Chinese Chemical Letters 26 (2015) 1031–1035

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Sensitive and enzyme-free detection for single nucleotide polymorphism using microbead-assisted toehold-mediated strand displacement reaction

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ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 30 December 2014
Accepted 7 April 2015
Available online 31 May 2015

Keywords:
Enzyme-free
Microbead-assisted
Toehold-mediated strand displacement
Point mutation

ABSTRACT

This report described a free-enzyme, convenient and inexpensive genotyping biosensor capable of detecting single nucleotide polymorphism at normal temperature based on the combination of toehold-mediated strand displacement reaction (toehold-SDR) and microbead-capture technique. The biosensor consists of a pre-hybridized strand formed by a reporter probe and a capture probe. In the presence of a mutant sequence, there is no toehold-mediated strand displacement and the reporter probe cannot be released from the pre-hybridized strand. Microbeads capture the fluorescent pre-hybridized strand through biotin–streptavidin interaction, so microbeads give out significant fluorescence signal, while there is no fluorescence in the solution. However, in the presence of a matched target, the strand displacement is effectively initiated and the reporter probe is released from pre-hybridized strand. After adding microbeads, the solution produces bright fluorescence, while microbeads have no obvious signal. Genotypes are identified conveniently according to the fluorescence intensity of the solution. The method provides a simple and inexpensive strategy to detect point mutation. Moreover, this biosensor shows the linear relationship in the range of 1–40 nmol/L and reaches a detection limit of 0.3 nmol/L.

1. Introduction

The sensitive and convenient detection of single nucleotide polymorphisms (SNPs) is important in biological studies, drug development and clinical diagnostics [1,2]. A series of SNP detection systems were developed, such as polymerase chain reaction amplification [3], gel electrophoresis [4], rolling circle amplification [5,6], strand-displacement of polymerase [7–9], locked nucleic acid (LNA)-based northern blot [10], specific enzymatic cleavage [11] and so on. However, these methods usually are enzyme-assisted complicated strategies [12]. It remains necessary and meaningful to develop a simple and enzyme-free strategy for SNPs detection at normal temperature.

The strand displacement reaction (SDR) can be initiated by a strand hybridization with the complementary single stranded overhang domains (known as toeholds) of prehybridized strands and progresses (toehold-SDR) through a branch migration process [13–15]. Toehold-SDR can take place without an enzyme at room temperature, and the kinetic rate can be controlled by adjusting the length and sequence composition of the toehold, which is used widely in analyzing SNPs as its enzyme-free, robustness, specificity and isothermality [16–18]. Li *et al.* combined gold nanoparticle with fluorescence anisotropy for the assay of SNP with the detection limit of 0.95 nmol/L based on toehold-mediated SDR [17] and Wang *et al.* combined quartz crystal microbalance and toehold-SDR for the detection of SNP with 0.3 nmol/L limit [18]. Until now, there has been no consensus for the best enzyme-free SNP detection method. There is a continuous need for new SNP detection methods to meet specific needs and situations.

In this work, we developed a novel microbead-assisted toehold-SDR biosensor to detect SNP for the first time. The biosensor consists of a pre-hybridized strand formed by a reporter probe and a capture probe. In the presence of a mutant sequence, there is no toehold-mediated strand displacement and the reporter probe cannot be released from the pre-hybridized strand. When adding microbeads, the fluorescent pre-hybridized strand is captured by

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http://dx.doi.org/10.1016/j.cclet.2015.05.036

microbeads, resulting in that the solution is no obvious fluorescence, while microbeads appear significant fluorescence. However, in the presence of a matched target, the strand displacement is effectively initiated and the reporter probe is released from pre-hybridized strand. After adding microbeads, the solution produces bright fluorescence, while microbeads have no obvious fluorescence signal. The target sequence is discriminated perfectly from the single-base mutant sequences.

2. Experimental

2.1. Materials

All synthesized and HPLC-purified sequences of oligos as depicted in Table 1 were commercially ordered from TaKaRa Bio Inc. (Dalian, China). The capture probe strand was 5′-biotin labeled sequence including 4-nt poly(T) spacer, 6-nt toehold region, and 20-nt template sequence. The single-base mismatched sequences were designed such that the mismatched site located in the sixth base of toehold region from 5′-end. The reporter probe was a 5′ FAM-labeled sequence complementary to the template strand of the capture probe. Streptavidin-coated microbeads were purchased from Sigma–Aldrich (MO, USA). All other reagents were of analytical grade. Deionized water was obtained from the Nanopure Infinity™ ultrapure water system (Barnstead/Thermolyne Corp., Dubuque, IA, USA).

Table 1
Sequence of oligos in this study.[a]

Name	5′–3′
Capture probe	Biotin – TTTTCACCTTGCCCCACAGGGCAGTAACG
Reporter probe	FAM – CGTTACTGCCCTGTGGGGC
Target I	CGTTACTGCCCTGTGGGGCAAGGTGA
Target II	CGTTACTGCCCTGTGGGGCAAGGTG
Target III	CGTTACTGCCCTGTGGGGCAAGGT
Target IV	CGTTACTGCCCTGTGGGGCAAGG
Mutant T	CGTTACTGCCCTGTGGGGC**T**AGGTG
Mutant G	CGTTACTGCCCTGTGGGGC**G**AGGTG
Mutant C	CGTTACTGCCCTGTGGGGC**C**AGGTG
Random DNA	CACGTTCTGTAGTAGGTTCAAGCTCA

[a] In the capture probe, the toehold is single underlined and the template is double underlined.

2.2. Detection procedure

The proposed method employed microbeads to capture biotin-label strands to produce signal difference through biotin–streptavidin interaction. Firstly, microbeads were washed twice in PBS solution as described before [19]. The detection process involved the following two major steps: (1) adding a target into the pre-hybridized strand between a capture probe and a reporter probe to perform the toehold-SDR; (2) capturing biotin-label strands using streptavidin-coated microbeads for the effective detection. Unless specified, the reaction buffer was used containing 200 nmol/L capture probe, 200 nmol/L reporter probe, 20 mmol/L Tris–HCl pH 7.4, and 15 mmol/L $MgCl_2$. Target II was added into the reaction buffer with the final concentration as indicated, which mixed and incubated for 30 min to perform a toehold-SDR. Then, microbeads were added to the mixture and incubated for 50 min with end-to-end shaking to capture biotin-label strands. Then, samples were kept quietly for 3 min and the fluorescence for the supernatant solution and the bottom of microbeads were detected, respectively. All procedures were performed at normal temperature.

2.3. Fluorescence measurement of the solution

The fluorescence intensities were recorded using F-7000 fluorescence spectrophotometer (Hitachi, Japan). The fluorescent spectra were measured using the spectrofluorophotometer. The excitation wavelength was 490 nm, and the spectra were recorded between 500 and 600 nm. The fluorescence emission intensity was measured at 520 nm.

2.4. Fluorescent detection of microbeads

Fluorescence of the microbead was achieved with a fluorescence microscope (Leica DM IRB, Leica Corp., Germany) equipped with a CCD camera (Leica DC 300F) and the corresponding quantitative values were obtained by calculating the gray scale of fluorescent particles with the software Image J [19,20].

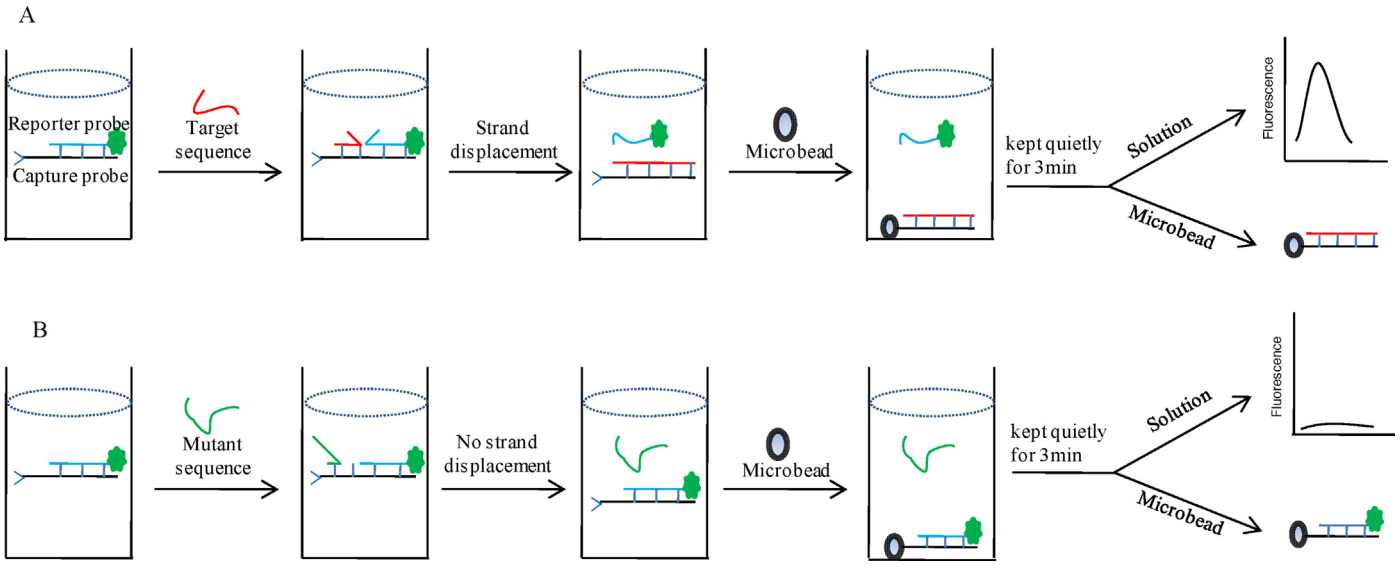

Fig. 1. The design of the microbead-assisted toehold-SDR for point mutations.