Highly efficient strand invasion by peptide nucleic acid bearing optically pure lysine residues in its backbone

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ABSTRACT
Chiral PNA monomers (PNA = peptide nucleic acid), in which nucleobases are attached to N-(aminoethyl)-D-lysine, were introduced to PNAs bearing pseudo-complementary nucleobases (2,6-diaminopurine and 2-thiouracil). When these highly cationic PNAs targeted double-stranded DNA, they invaded there much more efficiently than conventional pseudo-complementary PNAs composed of achiral PNA monomers. Although introduction of N-(aminoethyl)-D-lysine backbone was effective for promotion of strand invasion, L-isomer never promote it. Simple incorporation of lysine groups to the termini of PNA was also ineffective, indicating that introduction of positive charges into PNA backbone is important. Even highly G-C rich sequence, which conventional pseudo-complementary PNAs never invade, was successfully targeted based on this strategy.

INTRODUCTION
Many chemists and biochemists have been trying to develop artificial tools for sequence specific recognition of DNA, since they can be applied to regulation of biological functions in vivo and in vitro. Peptide nucleic acid (PNA), in which nucleobases are attached to α-nitrogen atom of N-(aminoethyl)-glycine, forms highly stable duplex with complementary DNA or RNA through Watson-Crick hydrogen bond. PNA is characterized by its ability to invade double-stranded DNA. When two PNA strands are complementary with both strands of double-stranded DNA and formation of PNA-PNA duplex is suppressed by incorporation of pseudo-complementary nucleobases (2,6-diaminopurine (D) and 2-thiouracil (U),) invasion complex called as “double-duplex invasion” is formed (Fig. 1a). Thus, double-duplex invasion is applicable to recognition of the specific site of double-stranded DNA. For example, we successfully prepared a man-made site-selective DNA cutter by combining Ce(IV)/EDTA complex (single-stranded specific DNA cutter) and PNA (Fig. 1b). However, double-duplex invasion of PNA never occurs in highly G-C rich sequences, since pseudo-complementary monomers of G and C are not available to date. New methodology other than pseudo-complementarity is necessary to solve this problem. In this study, positive charges are introduced to pseudo-complementary PNA by the use of chiral PNA monomers bearing N-(aminoethyl)-D-lysine backbone (Fig. 1c). These modified PNAs invade the target site in double-stranded DNA much more efficiently than conventional pseudo-complementary PNA.

RESULTS AND DISCUSSION
Strategy for promotion of strand invasion by using chiral PNA monomers is schematically presented in Fig. 2.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** (a) Double-duplex invasion of pseudo-complementary PNA into DNA. In these PNAs, 2-thiouracil (U) and 2,6-diaminopurine (D) are used in place of thymine and adenine. (b) Artificial restriction DNA cutter composed of Ce(IV)/EDTA and PNA. (c) Lys-PNA monomer used in this study.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2** Strategy for efficient strand invasion by the use of highly cationic PNA bearing chiral PNA monomer.
Formation of PNA-PNA duplex is suppressed by electrostatic repulsion induced by the lysine side-chains. On the other hand, PNA-DNA duplexes are stabilized by electrostatic attraction. As the result, strand invasion should be promoted by these chemical modifications.

Sequences of PNAs used in this study are shown in Table 1. Here, chiral monomer units are distinguished from conventional monomers by underlines (e.g., A vs. A and T vs. T). PNA1 and PNA2 bear one L-lysine residue (without nucleobase) at N-termini and another one at C-termini, and total charges in each strand are +3 at neutral pH. In PNA3 and PNA4, two chiral monomers are incorporated, and then total charges are +5. For the purpose of comparison, two lysine residues are attached to N- and C-termini in PNA5 and PNA6 (totally five positive charges in each strand).

Table 1 Sequences of pcpPNAs used in this study.

<table>
<thead>
<tr>
<th>PNA</th>
<th>sequence (bases)</th>
<th>chiral unit</th>
<th>cation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNA1</td>
<td>H-N(Lys)DGTGDUCATU(Lys)=-H</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>PNA2</td>
<td>H-(Lys)UCAGCDGTAD(Lys)=-NH</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>PNA3</td>
<td>H-N(Lys)DGTGDUCATU(Lys)=-H 2 (or Lys)</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>PNA4</td>
<td>H-N(Lys)UCAGCDGTAD(Lys)=-NH 2 (or Lys)</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>PNA5</td>
<td>H-N(Lys)DGTGDUCATU(Lys)=-H</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>PNA6</td>
<td>H-(Lys)UCAGCDGTAD(Lys)=-NH</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

a) D and U bear 2,6-diaminopurine and 2-thiouracil in place of conventional bases. Chiral units are underlined (e.g., A, T).

Upon addition of a pair of PNA3/PNA4 bearing two chiral PNA units derived from N-(aminoethyl)-D-lysine, a new band of smaller mobility appeared in the gel (lane 3, Fig. 3). Apparently, invasion complex was successfully formed by the use of these PNAs. Invasion efficiency of these PNAs is higher than PNA1/PNA2, which has no chiral PNA units (compare lanes 2 and 3). In contrast to notable promotion by incorporation of N-(aminoethyl)-D-lysine residues, its L-isomer never promote strand invasion. In the case of PNA5/PNA6 combination, serious aggregation took place and strand invasion did not occur although the numbers of positive charges in them are same as those in PNA3/PNA4 combination (+5 in each strand). Crucial importance of N-(aminoethyl)-D-lysine backbone is clearly evidenced. Furthermore, even highly G-C rich sequence (5'-GCGAGCGGCG-3'), which achiral PNAs never invade, could be targeted through introduction of N-(aminoethyl)-D-lysine backbones.

In order to shed light on the mechanism of efficient strand invasion, melting temperatures (Tm) of PNA-PNA duplexes and PNA-DNA duplexes were measured. Tm of PNA3-PNA4 duplex involving two A-T pairs (40.6°C) is notably lower than those of PNA1-PNA2 duplex (52.5°C; two A-T pairs in place of the two A-T pairs) and PNA5-PNA6 duplex (51.5°C; four additional lysine residues and two A-T pairs in place of the two A-T pairs). The stability of PNA-DNA hetero-duplex composed of PNA3 (76.0°C) or PNA4 (71.4°C) is much higher than that of PNA1 (60.0°C) or PNA2 (66.2°C), but nearly equal to that of PNA5 (77.7°C) or PNA6 (73.9°C). Thus, introduction of positive charges highly stabilize DNA-PNA heteroduplexes, but the positions and the modes of incorporation hardly affect the stability of PNA-DNA duplex. These results indicate that efficient strand invasion of PNA3/PNA4 combination is ascribed to both destabilization of PNA-PNA duplex and stabilization of PNA-DNA duplexes. Interestingly, incorporation of N-(aminoethyl)-lysine backbone is essential for destabilization of PNA-PNA duplex, and attachment of several lysine residues at PNA termini is ineffective for this purpose.

CONCLUSION

Chiral PNA monomers bearing N-(aminoethyl)-D-lysine backbone were introduced to pseudo-complementary PNAs, and strand invasion into double-stranded DNA was successfully promoted. These modified pcpPNAs invade even G-C rich sequences, which are never targeted by achiral pseudo-complementary PNAs. Promotion of strand invasion by chiral PNA monomers should be applicable to various purposes, and these attempts are now under way in our laboratories.

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REFERENCES