Chemistry Letters, 2024, **53**, upae234
https://doi.org/10.1093/chemle/upae234
Advance access publication 10 December 2024

Letter

Recognition of mismatched sites in double-stranded DNA by a pair of partially noncomplementary peptide nucleic acids

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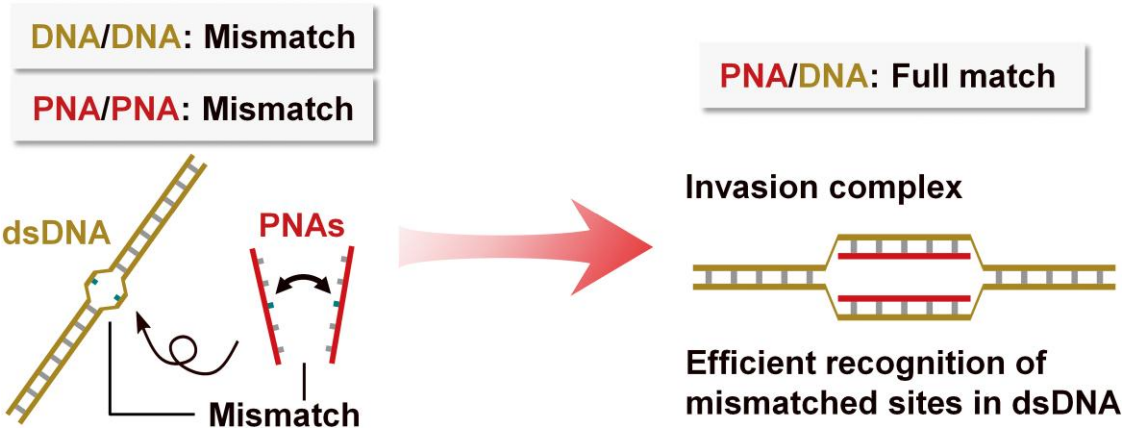
Abstract

We have successfully achieved efficient recognition of mismatched sites in double-stranded DNA through the formation of an invasion complex by using partially noncomplementary peptide nucleic acids (PNAs). Owing to mismatches between 2 PNAs used for invasion, the undesired PNA/PNA duplex, which inhibits invasion complex formation, was destabilized. This approach overcame an inherent challenge in PNA invasion, in particular, undesired PNA/PNA duplex formation resulting from PNA complementarity, thereby enhancing overall invasion efficiency.

Keywords: DNA recognition, mismatch, peptide nucleic acid.

Graphical Abstract



Peptide nucleic acid (PNA; Fig. 1a),[1] a synthetic DNA analog, exhibits exceptionally high nucleic acid binding affinity among artificial nucleic acids.[2] PNA can target specific sequences even in double-stranded DNA (dsDNA) by forming a unique PNA/DNA complex.[3] Two PNA strands invade dsDNA and bind to complementary DNA sequences, forming PNA/DNA duplexes that are more thermodynamically stable than the corresponding DNA/DNA duplexes (Fig. 1b).[4] This process, known as double-duplex invasion,[5] utilizes the high DNA binding affinity of PNA,[4] enabling direct recognition of DNA in its double-stranded form. Due to these characteristics, PNA is considered a leading molecule among artificial nucleic acids in invasion studies and is also expected to be applied in targeting specific gene sequences of genomic DNA.[6]

While PNA invasion is a very attractive methodology, there is a fundamental issue that the PNAs used for invasion are complementary to each other. The PNA/PNA duplex formed by these PNAs is even more stable than the corresponding PNA/DNA duplex,[7] and this undesired PNA/PNA duplex formation competes with invasion complex formation. To prevent the formation of undesirable PNA/PNA duplexes, nucleobase modifications have been introduced into PNA.[5,8] This special nucleobase modification, called pseudo-complementary nucleobases, decreases the stability of the PNA/PNA duplex, while

[Received on 14 November 2024; revised on 3 December 2024; accepted on 3 December 2024; corrected and typeset on 13 December 2024]

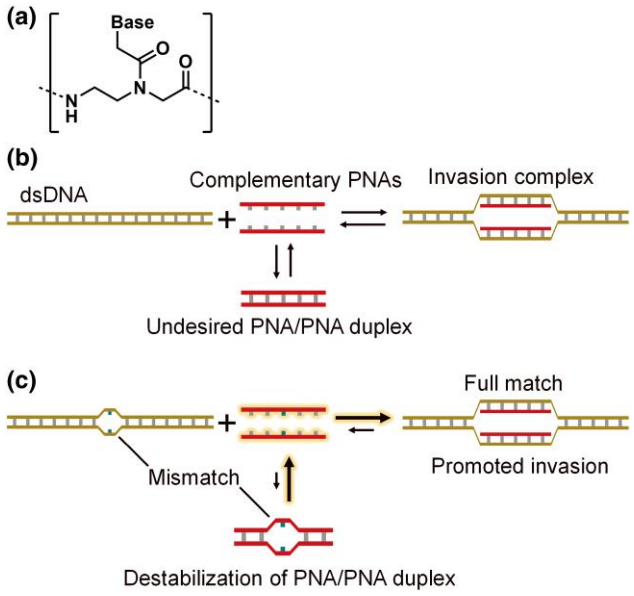


Fig. 1. a) Chemical structure of PNA. b) DNA recognition by PNA through the formation of a double-duplex invasion complex. c) The presence of a mismatch reduces competing PNA/PNA duplex formation and promotes the formation of the invasion complex.

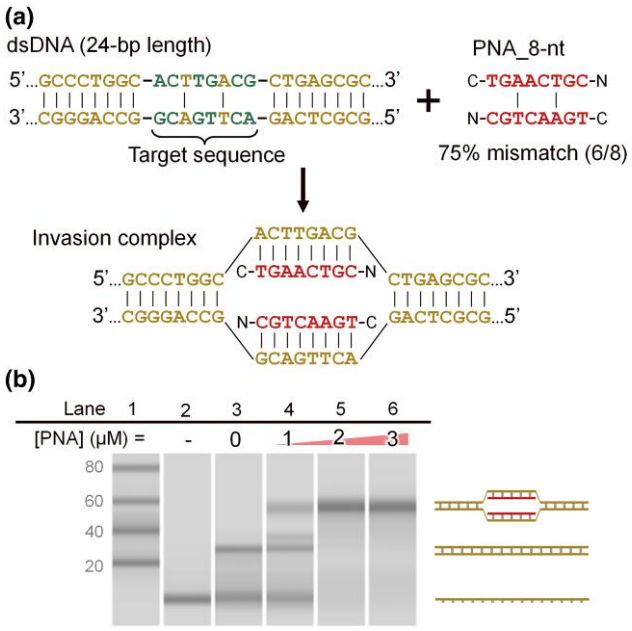


Fig. 2. a) Invasion complex formation between a 24 bp dsDNA target containing mismatches and its complementary PNA. PNA_8-nt hybridizes to both DNA strands, forming the invasion complex. b) EMSA showing that an invasion complex is formed between dsDNA and PNA containing mismatched base pairs. Lane 1: 20 bp DNA ladder marker; lane 2: 24-nt ssDNA; lane 3: 24 bp target dsDNA; lanes 4 to 6: dsDNA with PNA_8-nt. Conditions: [DNA] = 1 μM, [PNA] = 1 to 3 μM, [HEPES (pH 7.0)] = 5 mM, and [NaCl] = 100 mM at room temperature for 10 min.

maintaining the high stability of the PNA/DNA duplex. Without the pseudo-complementary nucleobase modification, the invasion complex does not form because the stable PNA/PNA duplex preferentially forms instead.[5,9]

In this study, we addressed the major intrinsic problem in PNA invasion, namely, that PNAs are complementary to each other, by targeting the mismatch sites in dsDNA. In targeting dsDNA containing mismatches, mismatches are also introduced between the two PNAs used for invasion (Fig. 1c). Destabilization of the PNA/PNA duplex caused by mismatches reduces competing duplex formation. By targeting the mismatch sites, the PNA design can be changed from fully matched to mismatched, facilitating effective invasion toward the target DNA. Additionally, destabilized dsDNA due to mismatches can also contribute to the efficient invasion complex formation. Consequently, mismatched dsDNA represents a promising target for invasion, and we evaluated the invasion efficiency at these mismatched sites.

Mismatch sites naturally occur in genomic DNA due to replication errors and chemical/physical damage under physiological conditions.[10] These mismatches can lead to harmful point mutations associated with disease, and the development of techniques to detect specific mismatched base pairs in dsDNA is important for diagnosing and treating such conditions. Various studies have extensively examined mismatches in dsDNA. For selective binding to mismatched base pairs, several small functional molecules and metal complexes have been developed.[11,12] For example, naphthyridine dimer and its analogs form hydrogen bonds with mismatched nucleobases and can recognize specific pair of mismatch bases.[11a,12b,12g] Rhodium-containing metal complexes can recognize mismatch sites through intercalation in dsDNA and can induce DNA cleavage at mismatch site.[12c] Compared to approaches employing small molecules that recognize structural features unique to mismatches, PNA is expected to exhibit high selectivity by directly recognizing specific mismatched sequences based on its complementarity. Thus, developing a mismatch recognition method based on PNA invasion could establish a novel modality for advanced mismatch studies and therapeutic strategies for related diseases.

First, we employed a dsDNA with an 8 bp noncomplementary region in the middle as a model of mismatched DNA (Fig. 2a and Supplementary Table S1). This target DNA was designed to be recognized by a single PNA strand for simplicity, unlike the typical setup in which 2 complementary PNAs are employed to form an invasion complex (Supplementary Table S2 and Supplementary Fig. S1). The PNA was designed to contain 6 mismatches out of 8 nucleotides when the PNA forms a self-duplex structure. The preannealed 24 bp DNA was incubated with an 8 nt PNA, and the invasion efficiency was evaluated using an electrophoretic mobility shift assay (EMSA) on a microchip electrophoresis system (SHIMADZU, MCE-202 MultiNA). Generally, the invasion complex exhibits lower mobility than the target DNA. As shown in Fig. 2b, the intensity of bands with reduced mobility increased with higher PNA concentrations. When there is an excess of PNA, only a single band with low mobility is present, which corresponds to the desired invasion complex. These results confirm that target DNA with a noncomplementary region can form an invasion complex similarly to that formed with fully complementary dsDNA targets. At a PNA concentration of 2 μM (lane 5), where 2 PNAs are available for each DNA strand, the band corresponding to the target DNA disappears. In most cases, an excess amount of nucleobase-modified PNA is required for efficient invasion complex formation. In contrast, in this case, using a 1:1 molar ratio of PNA and DNA achieved 100% invasion efficiency. Thus, intentional introduction of mismatches between PNAs effectively enhances invasion efficiency.

To assess the effect of mismatch percentage on invasion complex formation, a PNA with a lower mismatch percentage

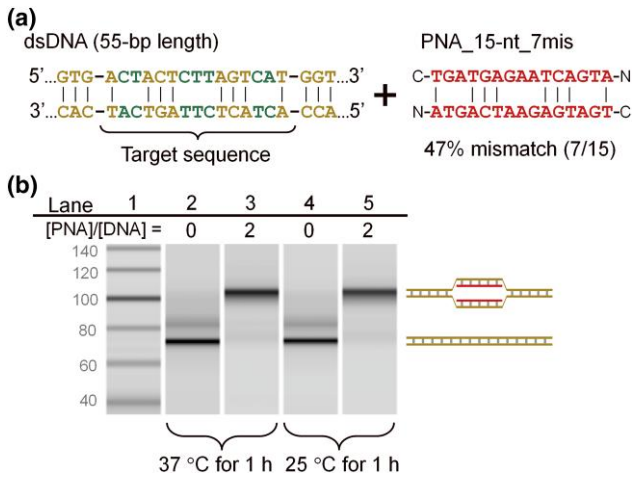


Fig. 3. a) A 55 bp dsDNA target containing 7 mismatched base pairs within target sequence and its complementary PNA (PNA_15-nt_7mis). b) EMSA analysis of the invasion complex formation between dsDNA and PNA containing 7 mismatches.[13] Lane 1: 20 bp DNA ladder marker; lanes 2 and 4: 55 bp target dsDNA; lanes 3 and 5: dsDNA with PNA_15-nt_7mis. Conditions: [DNA] = 500 nM, [PNA] = 1 μM, [HEPES (pH 7.0)] = 5 mM, and [NaCl] = 100 mM at 37 °C or 25 °C for 1 h.

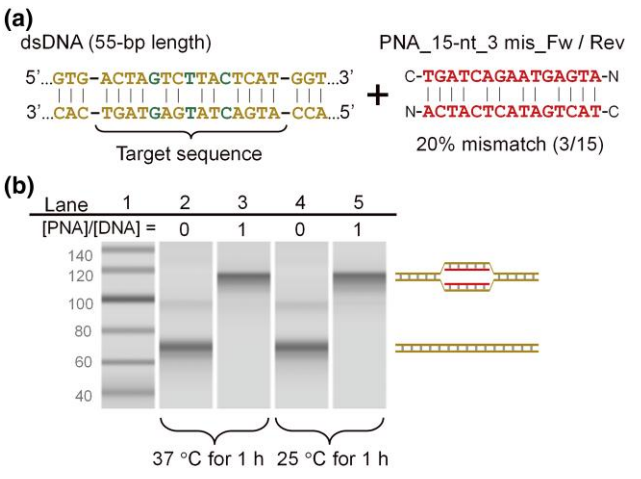


Fig. 4. a) A 55 bp dsDNA target and PNAs (PNA_15-nt_3mis_Fw and PNA_15-nt_7mis_Rev) containing 3 mismatched base pairs. b) EMSA analysis of the invasion complex formation between dsDNA and PNAs containing 3 mismatches.[13] Lane 1: 20 bp DNA ladder marker; lanes 2 and 4: 55 bp target dsDNA; lanes 3 and 5: dsDNA with PNA_15-nt_3mis_Fw and PNA_15-nt_3mis_Rev. Conditions: [DNA] = 500 nM, [each PNA] = 500 nM, [HEPES (pH 7.0)] = 5 mM, and [NaCl] = 100 mM at 37 °C or 25 °C for 1 h.

was examined. In this experiment, we used a 15 nt PNA and designed its sequence to introduce 7 mismatched base pairs (47% mismatch) in the target site. In the previous study with 24 bp DNA, a band corresponding to single-stranded DNA was observed. Therefore, the target DNA length was increased to 55 bp to enhance stability. After the PNA and DNA mixture was incubated, invasion complex formation was evaluated (Fig. 3a). Despite the reduction in mismatch percentage from 75% (6 mismatched bases out of 8 in the previous examination) to 47%, invasion complex formation was still observed, indicating the robustness of PNA binding to mismatched sites (Fig. 3b). These findings further support the effectiveness of PNA in selectively recognizing mismatched DNA sequences.

We further reduced the number of mismatches and examined the DNA recognition of PNA containing 3 mismatches out of 15 base pairs (Fig. 4a). Here, we used 2 different PNA strands, in accordance with established procedures for double-duplex invasion, to bring the situation closer to practical conditions. The target DNA band disappeared when an equimolar amount of PNA was used, indicating that the PNA strands fully bound to and recognized the target DNA (lanes 3 and 5 in Fig. 4b). Thus, even with a sequence containing 3 mismatched base pairs out of 15, we demonstrated that PNA can effectively recognize mismatched DNAs. We further evaluated the formation of invasion complexes at shorter incubation times of 10 min and confirmed complete formation of invasion complex (Supplementary Fig. S2).

To better understand the contribution of introducing mismatches to the destabilization of the PNA/PNA duplexes, we measured the T_m values of PNA/PNA duplexes with 7 and 3 mismatches. The duplex with 7 mismatches exhibited T_m values of 59 °C for cooling and 65 °C for heating, while the duplex with 3 mismatches exhibited T_m values of 56 °C for cooling and 66 °C for heating. This hysteresis is likely caused by the presence of multiple mismatches in the sequence. Both T_m values were lower than the reported value for a fully matched 15 bp PNA/PNA duplex, which exceeded 90 °C.[9a] Although T_m values are known to depend on the sequence, the substantial difference between the fully matched and mismatched sequences is evident. These results support our concept that mismatch-induced duplex destabilization is effective in promoting PNA invasion.

In conclusion, the presence of mismatches between PNAs weakens the interaction between PNA strands used for invasion and simultaneously destabilizes the target DNA. These instabilities promote the formation of invasion complex and enhance overall invasion efficiency. Efficient invasion is facilitated when both dsDNA and PNA duplex contain mismatches, respectively, while maintaining full complementarity between the target DNA and invading PNA strands. By targeting mismatched sites in dsDNA, we have eliminated a major problem in PNA invasion, namely, the undesired formation of a PNA/PNA duplex due to complementarity between PNA strands.

Our findings underscore the potential of mismatched sequences as effective targets for PNA invasion, supporting a promising strategy for selective mismatch recognition in dsDNA. Unlike previous studies using small molecules for mismatch recognition, our approach utilizes specific base-pair complementarity between PNA and mismatched sequences in dsDNA. This selectivity, driven by specific nucleobase pairing, enables precise sequence recognition and allows flexibility in designing PNA for various target sequences. The mismatch detection system proposed in this study could be useful for detecting multiple mismatches, such as those in dsDNA or dsRNA produced by trinucleotide repeat regions. Furthermore, given that PNA can be readily modified with fluorescent dyes, fluorescence-based detection systems such as Förster (or Fluorescence) Resonance Energy Transfer could be easily adapted to identify mismatch sites. By combining our method with advanced diagnostic technologies beyond electrophoresis, it is possible to establish a rapid and sensitive detection system for mismatched DNA.

Acknowledgments

M.S. would like to thank the *Nagoya University Interdisciplinary Frontier Fellowship*, supported by JST and

Nagoya University. We thank Dr. Hiromu Kashida for his valuable insights, which contributed to the development of this study.

Supplementary data

Supplementary material is available at *Chemistry Letters* online.

Funding

This work was supported by JSPS KAKENHI Grant Numbers JP22K05350 to Y.A., JP22KJ1567 to M.S. from the Ministry of Education, Culture, Sports, Science and Technology (Japan). This work was also partially supported by a National Institutes of Health grant (1R21AI180006-01) to Y.A., Foundation of Public Interest of Tatematsu to Y.A., the Noguchi Institute to Y.A., and the Iwadare Scholarship to M.S. from the Iwadare Scholarship Foundation.

Conflict of interest statement. None declared.

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13. The salt concentration in the buffer causes a slight shift in the mobility of the bands. Under low salt conditions, electrophoresis confirmed that the dsDNA targets match the size of the DNA ladder marker (data not shown).