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Highly sensitive and multiple DNA biosensor based on isothermal strand-displacement polymerase reaction and functionalized magnetic microparticles

Ming Luo, Ningxing Li, Yufei Liu, Chaohui Chen, Xia Xiang, Xinghu Ji*, Zhike He*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan, Hubei 430072, PR China

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ABSTRACT

A universal, highly sensitive and selective chemiluminescence (CL) imaging method has been developed for high throughput detection of DNA. After molecular beacon (MB) hybridized with the target DNA, the biotin-labeled primer was attached to a magnetic microparticle (MMP) surface by hybridization with the stem part of the MB to initiate a polymerization of DNA strand, which led to the release of the target and another polymerization cycle. Thus the polymerization produced the multiplication of biotin-labeled primer on the surface of MMPs. Sequentially, the horseradish peroxidase (HRP) was conjugated to MMPs surface through the biotin–streptavidin reaction. Then, the conjugated HRP was determined by the CL imaging method. This proposed method could detect the sequence-specific DNA as low as 0.4 pM and discriminate perfectly matched target DNA from the mismatch DNAs. All in all, this proposed method exhibited an efficient amplification performance, and would open new opportunities for sensitive and high throughput detection of DNA.

1. Introduction

The highly sensitive detection of DNA hybridization is of great demand in gene profiling, drug screening, clinical diagnostics and environmental analysis, food safety and a variety of biomedical studies (Fan et al., 2006; Turner et al., 2009; Luo et al., 2012a). Motivated by this demand, many signal amplification strategies have been employed to produce stronger signal in various DNA biosensors. To achieve strong signals, a series of functionalized nanoparticle probes, such as quantum dots (Chan and Nie, 1998; Chan et al., 2002), dye-doped nanoparticles (Zhao et al., 2003; Tan et al., 2004), and nanoparticles (Li et al., 2008; Song et al., 2010) whose surfaces were modified with many signal molecules, have been developed to enhance recognition events of targets and significantly lower the detection limit for DNA analysis. However, there are some limitations involved in using functionalized nanoparticles as signal probes, such as an extra functionalized process on the surface of these nanoparticles may affect the analytical accuracy and reproducibility. Furthermore, it is time-consuming to synthesize such functionalized nanoparticle probes, and the functionalized process is also tedious and complicated.

Meanwhile, signal amplification strategy based on nuclease, such as hybridization chain reaction (HCR) (Huang et al., 2011; Bi et al., 2013b), rolling circle amplification (RCA) (Cheng et al., 2010; Bi et al., 2010a), loop-mediated amplification (Hsieh et al., 2012) and the target DNA recycling amplification with endonuclease (Bi et al., 2010b; Chen et al., 2011; Jang et al., 2009) or exonuclease (Zuo et al., 2010; Luo et al., 2012b) have also been employed to improve the sensitivity of various DNA biosensors. Recently, isothermal strand-displacement polymerase reaction (ISDPS) has attracted considerable attention in target recycling amplification (Ye et al., 2003). This technique uses lengthening of a new strand to replace the target sequence, and thus releases the target to initiate a new polymerization reaction. Because ISDPS does not require a specific recognition site, it can be conveniently used for designing DNA biosensors. Wang's group reported a method for amplified detection of DNA based on the inherent signal-transduction mechanism of the hairpin fluorescence probe and strand-displacement property of polymerase (Guo et al., 2009). In addition, Ju's group developed several biosensors based on polymerase for the sensitive DNA detection (Dong et al., 2012; Gao et al., 2012, 2013). For example, they designed a novel dual signal amplification strategy which combined the ISDPS technique with nanoparticles-based signal amplification for electrochemical detection of the target DNA (Gao et al., 2012). Due to the extensive applications of DNA detection, novel sensitive DNA detection methods based on signal amplification strategies with high throughput are continually needed (Luo et al., 2012a,b; Xiang et al., 2013).

* Corresponding authors. Tel.: +86 27 68756557; fax: +86 27 68754067.
E-mail address: zhkhe@whu.edu.cn (X. Ji).

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Chemiluminescence (CL) imaging (Bi et al., 2013a; Dong et al., 2013; Yang et al., 2008, Zong et al., 2012), a powerful analytical technique, has been proved of particular utility in high throughput detection because it does not need external light source. The system of the horseradish peroxidase (HRP)-catalyzed luminol-*p*-iodophenol (PIP)-H_2O_2 reaction with PIP as the enhancer, allowing a higher sensitivity and steady-state light signal, is suitable for CL imaging detection. For example, our previous work developed a CL imaging biosensor for the DNA detection (Li and He, 2009). However, in order to expand the proposed method to detect various DNA sequences simultaneously, different capture DNA-modified MMPs were needed.

Herein, we reported a CL imaging method for amplified and high throughput detection of DNA based on functionalized magnetic microparticles (MMPs) and strand-displacement property of polymerase (Fig. 1). As shown in Fig. 1A, the molecular beacon (MB) acted as a template of polymerization reaction, while the target acted as a trigger of polymerization reaction. The activation of this DNA detection system was based on the conformational change of the MB induced by hybridization between the MB and the target DNA. In this method, the target DNA was displaced in the process of the polymerization reaction and then hybridized to another MB. Thus, in essence, this method allowed hybridization, polymerization reaction and displacement to occur cycle-after-cycle, producing, at the same time, an amplified CL signal sufficient to indicate the presence of trace amount of the target DNA. This sensing platform, however, needed to design specific functionalized MMPs for every target DNA. In order to resolve this apparent limitation, we used the short primer functionalized MMPs as a versatile probe (Fig. 1B). Only need to change MB, this biosensor could be expanded for the simultaneous detection of various DNA sequences in a microarray. In short, combined with the inherent advantages of CL imaging method, this new ISDPS-based biosensor could be widely applied to sensitive and high throughput DNA detection.

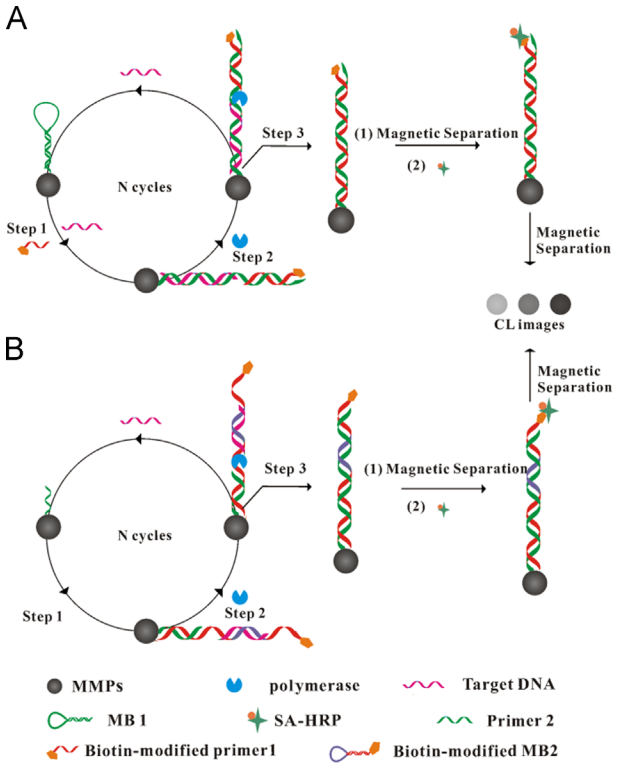

Fig. 1. Schematic representation of chemiluminescence (CL) imaging DNA detection relying on ISDPR and functionalized MMPs.

2. Materials and methods

2.1. Chemicals and reagents

The polymerase Klenow fragment exo- and the mixture of deoxyribonucleotides (dNTPs) were obtained from Fermentas Biotechnology Co. Ltd. (Canada). The carboxylated MMPs (1.02 μm, 10 mg mL^{-1}) were obtained from Invitrogen (Norway). Streptavidin-horseradish peroxidase (SA-HRP), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Sigma Chemical Co. Ltd. (USA). The other chemical reagents were all analytical reagent grades and purchased from Sigma Chemical Co. Ltd. (USA). The HRP substrate kit was purchased from Millipore. Polystyrene microplates (Costar) were used to perform CL imaging detection. DNA hybridization buffer was PBS (137 mM NaCl, 10 mM $MgCl_2 \cdot 12H_2O$, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4, pH 7.4). The washing buffer was 10 mM PBS (0.1% (w/v) Tween-20, pH 7.4). All oligonucleotides with different sequences were synthesized and high-performance liquid chromatography (HPLC) purified by Sangon Biotechnology Co. Ltd. (Shanghai, China) and was stored in 10 mM Tris–HCl buffer (1 mM EDTA, pH=8.0). The sequences of the oligonucleotides were as follows:

The DNA sequences of Fig. 1A:

Molecular beacon (MB1): 5/-NH_2-GCT CGG CAT GAC CAC ATC ATC CAT ACA TGC CGA GC-3/
Target DNA for MB1 (HT1): 5/-TAT GGA TGA TGT GGT-3/
Primer DNA for MB1 (Primer1): 5/-biotin-GCT CGG C-3/

Mismatched target sequences for MB1:

Single-base mismatched (MT1): 5/-TAT GGA TAA TGT GGT-3/
Two-base mismatched (MT2): 5/-TAT GTA TGA TTT GGT-3/
Three-base mismatched (MT3): 5/-TAT TGA TAA TGC GGT-3/

The DNA sequences of Fig. 1B:

Molecular beacon (MB2): 5/-Biotin-GTG CTC GAG TCG AAT ACC ACA TCA TCC ATA TAC GAC TCG AGC AC-3/
Target DNA for MB2 (HT2): 5/-TAT ATG GAT GAT GTG GTA TT-3′
Primer DNA for MB2 (Primer2): 5/-NH_2-TTT TTT GTG CTC GAG-3/

Mismatched target sequences for MB2:

Single-base mismatched (MT1): 5/-TAT ATG GAT TAT GTG GTA TT-3/
Two-base mismatched (MT2): 5/-TAT ATG TAT GAT GCG GTA TT-3/
Three-base mismatched (MT3): 5/-TAT ACG GAT TAT GTA GTA TT-3′

The CL images were acquired with a ChemiDoc XRD system (Bio-Rad). A HZQ-F160 temperature oscillation incubator (Shanghai Anting Scientific Instrument Co. Ltd.) was used to control the temperature of the reaction. Ultrapure water was produced by a Millipore-Q Academic purification set (Millipore, Bedford, MA, USA). A pB-10 potentiometer (Sartorius) was used to measure pH of solutions.

2.2. Modification of MMPs

The conjugates between MMPs and DNA sequences (MB1 or Primer2) were prepared using a modified protocol suggested by the manufacturer (Invitrogen). Briefly, 0.5 mL of 10 mg mL^{-1} carboxyl-modified MMPs suspension was washed two times with 1 mL of the