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Aptamer proximity recognition-dependent strand translocation for enzyme-free and amplified fluorescent detection of thrombin via catalytic hairpin assembly

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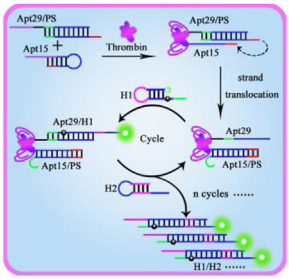
HIGHLIGHTS

- Enzyme-free and amplified fluorescent detection of low level of thrombin is reported.
- Target-induced strand translocation leads to catalytic hairpin assembly amplification.
- Sensitive detection of thrombin in serum samples has been demonstrated.

GRAPHICAL ABSTRACT



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ABSTRACT

By coupling a new aptamer proximity recognition-dependent strand translocation strategy with catalytic hairpin assembly (CHA) signal amplification, we have developed a simple and sensitive method for detecting thrombin in human serums. Simultaneous binding of two engineered aptamer probes to the thrombin target significantly increases the local concentrations of the two probes and facilitates the translocation of a ssDNA strand from one of the probes to the other through toehold mediated strand displacement. Such a strand translocation leads to the generation of a ssDNA tail in the aptamer sequence for subsequent initiation of the assembly of two fluorescently quenched hairpins into many DNA duplexes via CHA. The formation of the DNA duplexes thus results in significant fluorescence recovery for amplified detection of thrombin down to 8.3 pM. The developed method is highly selective to the thrombin target against other interference proteins due to the dual recognition mode, and can be employed to monitor thrombin in human serum samples. With the advantage of simplicity, sensitivity and selectivity, this method can be a universal non-enzymatic and nanomaterial-free amplified sensing platform for detecting different protein molecules.

1. Introduction

Aptamers are a special class of functional DNA or RNA oligonucleotides originated from random-sequence nucleic acid libraries via an in vitro selection process termed as systematic evolution of ligands by exponential enrichment (SELEX) [1–3].

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Aptamers can fold into specific well-defined secondary or tertiary structures and possess strong affinity and specificity to a wide of target molecules ranging from organic and inorganic compounds to proteins and even entire cells [4–6]. Emerging as promising alternative recognition elements to traditional antibodies and other biomimetic receptors, aptamers have attracted increasing attention due to their superior merits such as small size, low cost, high binding affinity, simple modification and good thermal stability [7,8]. Consequently, aptamers have been widely utilized to construct different types of biosensors for signal transduction, drug delivery and diagnostic applications [9,10]. These aptamer-based biosensors commonly rely on target-triggered structural change of the aptamer sequences to recognize different parts of the target molecules, such as thrombin [11], cocaine [12] and platelet-derived growth factor [13].

Thrombin, a multifunctional protease in bloodstream, plays significant roles in various crucial physiological and pathological processes, such as blood coagulation, thrombosis and angiogenesis [14]. It can convert soluble fibrinogen into insoluble fibrin that forms the fibrin gel in many coagulation-related reactions [15], and the change of its concentration is related to certain diseases. For instance, thrombin is almost absent in the blood of healthy individuals while low micromolar concentration of thrombin can be generated during the coagulation process and even low nanomolar can be reached in early hemostatic progress [16]. Moreover, high-picomolar level of thrombin can be found in the blood of patients related to coagulation abnormalities out of the hemostatic process [17]. Therefore, it is of great significance to detect thrombin with high sensitivity and selectivity. To date, many detection methods have been developed to identify the recognition interaction between the detection probes and thrombin [18–20]. Most recognition interactions strongly compromise the detection sensitivity because one target could only cause one quenched signal probe to recovery fluorescence. In recent years, different aptamer-based signal amplification strategies for the detection of thrombin, including rolling circle amplifications [21,22], nanomaterial-assisted amplifications [23,24] and enzyme-assisted recycling amplifications [25,26] have been successfully established. Despite the fact that these amplified aptasensors have indeed enhanced the signal output, sophisticated material synthesis and complicated thermal-cycling steps however resulted in complex bioassay procedures, which significantly reduces the simplicity of the analytical methods for protein detection.

To address these problems, the breakthroughs and advances in molecular programming of non-enzymatic nucleic acid circuits for both amplifying and transducing signals have been previously proposed [27–29]. In particular, as an isothermal, homogeneous and enzyme-free signal generation strategy for amplified detection of biomolecules, catalytic hairpin assembly (CHA) has successfully achieved significant signal amplification [30]. In a typical toehold-mediated strand displacement CHA process, two rationally designed hairpins are initially unable to interact with each other, and only an additional initiator DNA input can bring a toehold domain and a branch-migration domain into close proximity to form a duplex and catalyze the CHA reaction. Taking consideration of the convenience and sensitivity of CHA, it has been extensively used as new signal amplification means to construct different biosensors for detecting nucleic acid and small molecule targets [31–34]. However, it is still of challenge to expand CHA for detecting proteins due to the lack of a universal strategy to construct the protein-responsive CHA.

In this regard, based on a new aptamer proximity recognition-dependent strand translocation strategy, we report on herein a simple and sensitive fluorescent approach for the detection of thrombin through CHA-mediated signal amplification. The proximity recognition involves simultaneous binding of two probes to the same target molecule at distinct binding sites [35,36]. Such proximity recognition can significantly increase the local concentrations of the two probes, thereby facilitating the ligation of two DNA strands [37] or the assembly of DNA [38] for amplified detection of different target molecules. In our method, two well-characterized aptamers [39,40], denoted as Apt15 and Apt29, can respectively bind to the distinct fibrinogen and heparin sites of the target thrombin simultaneously in a proximity recognition format. Such bindings can increase the local concentrations of the two aptamers and cause the translocation of a strand from the Apt29 duplex to Apt15. The strand translocation thus leads to the formation of a ssDNA tail of Apt29 to trigger subsequent CHA, which unfolds the fluorescently quenched hairpins to generate many DNA duplexes. The cyclic unfolding of the fluorescently quenched hairpins induced by CHA thus generates substantially amplified fluorescence recovery for highly sensitive detection of thrombin.

2. Experimental

2.1. Materials and reagents

Thrombin, lysozyme, mouse immunoglobin G (IgG), normal human serum and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffer, sodium chloride (NaCl), magnesium chloride ($MgCl_2$), potassium chloride (KCl) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The normal human serum was used as received and first diluted 10 folds with Tris-HCl buffer (pH 7.4, 5 mM KCl, 10 mM $MgCl_2$, 100 mM NaCl) and then centrifuged at 6000 rpm for 20 min. Next, three concentrations of thrombin were spiked into the pretreated human serum for detection. All synthetic oligonucleotides listed below were ordered from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China): Apt29: 5'-AGT CTA GGA TTC GGC GAG GGT TAA TTTTT AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3'; Apt15: 5'-CAA CCA GTC TAG GAT TCG GCG AGA GAC TGG TTG GTG TGG TTG G-3'; A1: 5'-CTG GTT GGT GTG GTT GGC AAC CAG TCT AGG ATT CGG CGA G-3'; A2: 5'-CAA CCA GTC TAG GAT TCG GCG AGC TAG ACT GGT TGG TGT GGT TGG-3'; Protection strand (PS): 5'-TTA ACC CTC GCC GAA TCC TAG ACT GGT TG-3'; H1: 5'-TTA ACC CT (Dabcyl) C GCC GAA TCC TAG ACT CCA TGT GTA GAA GTC TAG GAT TCG GCG (FAM) -3'; H2: 5'-CCT AGA CTT CTA CAC ATG GAG TCT AGG ATT CGG CGC CAT GTG TAG A-3'.

2.2. Probe preparation for thrombin recognition

Apt29 (5 μM) and the protection strand (PS, 5 μM) were mixed and heated to 90 °C for 5 min, followed by cooling down to room temperature for 1 h to form the Apt29/PS duplexes. Apt15, H1 and H2 were also separately treated with the same thermal annealing step.

2.3. Amplified fluorescent detection of thrombin

Briefly, different concentrations of thrombin (or the nonspecific proteins) were incubated with the reaction mixture containing Apt15 (50 nM), Apt29/PS (50 nM), H1 and H2 (each at 200 nM) in Tris-HCl buffer at 37 °C for 100 min. The same reaction mixture without thrombin was used as the blank control. After that, the fluorescence emissions of the mixtures were recorded on a RF-5301 P C fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) with a Xenon lamp excitation source at 150 W and the excitation wavelength of 488 nm.

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