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Label-free chemiluminescent aptasensor for platelet-derived growth factor detection based on exonuclease-assisted cascade autocatalytic recycling amplification

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ABSTRACT

Here an exonuclease III (Exo III)-assisted cascade autocatalytic recycling amplification (Exo-CARA) strategy is proposed for label-free chemiluminescent (CL) detection of platelet-derived growth factor BB (PDGF-BB) by taking advantage of both recognition property of aptamer and cleavage function of Exo III. Functionally, this system consists of a duplex DNA (aptamer–blocker hybrid), two kinds of hairpin structures (MB_1 and MB_2), and Exo III. Upon recognizing and binding with PDGF-BB, aptamer folds into a close configuration, which initiates the proposed Exo-CARA reaction (*Recyclings* $I \rightarrow II \rightarrow III \rightarrow II$). Finally, numerous "caged" G-quadruplex sequences on $DNAzyme_1$ and $DNAzyme_2$ release that intercalate hemin to catalyze the oxidation of luminol by H_2O_2 to generate an amplified CL signal, achieving excellent specificity and high sensitivity with a detection limit of 6.8×10^{-13} M PDGF-BB. The proposed strategy has the advantages of simple design, isothermal conditions, homogeneous reaction without separation and washing steps, effective-cost without the need of labeling, and high amplification efficiency, which might be a universal and promising protocol for the detection of a variety of biomolecules whose aptamers undergo similar conformational changes.

1. Introduction

The detection of nucleic acids and proteins plays significant role in investigating their functions for the development of molecular diagnostic (Adams et al., 2012). To achieve determination of these biomolecules in complex systems, much effort has been devoted to the development of amplification strategies to improve detection sensitivity and specificity, especially isothermal amplification techniques (Kim and Easley, 2011). In comparison with polymerase chain reaction (PCR) that requires thermal cycling, isothermal amplification has the advantages of easy operation, cost effective, and more tolerant to inhibitory components from a crude sample. So far, various isothermal amplification strategies have been developed for biomolecules detection, such as rolling circle amplification (Zhao et al., 2008), strand-displacement reaction (Krishnan and Simmel, 2011), helicase-dependent amplification (Vincent et al., 2004), recombinase polymerase amplification (Rohrman and Richards-Kortum, 2012). Recently, exonuclease III (Exo III) assisted target recycling strategies have been reported for amplified detection of DNA (Liu et al., 2013; Zuo et al., 2010; Bi et al., 2012). Exo III can catalyze the stepwise removal of mononucleotides from 3′-hydroxyl terminus of double-stranded DNA when substrate is a blunt or recessed 3′-terminus, while shows limited activity on single-stranded DNA or duplex DNAs with a protruding 3′ end (Zuo et al., 2010). Thus, as an advantage over nicking endonucleases, Exo III is sequence-independent that does not require a specific recognition site, providing a more versatile platform for biomolecules detection (Freeman et al., 2011; Liu et al., 2012; Xu et al., 2012).

Aptamers are single-stranded oligonucleotides that can be isolated in vitro from random sequence libraries through a process termed SELEX (selective evolution of ligands by exponential enrichment) (Famulok et al., 2007). Advantages of aptamers over antibodies include ease synthesis and modification, high stability, unlimited shelf-life, and so on (Li et al., 2010). So far, aptamers have been selected and synthesized for a broad range of analytes,

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including small molecules, metal ions, proteins, and even cells (Tang et al., 2007; Wu et al., 2011). Aptamers can fold into distinct secondary structures and function as receptors for target molecules with high affinity and specificity, which thus have been employed to design variously novel biosensors for signal amplification (i.e., aptasensors) (Iliuk et al., 2011; Nutiu and Li, 2003; Xue et al., 2012, 2010). To achieve rapid, simple and convenient detection of biomarkers, label-free strategies have attracted considerable interest for the development of aptasensors. The main advantage of label-free methods is that they can effectively avoid expensive and tedious labeling procedures, which can retain the highest activity and affinity of the recognition elements (Luo and Davis, 2013; Ma et al., 2013). Particularly, DNA-mediated homogeneous binding assays which perform in solution containing the specimen and all reagents without the need of immobilization, separation or washing steps can minimize the effects of contamination (Sassolas et al., 2011; Zhang et al., 2013). Thus, such homogeneous assays are the most promising techniques for molecular diagnostics and point-of-care applications.

Herein, an isothermal, homogeneous, and label-free chemiluminescent (CL) aptasensor has been developed for the detection of platelet-derived growth factor BB (PDGF-BB) that is known to be related to tumor transformation, growth and progression (Heldin and Westermark, 1990) based on Exo III-assisted cascade autocatalytic recycling amplification (Exo-CARA), combing with the induced formation of G-quadruplex for amplified signal transduction. Since this design is simply based on nucleic acid hybridization for 3′→5′ exodeoxyribonuclease activity of Exo III, it can be generally applied to other aptamer-based strategies for label-free detection of various analytes.

2. Experimental section

2.1. Chemicals

Olignoculeotides used in the present study were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences are listed in Table S1. Exonuclease III (Exo III) was purchased from MBI Fermentas (Canada). Recombinant human platelet-derived growth factor BB (PDGF-BB human) was ordered from Prospec-Tany TechnoGene Ltd. (Israel), which was dissolved in 4 mM HCl containing 0.2% bovine serum albumin (BSA). Hemin, 4-(2-hydroxyethyl)piperazine-1 ethanesulfonic acid sodium salt (HEPES) and luminol were obtained from Aladdin Chemistry Co. Ltd (China). A hemin stock solution (5.0×10^{-3} M) was prepared in DMSO and stored in the dark at −20 °C. Double-distilled, deionized ultrapure water was used in all experiments. All regents were of analytical grade and used without further purification.

2.2. Exo III-assisted cascade autocatalytic recycling amplification (Exo-CARA) for PDGF-BB detection

Firstly, the DNA solutions of aptamer (2.0×10^{-6} M) and blocker (2.0×10^{-6} M), MB_1 (1.0×10^{-6} M), MB_2 (1.0×10^{-6} M) were respectively heated to 90 °C for 10 min and allowed to gradually cool to room temperature. Then, a 10 μL of different concentrations of PDGF-BB was incubated with 10 μL of aptamer–blocker solution (1.0×10^{-6} M) at 37 °C for 15 min to perform the aptamer–target binding reaction. The amplification process was performed by adding 10 μL of MB_1 (1.0×10^{-6} M), 10 μL of MB_2 (1.0×10^{-6} M), 4.5 μL of 10 × reaction buffer, 50 units of Exo III, followed by incubating at 25 °C for 30 min. The resulting products were incubated with 50 μL of hemin (1.0×10^{-8} M) in 25 mM HEPES buffer (pH 7.4, 20 mM KCl, 200 mM NaCl, 1% DMSO (v/v), 0.05% Triton X-100 (w/v)) for 30 min to induce the liberated $DNAzyme_1$ and $DNAzyme_2$ to fold into G-quadruplex-hemin complexes.

The CL signals were monitored on a Centro LB942 luminometer (Berthold, Germany). Briefly, corresponding samples and 50 μL of luminol (5.0×10^{-4} M) were firstly added to 96-well plate. And then, 50 μL of H_2O_2 (5.0×10^{-4} M) was introduced to each well through automatic injector of the CL equipment. The CL kinetics was recorded for 2.5 min in 0.5 s intervals. The negative high voltage of luminometer was 1200 V.

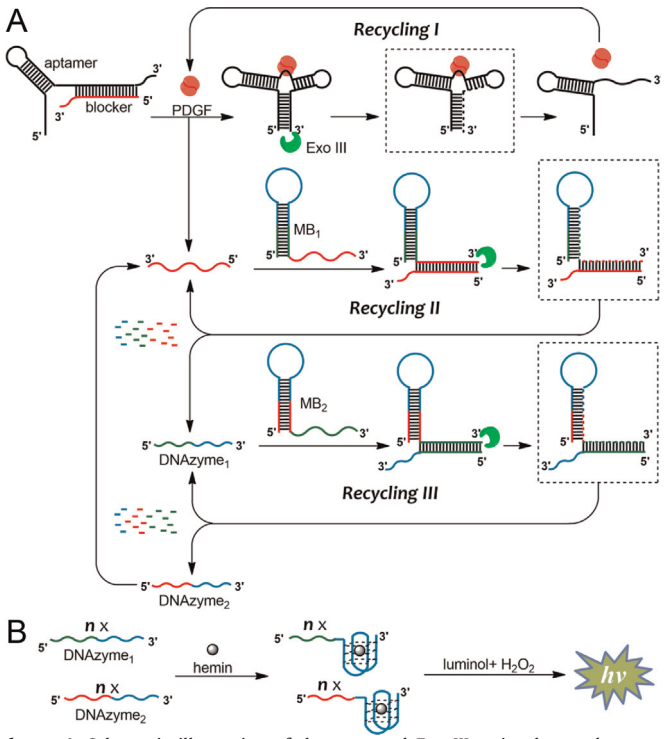
2.3. Nondenaturing polyacrylamide gel electrophoresis

The Exo III-assisted amplification processes were characterized by 12.5% native polyacrylamide gel electrophoresis. The samples were added to 3 μL of loading buffer. Electrophoresis was carried out in 1 × Tris–acetate–EDTA (TAE) at 120 V constant voltage for 1 h at room temperature. After staining gels with ethidium bromide (EB, 0.5 μg/mL) for 30 min, the visualization and photography were performed using a digital camera under UV illumination.

3. Results and discussion

3.1. Principle of the proposed Exo-CARA

The principle of our proposed Exo-CARA strategy for PDGF-BB detection is shown in Scheme 1. This system consists of an aptamer–blocker hybrid, MB_1, MB_2, and Exo III. Blocker DNA that 3′ terminus with four bases non-complementary to aptamer plays significantly roles in not only preventing aptamer from uncontrolled folding into an active configuration in the absence of targets, but also resisting the activity of Exo III. In addition, MB_1 and MB_2 self-hybridize to form stem-loop structures that contain Exo III-resistant 3′ protruding terminus. As a result, the sequences



Scheme 1. Schematic illustration of the proposed Exo III-assisted cascade autocatalytic recycling amplification (Exo-CARA) strategy (*Recyclings I→II→III→II*) for label-free CL detection of PDGF-BB. (For interpretation of the references to color in this scheme, the reader is referred to the web version of this article.)