the ARCUT, Ce(IV)/EDTA, hydrolyzes the phosphodiester linkages in these single-stranded portions to give site-selective scission of the double-stranded DNA, since this complex preferentially hydrolyzes single-stranded DNA over double-stranded DNA.[24−27]

The sequences and lengths of pcPNA strands are almost freely chosen, and thus, in principle, use of ARCUTs has the potential to cut even huge genomic DNA at any desired site. To date, however, little information has become available on the following three important factors: (i) how high the fidelity of the ARCUT is, (ii) which step (invasion or scission) really governs the fidelity of the scission, and (iii) which nucleobases in the DNA interact with the pcPNA strands in the ARCUT. The fidelity of the ARCUT [factor (i)] is crucial, especially in the manipulation of huge DNA, since the target sequence must be precisely differentiated from similar sequences that are different in only one or two base pairs. Factors (ii) and (iii) are essential for a detailed mechanistic understanding of the scission and more precise molecular design. In this paper, one base pair at the target site of a DNA substrate is systematically changed to another base pair, and the scission efficiency by an ARCUT is evaluated. Independently, the effects of mismatches on the PNA invasion processes are analyzed by gel-shift assays and melting temperature (T_m) measurements. In terms of these results, factors (i)−(iii) are addressed, and the origin of the fidelity of the ARCUT is clarified. Furthermore, the essential roles of high ionic strengths, which are necessary for the site-selective scission, are interpreted in terms of the stability of invasion complexes. The potential of using ARCUTs for manipulation of huge DNA is discussed.

Results and Discussion

Mismatch Recognition by an ARCUT in Site-Selective Scission of Double-Stranded DNA. One base pair at the target scission site was systematically changed to another base pair. The target scission site, which was arbitrarily chosen, was located near the middle of a 408 base pair (bp) double-stranded DNA (the whole sequence of the DNA is provided in Figure S1 in the Supporting Information). As shown in Figure 2a, pcPNA1 is complementary to T1821−C1835 in the upper strand of the parent DNA, and pcPNA2 is complementary to G1826−T1840 in the lower strand.[16] Accordingly, in the double-duplex invasion complex of the parent DNA with pcPNA1 and pcPNA2, C1836−A1840 of the upper strand and A1821−A1825 of the lower strand are kept single-stranded. The phosphodiester linkages at these sites were selectively hydrolyzed by Ce(IV)/EDTA, since this complex preferentially hydrolyzes single-stranded DNA over double-stranded DNA.[24]

When one base pair in the T/A1821−A/T1840 range was changed to another base pair, mismatches between the DNA and pcPNA1/pcPNA2 were introduced at the corresponding invasion site. The fidelity of the ARCUT was evaluated in terms of the magnitudes of the changes in scission efficiency. The position of the single base-pair alteration can be placed into one of the following two categories: (i) the central double-invasion region (C/G1826−C/G1835), in which both of the pcPNA strands are complementary to the corresponding DNA strands, and (ii) the flanking single-invasion region (T/A1821−T/A1825 plus C/G1836−A/T1840), in which one of the two pcPNAs is complementary to its counterpart DNA strand and the other DNA strand is kept single-stranded.

1. Mismatch-Recognition Activity in the Central Double-Invasion Region. When the DNA substrate was the parent one, which was completely complementary to pcPNA1 and pcPNA2, the site-selective scission occurred at the target site and only two scission bands were detected (lane 2 in Figure 2a), as reported previously (the gel was stained with a dye).[13] In lanes 3−5, the C/G pair at site 1829 of the parent DNA was changed to A/T, T/A, and G/C pairs, respectively, and two mismatches (one between each pcPNA and its corresponding DNA strand) were introduced to the double-invasion region. In all of these lanes, no scission bands were observed.[28] The

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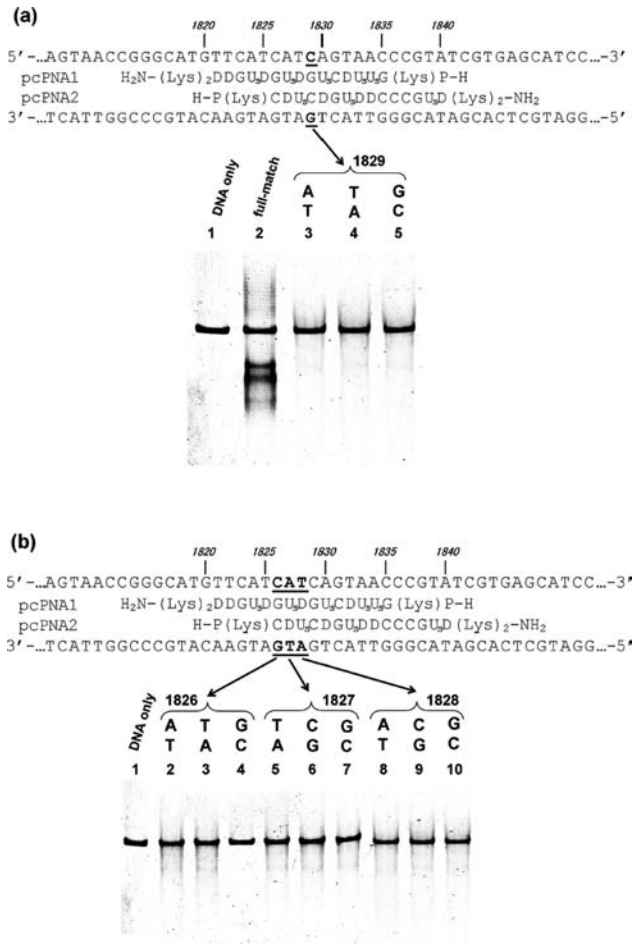

Figure 2. Mismatch recognition in the central double-invasion region for site-selective scission by the ARCUT. (a) Lane 1, without Ce(IV)/EDTA (DNA only); lane 2, fully matched DNA; lanes 3−5, the C/G pair at the 1829 site was changed to another base pair as indicated. (b) Lane 1, without Ce(IV)/EDTA (DNA only); lanes 2−10, one of the three underline base pairs was changed to another base pair. Reaction conditions: [DNA] = 20 nM, [pcPNA1] = [pcPNA2] = 100 nM, [Ce(IV)/EDTA] = 50 μM, [NaCl] = 100 mM, and [HEPES] = 5 mM at pH 7.0 and 50 °C for 14 h. In pcPNA1 and pcPNA2, L-phosphoserine (P) was attached to the N-termini to promote DNA scission.[16]

scission was also nil when the base pair at site 1826, 1827, or 1828 was replaced with another base pair (lanes 2−10 in Figure 2b). Apparently, the ARCUT completely distinguishes the alteration of only one base pair in the central double-invasion region and hydrolyzes only the completely complementary DNA at the target phosphodiester linkages. All of the nucleobases of the DNA in this region thus formed stable Watson−Crick base pairs with the corresponding pcPNA strands.

2. Mismatch-Recognition Activity in the Flanking Single-Invasion Region. In lanes 9−11 of Figure 3, the T/A pair at the 1825 site, which is next to the central double-invasion region, was changed to another base pair. The scission hardly occurred for any of the base-pair changes. The fidelity was also satisfactory for the change of C/G1823 to A/T or G/C, although very faint scission bands were perceivable (lanes 6 and 8). However, the change of C/G1823 to T/A resulted in rather strong scission bands (lane 7). This exceptionally poor recognition of the mismatches is ascribed to the formation of a wobble base

(28) The scission bands in lanes 3−5, if present, were at least 100-fold weaker than those in lane 2.

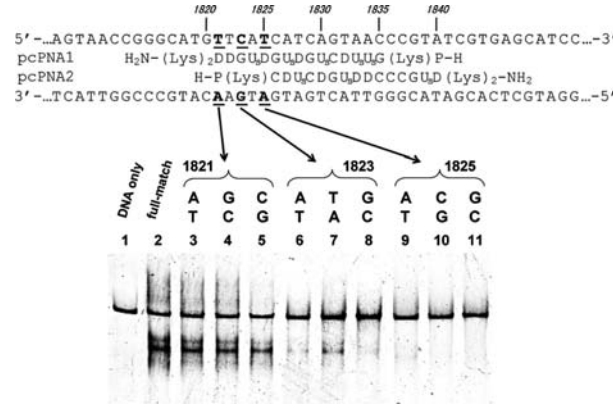

Figure 3. Mismatch recognition in the flanking single-invasion region for site-selective scission by the ARCUT. Lane 1, without Ce(IV)/EDTA (DNA only); lane 2, fully matched DNA; lanes 3−11, one base pair in the flanking single-invasion region (underlined) was changed to another base pair as indicated. The reaction conditions were the same as described for Figure 2.

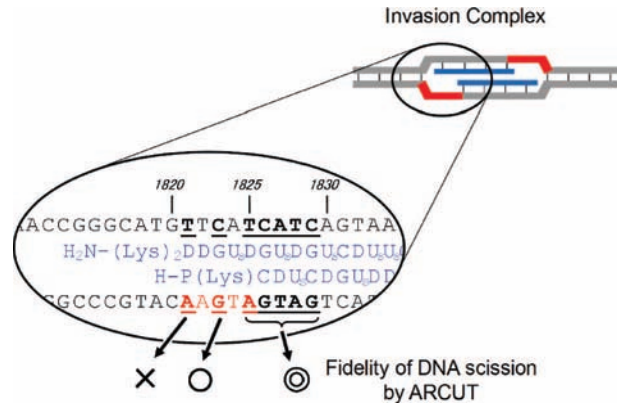

Figure 4. Summary of the study of the fidelity of the ARCUT in its site-selective scission of the DNA. The double circle, single circle, and cross indicate high, moderate, and poor fidelities, respectively.

pair between the T1823 in the upper DNA strand and the confronting G in pcPNA1, which gives rise to a rather stable double-duplex invasion complex. This argument is more directly confirmed by the gel-shift assay of the invasion complex formation (see lane 7 in Figure 5b), as described below. In the base-pair change of T/A1821 (at the edge of the flanking region), the recognition by the ARCUT is poor, and the site-selective scission occurred efficiently irrespective of the kind of incorporated base pair (lanes 3−5 of Figure 3).

The results of these analyses are summarized in Figure 4. The fidelity of the ARCUT is almost complete with respect to (i) all of the base pairs of the substrate DNA in the central double-invasion region and (ii) the flanking base pairs next to the central double-invasion region. In these positions, no scission occurred even when a wobble pair could be formed (see lane 4 of Figure 2a and lane 3 of Figure 2b). The fidelity is also notable in the flanking single-invasion region as long as the base pair is the third (or probably the second base pair also) from the central double-invasion region. These sites are the primary interaction sites between the DNA and the pcPNAs in the invasion complex.

Mismatch Recognition in Invasion Complex Formation under the Scission Conditions. In order to shed light on the origin of the fidelity of the ARCUT, formation of the invasion complexes of pcPNA1/pcPNA2 with the DNAs used above was

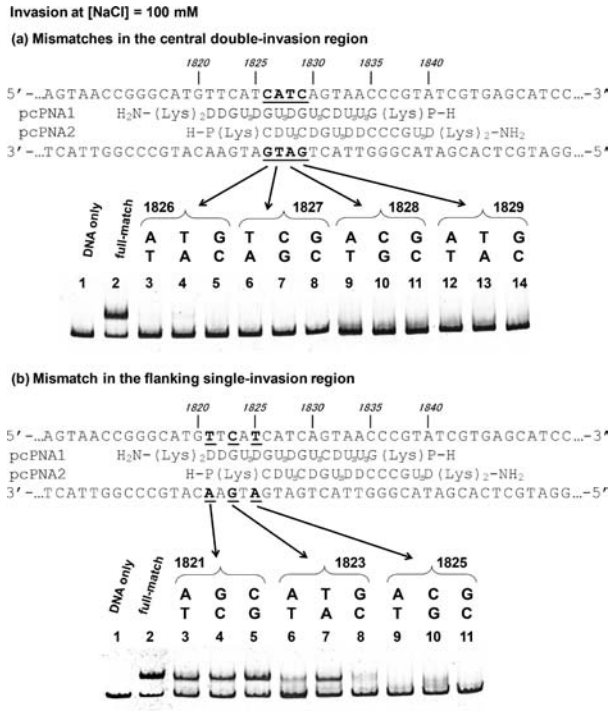

Figure 5. Gel-shift assays for the formation of invasion complexes under the conditions for the ARCUT scission ([NaCl] = 100 mM). (a) Lane 1, DNA only; lane 2, fully matched DNA; lanes 3−14, one base pair in the central double-invasion region (underlined) was changed to another base pair. (b) Lane 1, DNA only; lane 2, fully matched DNA; lanes 3−11, one base pair in the flanking single-invasion region (underlined) was changed. The concentrations of the reagents were the same as those for Figure 2.

investigated by gel-shift assays under the conditions of ARCUT scission (in the presence of 100 mM NaCl). Figure 5a shows the results for the base-pair changes at sites 1826, 1827, 1828, and 1829 in the central double-invasion region. Irrespective of the positions and kinds of the two mismatches between the pcPNA strands and the DNA (one for each of the pcPNA/DNA pairs), the invasion complex was never formed (lanes 3−14). A mismatch at site 1825 in the flanking single-invasion region was also critical, and formation of the invasion complex was almost completely inhibited (lanes 9−11 in Figure 5b). In order to form the invasion complex under the conditions used for the ARCUT reactions, the sequences of DNA at these sites had to be complementary to the pcPNA1/pcPNA2 combination. The recognition of a mismatch at 1823 (due to the change of C/G to A/T or G/C) was also satisfactory, although small bands assignable to invasion complexes were detectable (lanes 6 and 8).

In contrast to these satisfactory recognitions, the change of C/G1823 to T/A provided a rather stable invasion complex (lane 7 of Figure 5b). As described above, a wobble base pair is formed between the T1823 in the upper strand of this DNA and the G in pcPNA1, and this interaction provides additional stability to the invasion complex. Accordingly, scission by the ARCUT is rather efficient here (see lane 7 of Figure 3). The base pair at site 1821 (at the end of the flanking region) was hardly recognized, and the invasion complex was efficiently formed irrespective of the kind of base pair (lanes 3−5). The terminal portions in the flanking region of the double-duplex invasion complex breathe rather vigorously and are unfavorable for strict recognition.

All of these results are completely consistent with the results for the DNA scission by the ARCUT in Figures 2 and 3. The site-selective scission occurs only when the invasion complex is effectively formed. With the completely complementary pcPNA strands, both the invasion and DNA scission are efficient. With one or two mismatches between the pcPNA strands and the DNA, the invasion efficiency is greatly decreased, and accordingly, the scission becomes inefficient. Thus, the scission fidelity of an ARCUT is governed by the efficiency of invasion complex formation.

Analysis of the Thermal Stability of DNA/PNA Duplex Formation Relevant to the Invasion Process. Table 1 lists the T_m values for the DNA/PNA duplexes that are relevant to the invasion complexes formed in the ARCUT reactions. Each of the 15-mer portions of the two strands of DNA in the invasion site was combined with a 15-mer PNA having zero or one mismatch with respect to the DNA strand. The T_m values were measured in the presence of 100 mM NaCl, where the ARCUT reactions in Figures 2 and 3 were achieved. As expected, the fully matching PNA/DNA pairs showed the highest T_m values of 78.2 and 83.9 °C. When a mismatch existed at position 1829 (near the middle of the double-invasion region), the T_m values of the two DNA/PNA duplexes were substantially decreased. The differences between these T_m values and those for the fully matching DNA/PNA combinations (i.e., the ΔT_m values) were 5.9−13.4 °C, depending on the kind of mismatch introduced. Here, one mismatch was incorporated into each of the DNA/PNA duplexes, and these mismatches destabilized the invasion complex cooperatively. With a mismatch at site 1826 (the end of double-invasion region), the ΔT_m values for the duplexes between the upper DNA strands and the PNA are also quite large (7.6−12.6 °C). Single base-pair changes were clearly discriminated, although the corresponding ΔT_m values for the lower DNA strands were smaller (0.4−1.3 °C). Thus, when a single base-pair change is made in the double-invasion region, the invasion complex is almost completely destroyed. For a single base-pair change at site 1823 (in the middle of the single-invasion region), the mismatch-induced destabilization of the duplex of one DNA strand was also notable (the ΔT_m values were 6.6−8.6 °C). However, the other DNA strand remained single-stranded irrespective of the presence or absence of the single base-pair change. Accordingly, the destabilization of the invasion complex due to the base-pair change was not so significant, and thus, the mismatch recognition in double-duplex invasion for this case is less strict than that in double-invasion region. The mismatch at the end of the single-invasion region (position 1821) hardly decreases the stability of the duplex (the ΔT_m values were 0.2−0.5 °C). Thus, the invasion complexes are efficiently formed in spite of the mismatches.

All of these results are completely consistent with the fidelity of the ARCUT. When the DNA/PNA duplexes are unstable, the energy gain due to the formation of these heteroduplexes cannot sufficiently compensate for the energy loss due to the destruction of the DNA/DNA duplex, and formation of the invasion complex is inefficient (it should be noted that the double-duplex invasion is accompanied by local dissociation of the DNA/DNA duplex). Accordingly, scission by the ARCUT hardly occurs.

Essential Roles of NaCl for High Fidelity of the ARCUT. As shown previously,[13] addition of considerable amounts of NaCl (e.g., 100 mM) to the reaction mixtures was necessary for the ARCUT to achieve clear-cut site-selective DNA scission. Otherwise, nonspecific DNA scission by Ce(IV)/EDTA occurred

Table 1. Melting Temperatures of DNA/PNA Duplexes at the Invasion Site

(a) upper DNA/pcPNA1 duplex

Base-pair change pattern		Sequence of DNA (5'-3')	T_m (°C)	ΔT_m (°C)
Fully-matched DNA		5′-TTCATCATCAGTAAC-3′	78.2	–
C/G1829	**A**/T	5′-TTCATCAT**A**AGTAAC-3′	66.3	11.9
	T/A	5′-TTCATCAT**T**AGTAAC-3′	71.2	7.0
	G/C	5′-TTCATCAT**G**AGTAAC-3′	64.8	13.4
C/G1826	**A**/T	5′-TTCAT**A**ATCAGTAAC-3′	66.2	12.0
	T/A	5′-TTCAT**T**ATCAGTAAC-3′	70.6	7.6
	G/C	5′-TTCAT**G**ATCAGTAAC-3′	65.6	12.6
C/G1823	**A**/T	5′-TT**A**ATCATCAGTAAC-3′	69.6	8.6
	T/A	5′-TT**T**ATCATCAGTAAC-3′	71.6	6.6
	G/C	5′-TT**G**ATCATCAGTAAC-3′	70.0	8.2
T/A1821	**A**/T	5′-**A**TCATCATCAGTAAC-3′	77.7	0.5
	G/C	5′-**G**TCATCATCAGTAAC-3′	78.0	0.2
	C/G	5′-**C**TCATCATCAGTAAC-3′	77.9	0.3

(b) lower DNA/pcPNA2 duplex

Base-pair change pattern		Sequence of DNA (5'-3')	T_m (°C)	ΔT_m (°C)
Fully-matched DNA		5′-TACGGGTTACTGATG-3′	83.9	–
C/G1829	A/**T**	5′-TACGGGTTACT**T**ATG-3′	77.9	6.0
	T/**A**	5′-TACGGGTTACT**A**ATG-3′	77.7	6.2
	G/**C**	5′-TACGGGTTACT**C**ATG-3′	78.0	5.9
C/G1826	A/**T**	5′-TACGGGTTACTGAT**T**-3′	83.5	0.4
	T/**A**	5′-TACGGGTTACTGAT**A**-3′	83.1	0.8
	G/**C**	5′-TACGGGTTACTGAT**C**-3′	82.6	1.3

(see Figure S2 in the Supporting Information). It was proposed that the salt stabilizes the DNA duplex and suppresses undesired scission at sites other than the target one.

In order to clarify the roles of the salt more precisely, invasion complex formation in the absence of NaCl was investigated using gel-shift assays (Figure 6). Surprisingly, the mismatch-recognizing activity in the invasion process was very poor under these conditions. Even when one base pair in the central double-invasion region (at position 1826, 1827, 1828, or 1829) was changed to another base pair, resulting in the introduction of two mismatches between the DNA strands and the pcPNA additives, double-duplex invasion in most cases occurred to a notable extent (Figure 6a). Similar results were obtained when one base pair in the flanking single-invasion region was changed (Figure 6b).[29] These results indicate that in the absence of NaCl, mismatch recognition in double-duplex invasion is never strict enough to differentiate single-base-pair alteration at the target site. When NaCl is absent, the DNA/DNA duplex is intrinsically unstable, and thus, even unstable invasion complexes involving mismatches can be competitively formed. Only when a considerable amount of NaCl is added to the reaction mixtures is the formation of invasion complexes involving mismatches prevented and the fidelity of the ARCUT satisfactorily high. Thus, the NaCl plays the following two essential roles in DNA scission by the ARCUT: (i) promotion of sequence recognition in double-duplex invasion and (ii) suppression of nonspecific DNA scission by Ce(IV)/EDTA through stabilization of the DNA duplex. It is noteworthy that the fidelity of the ARCUT is also sufficiently high when 100 mM KCl is added to the mixture in place of 100 mM NaCl (data not presented).

Conclusion

Recently developed artificial restriction DNA cutters (ARCUTs) show site-selective scission of double-stranded DNA substrates only when the target site is completely complementary to the pcPNA additives. In the double-duplex invasion complex used in this study, a 5 bp single-invasion region flanked each side of the central double-invasion region (10 bp).[30] According to the analysis in this paper, the recognition by the ARCUT of base-pair changes in the central 10 bp region is complete, showing the formation of strong Watson−Crick base pairs there. Even the potential formation of wobble pairs is strictly excluded. In the flanking single-invasion regions, however, the interactions are weaker, and only two or three base pairs in each of the franking regions are precisely recognized. Accordingly, this ARCUT can recognize an ~14−16 base-pair sequence strictly. Statistically, this scission site appears only once in every 4^{14}−4^{16} [$(2.7-43) \times 10^8$] base-pair sequences, which is comparable to the size of many genomes. The specificity can be improved, if necessary, by increasing the length of the central double-invasion region. Thus, the use of ARCUTs has great feasibility as a

(29) The gel mobilities of the invasion complexes in Figure 6 differed from lane to lane. It is probable that the magnitude of the bending of the substrate DNA induced by the invasion is dependent on the kind of mismatch incorporated at the invasion site.

(30) It should be noted that the invasion complex has pseudo-C_2 symmetry when the difference in sequence is neglected (see Figure 1).

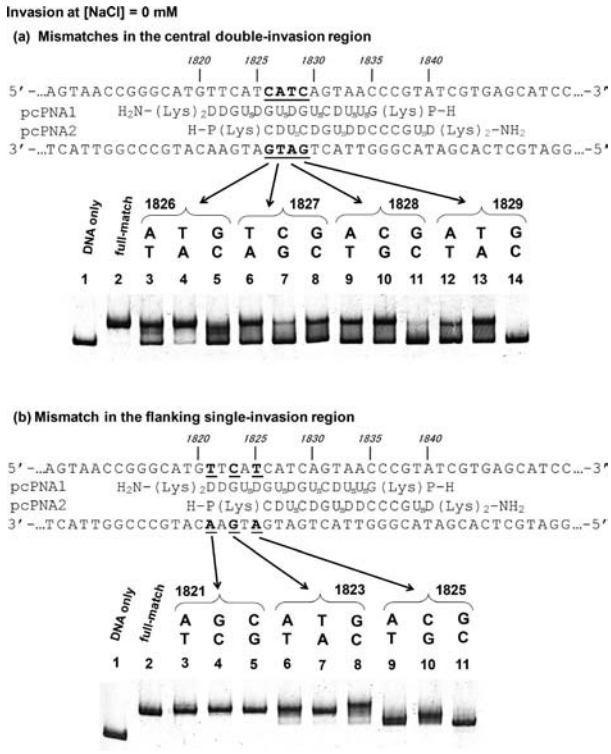

Figure 6. Gel-shift assays for the formation of invasion complexes in the absence of NaCl. (a) Lane 1, DNA only; lane 2, fully matched DNA; lanes 3−14, one base pair in the central double-invasion region (underlined) was changed to another base pair as indicated. (b) Lane 1, DNA only; lane 2, fully matched DNA; lanes 3−11, one base pair in the flanking single-invasion region (underlined) was changed to another base pair. The concentrations of the reagents are the same as those described for Figure 2, except that [NaCl] = 0 mM.

powerful tool for the manipulation of genomic DNAs, which are too huge to be treated by naturally occurring restriction enzymes. This high fidelity of ARCUTs is achieved under rather high salt concentrations (e.g., 100 mM NaCl or KCl), which are relevant to the conditions in cells.

The fidelity in the site-selective scission by ARCUTs is governed by the fidelity in the PNA invasion process. Accordingly, most of the conclusions presented above will be applicable to the fidelity analysis in various enzymatic and nonenzymatic reactions using double-duplex invasion.

Experimental Section

Synthesis of pcPNAs. All of the pcPNAs were prepared by Boc chemistry, purified by reversed-phase HPLC, and characterized by MALDI-TOF mass spectrometry (Bruker, AutoFLEX; the MALDI-TOF data are presented in Table S1 in the Supporting Information). The Boc-protected pcPNA D and U_s monomers were synthesized according to the literature.[12] In order to introduce a monophosphate group to the N-terminus of pcPNA, *N*-Fmoc-*O*-benzyl-L-phosphoserine (Novabiochem) was reacted in the final step of the pcPNA synthesis.

Preparation of DNA Substrates. The parent fully matched DNA substrate (408 bp, T1651−T2058 of pBR322 plasmid DNA) was synthesized by PCR using the following two primers: 5′-TGCACCATTATGTTCCGGATCTG-3′ and 5′-AAGCTCATCAGCGTGGTCGTG-3′. In order to alter one base pair in this DNA, mutated double-stranded DNA was first prepared from pBR322 by overlapping PCR using the corresponding primers and then inserted into pBR322 by a conventional method. After cloning in JM109 (Toyobo) was performed, the plasmid was purified using a QIAprep Spin Miniprep Kit (Qiagen). The T1651−T2058 portion of the resultant mutated plasmid was amplified with the two primers presented above.

DNA Scission by the ARCUT. The invasion complexes were formed by mixing the DNA substrate with 1:1 mixtures of the pcPNA additives at 50 °C and pH 7.0 for 1 h under the conditions [DNA] = 20 nM, [pcPNA1] = [pcPNA2] = 100 nM, and [HEPES] = 5 mM and then adding NaCl to a final concentration of 100 mM. The DNA scission was started by adding Ce(IV)/EDTA complex. Scission conditions: [DNA] = 20 nM, [pcPNA1] = [pcPNA2] = 100 nM, [HEPES] = 5 mM, [NaCl] = 100 mM, and [Ce(IV)/EDTA] = 50 μM at pH 7.0 and 50 °C for 14 h. After the reaction, a *N*,*N*,*N*′,*N*′-ethylenediaminetetrakis(methylenephosphonic acid) aqueous solution adjusted to pH 7.0 was added to the mixture to stop the reaction. The mixture was further incubated at 50 °C for 1 h and then subjected to 5% nondenaturing PAGE. The bands were stained with GelStar (Lonza) and analyzed on a FujiFilm FLA-3000G fluorescent imaging analyzer.

Gel-Shift Assay for the Formation of the Invasion Complex. The DNA substrate and 1:1 mixtures of the pcPNA additives were incubated at 50 °C and pH 7.0 for 1 h, as described above. These specimens were subjected to the assay either directly or after addition of 100 mM NaCl. The gel-shift assay was carried out using 5% nondenaturing PAGE. The bands were stained with GelStar and quantified with a FujiFilm FLA-3000G fluorescent imaging analyzer.

Measurement of T_m Values for DNA/PNA Duplexes. Absorbance-versus-temperature curves were measured at 260 nm with a JASCO V-530 UV−vis spectrophotometer. The heating rate was 1.0 °C/min. Measurement conditions: [DNA] = [pcPNA] = 1 μM, [HEPES] = 5 mM, and [NaCl] = 100 mM.

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Supporting Information Available: Entire sequence of the 408 bp fully-matched DNA substrate, nondenaturing PAGE assay for DNA scission by ARCUT in the absence of NaCl, and MALDI-TOF MS data for the two PNAs used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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