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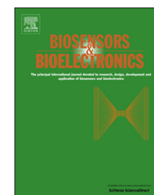

Fast and quantitative differentiation of single-base mismatched DNA by initial reaction rate of catalytic hairpin assembly

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ABSTRACT

The widely used catalytic hairpin assembly (CHA) amplification strategy generally needs several hours to accomplish one measurement based on the prevailingly used maximum intensity detection mode, making it less practical for assays where high throughput or speed is desired. To make the best use of the kinetic specificity of toehold domain for circuit reaction initiation, we developed a mathematical model and proposed an initial reaction rate detection mode to quantitatively differentiate the single-base mismatch. Using the kinetic mode, assay time can be reduced substantially to 10 min for one measurement with the comparable sensitivity and single-base mismatch differentiating ability as were obtained by the maximum intensity detection mode. This initial reaction rate based approach not only provided a fast and quantitative differentiation of single-base mismatch, but also helped in-depth understanding of the CHA system, which will be beneficial to the design of highly sensitive and specific toehold-mediated hybridization reactions.

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1. Introduction

The ability for rapid detection and differentiation of sequence-specific DNA with high selectivity and sensitivity is highly desirable in various applications of life sciences, such as point-of-care diagnosis, high-throughput pathogenic agent identification and food safety monitoring (Bi et al., 2010; Dai and Kool, 2011; Huang et al., 2012; Kang et al., 2010; Lermo et al., 2007; Nishizawa et al., 2006; Szemes and Bonants, 2005; Xie et al., 2004). To realize the highly sensitive DNA detection, assorted amplification strategies have been developed in the past decades (Duan et al., 2007; Kim and Easley, 2011; VanGuilder et al., 2008; Wang et al., 2011; Zhang et al., 2009). Isothermal amplification technique is but one promising approach as an alternative to the PCR-based amplification, which greatly simplifies the point-of-care or high throughput analysis without the need of thermo cycling apparatus (Gill and Ghaemi, 2008; Kim and Easley, 2011). Amongst the isothermal techniques, enzyme-involved methods, such as nucleic acid sequence-based amplification (NASBA) (Compton, 1991), rolling circle amplification (RCA) (Lizardi et al., 1998), loop-mediated isothermal amplification (LAMP) (Notomi, 2000), strand displacement amplification (SDA) (Walker et al., 1992), single primer isothermal amplification (SPIA) (Kurn et al., 2005), and helicase-dependent amplification (HAD) (Chang et al., 2012) have been used for signal amplification in nucleic acids detections. However, these enzyme-based methods are sensitive to reaction and storage condition changes, costly due to the complicated procedure and required specific laboratory setting (Zheng et al., 2012a). Nucleic acids have been extensively used as the fuel for signal amplifications in assorted applications (Huang et al., 2013; Jiang et al., 2013; Niu et al., 2012). Nucleic acid-based, enzyme-free amplification strategies have the advantage of operating under broad experimental conditions that are not favorable for enzyme and endorsing the rational and modular adaptation for various analytes of interest (Li et al., 2011). Effective nucleic acid-based, enzyme-free amplification strategies for DNA detection have been demonstrated via target recycling approaches primarily based on hybridization and strand displacement reactions, such as the hybridization chain reaction (HCR) (Dirks and Pierce, 2004), the entropy-driven catalysis (Zhang et al., 2007), and the catalytic hairpin assembly (CHA) (Yin et al., 2008).

As an enzyme-free signal amplification technique triggered by single-stranded DNA (ssDNA), CHA has seen wide applications including the detection of nucleic acids (Huang et al., 2012; Jiang et al., 2012; Zheng et al., 2012a), thrombin (Zheng et al., 2012b), and adenosine (Fu et al., 2013; Li et al., 2011), as well as probing spatial organization of DNA strands in HCR (Li et al., 2012b). Furthermore, because of its excellent modularity and robustness,

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CHA has been integrated with other experimental designs to convert analytical binding events to multiple detection modalities, including fluorescent, colorimetric, and electrochemical signals (Li et al., 2011, 2013; Ren et al., 2011). Recently CHA was also reported to function as a specific signal transducer for isothermal nucleic acid amplification reactions, such as RCA, SDA, and LAMP, to accomplish highly specific and real-time detections of amplified products (Jiang et al., 2013; Li et al., 2012a). However, several hours are usually necessary to obtain the final signal even with the assistance of a toehold domain design (Fu et al., 2013; Huang et al., 2012; Zheng et al., 2012b), which is not favorable for the application in point-of-care and high throughput analyses. There have been kinetic and thermodynamic studies of DNA strand displacement based reactions, which provide insights for programmable and efficient circuit designs (Elbaz et al., 2010; Green et al., 2006; Huang et al., 2012; Li et al., 2011; Ren et al., 2011; Soloveichik et al., 2010; Srinivas et al., 2013; Venkataraman et al., 2007; Zhang and Seelig, 2011). However, the significant kinetic difference between the target DNA strand and the mismatch-in-toehold DNA strand in strand displacement reaction (Li et al., 2011; Panyutin and Hsieh, 1993, 1994; Zhang and Winfree, 2009), especially the kinetic performance based on CHA reaction, has not been very well investigated and applied to the detection of single-nucleotide polymorphism.

Based on the CHA circuit design, we in this work developed an initial reaction rate detection mode for rapid and quantitative differentiation of single-base mismatch by taking advantage of the kinetic difference in the toehold region, which greatly reduced the time needed for data acquisition. A 30-nucleotide (nt) p53 gene fragment including the mutation hotspot R273H (Levine et al., 1991) was used as the model target to demonstrate the feasibility of this detection mode. With this kinetic approach, one measurement can be accomplished in less than 10 min with a reasonably good differentiating factor and sensitivity. This mode also provided a quick approach for DNA detection, which will be beneficial to the point-of-care detection. With elaborated experimental design, this proposed detection mode can be applied to other targets which can initiate and catalyze the CHA reaction, thus more application examples will be expected to be demonstrated in the future.

2. Experimental section

2.1. Materials

Fetal bovine serum and 40% acrylamide/*N*,*N*′-methylenebisacrylamide (29:1) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). *N*,*N*,*N*′,*N*′-Tetramethyl ethylenedamine (TEMED) and tris (hydroxymethyl)-aminomethane (Tris) were obtained from Sigma-Aldrich (St. Louis, MO). Ammonium persulphate was from Beijing Chemical Works (Beijing, China). GelSafe stain was from Yuanpinghao Biotech Co., Ltd. (Tianjin, China). All other reagents were of analytical grade and used without further purification. Wahaha® purified water was used throughout the study.

All oligonucleotides as listed in Table S1, including 6-FAM (6-carboxy-fluorescein) labeled ssDNA, were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). All oligonucleotide stock solution were prepared in Tris–HCl buffer (20 mM Tris, 300 mM NaCl, 5 mM $MgCl_2$; pH 7.6) and stored at 4 °C. The oligonucleotides were incubated at 95 °C for 5 min and cooled to 25 °C before use.

2.2. General procedure for fluorescence measurements

Fluorescence measurements were conducted with F-7000 fluorescence spectrometry (Hitachi, Japan) with wavelengths at 490 nm for excitation and 518 nm for emission and a water-bath circulator to maintain the temperature. In a typical experiment, the target (the catalyst in the CHA system) was added to the fuel solution (hairpin A and hairpin B) with concentrations as indicated and mixed by swirling for 10 s, and the time-dependent fluorescence of the mixture was recorded. Fluorescence intensity at each experiment was normalized as the ratio of the fluorescence to the reagent blank for the concentration correction. The initial reaction rate was calculated and presented as the slope of the normalized fluorescence intensity versus time during the first 5 – 10 min.

2.3. Calculation of the differentiating factor (DF)

In our work, the differentiating factor was calculated as the initial reaction rate ratio between the complementary and single-base mismatched strands. In cases of comparison, the differentiating factor was also calculated as the ratio of recovered fluorescence between the complementary and single-base mismatched strands, which was adopted primarily in the literature (Fu et al., 2013; Huang et al., 2012; Zheng et al., 2012b).

2.4. Native polyacrylamide gel electrophoresis

In a typical experiment, the reaction mixture contained 3.3 μM hairpin A, 3.3 μM hairpin B, and 0.67 μM target strand (I) were incubated at room temperature for 4 h. The native polyacrylamide gels electrophoresis (12%) separation was carried out at 110 V in 1 × TBE buffer (90 mM Tris, 90 mM boric acid, and 2 mM EDTA; pH 8.0) for 1.5 h. After electrophoretic separation, PAGE gels containing DNA were stained using GelSafe Dye, and imaged by the Tanon 1600 imager (Tanon, China).

3. Results and discussion

3.1. The rationale and characterization of the model CHA circuit

A simple CHA circuit was designed for target recycling as illustrated in Scheme 1. For clarification and simplicity, the DNA molecule was grouped into numbered domains, and complementary domains were denoted after the same number with an asterisk. As fuel strands, A and B were designed to form hairpin structures. The sticky end region (domain 1*) of hairpin A served as a toehold to initiate the hybridization of strand I with hairpin A. Hairpin B was labeled with the fluorophore FAM at 5′-end and the quencher Dabcyl at the 3′-end, such that fluorescence was quenched with the hairpin configuration. When strand I was introduced, hybridization of strand I with hairpin A exposed the domain complementary to that of hairpin B (domain 6). Through the strand displacement, fluorescence was recovered by the formation of product A · B, and strand I was liberated to trigger another cycle of CHA reaction. In this way, the quantitative detection of strand I can be achieved by measuring fluorescence of the product A · B. Generally, the reaction needed hours to reach the maximum fluorescence signal, which made this approach less realistic when fast analysis was required. To circumvent this problem, we proposed an initial reaction rate detection mode for fast quantitative detection of strand I and single-base mismatch based on the significant kinetic difference between the complementary and mismatched ssDNA, for it has been reported (Panyutin and Hsieh, 1993, 1994; Zhang and Winfree, 2009) that the mismatched DNA may impede the toehold-mediated strand displacement reaction by at least one order of magnitude.

The 9-nt bulge loop structure in hairpin A was designed to serve as the sequestered toehold to facilitate a fast strand displacement reaction between I · A and hairpin B without compromising the generality of the design for sequence-specific DNA

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