Polycation-accelerated strand exchange (PASE) for SNPs typing

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ABSTRACT
In the previous study (Chem. Eur. J., 7, 176 (2001), J. Am. Chem. Soc., 124, 12676 (2002)), we demonstrated that the cationic comb-type copolymer (PLL-g-Dex, CCC) composed of a cationic poly(L-lysine) backbone and water-soluble side chains of dextran accelerated the DNA strand exchange reaction between double helical DNA and its homologous single strand. In this study, Polycation-Accelerated Strand Exchange (PASE) assay was applied to discriminate single-base mismatch in DNA sequence such as single nucleotide polymorphisms (SNPs). We could discriminate the 20mer DNA with a single-base mismatch from the full matched DNA with high efficacy by PASE.

INTRODUCTION
Increasing demands have been placed on the rapid, reliable and inexpensive genotyping method to detect subtle differences in DNA sequences such as single nucleotide polymorphisms (SNPs). To date various methods for highly sensitive analysis of nucleic acid sequences have been reported. Most popular principle for genetic diagnosis is probe hybridization between sample and probe single-stranded nucleic acids. However, discrimination of single-base mismatch in nucleic acid sequence has been rarely achieved or limited to short sequences because thermodynamic difference between the resulting hybrids with and without single-base mismatch was not sufficient. Careful optimization in probe design and operating conditions was needed, making the method difficult for high-throughput applications. In this study, we have focused on strand exchange reaction between double stranded (ds) DNA probe and single-stranded (ss) target nucleotides as a novel format for detection of single-base mismatches. Compared with the conventional probe hybridization between ss DNAs, not only thermodynamic stabilities of the resulting duplex but also the kinetics of the reaction was expected to reflect the presence of a mismatched base. However a drawback of the assay could be an extremely slow rate of the strand exchange reaction. We have interested in artificial polymer (Fig. 1b) that is capable of accelerating breakage and re-association of base pairs and acting as a nucleic acid chaperone.

Figure 1. a) Experimental schematic of single-base mismatch detection and b) Structural formula of cationic comb-type copolymer (PLL-g-Dex, CCC)
RESULTS AND DISCUSSION

Detection of single-base mismatch by PASE.

The experimental schematic of the single-base mismatch detection by PASE is depicted in Fig. 1a. We used fluorescence resonance energy transfer (FRET) to determine strand exchange reaction with the punctuality to a second. The experimental procedure is almost same as that of previous report1. Briefly, strand exchange reaction was initiated by adding a complementary non-labeled ssDNA to FITC-, TAMRA-labeled (FT-) duplex whose FITC emission was quenched by TAMRA (Fig. 1a). As reaction goes on the quenched fluorescence of FITC is recovered. We assessed the effect of single-base mismatch on the strand displacement kinetics. Time courses of the reactions are depicted in Fig. 2. While the strand exchange reaction of 20 bp ds probe even with perfectly homologous 20 mer ss target hardly proceeded in the absence of CCC, it occurred actively in the presence of CCC (Fig. 2). As expected, the single-base mismatch in the ss target significantly retarded the reaction even in the presence of CCC. Hence we can discriminate between the mismatched and the full-matched sequences within a few minutes at 37°C.

Simultaneous discrimination of mismatches on the 96-well microplate.

Figure 3. Simultaneous discrimination between ds DNA probe and ss DNA targets, having various mismatches by PASE. Sequences of ss DNA targets: 3'-AGTATTAGTCXGTATGGTGT-5', X=G(full match), T, A, C (mismatch), ds DNA probe: 3'-fluorescein isothiocyanate (FITC) labeled DNA (5'-TCATAATCACGACCACA-3'-FITC) and its complementary 5'-carboxytetramethylrhodamine (TAMRA) labeled DNA (3'-AGTATTAGTCGGTATGGTGT-5'-TAMRA).

The PASE was then challenged with diverse mismatches in various sequences. We have used a 96-well microplate to examine various single-base mismatches simultaneously. Strand exchange reactions of ds DNA probes (12 nM) with ss DNA targets (12 nM) performed in a 96-well black plate for 10 min at 25°C or 30°C or 37°C in the absence or presence of 20 nM CCC (CCC / DNA charge ratio=2) in PBS buffer. Regardless of mutation types ss targets having single-base mutation could be distinguished from the full matched ss DNAs within 10 min at 30°C (Fig. 3). Furthermore, mismatch discrimination was attained at temperatures ranging 37°C to 25°C (data not shown).

In conclusion, we could discriminate the single-base mismatch in 20mer from full matched target with high efficacy by PASE. PASE could be applied to various format of DNA analyses (ex. solid-surface or colloidal DNA arrays).

REFERENCES