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Enzyme-free fluorescence aptasensor for amplification detection of human thrombin via target-catalyzed hairpin assembly

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ABSTRACT

Aptamers have many advantages, such as simple synthesis, good stability, high binding affinity and wide applicability, making them suitable candidates for protein detection. Since the disease-related protein may be present in very small amounts in biological samples, the development of amplification paths for aptasensors is essential. In this paper, we develop a simple and enzyme-free amplified aptasensor for protein detection via target-catalyzed hairpin assembly. This aptasensor contains two DNA hairpins termed as H1 and H2. H1, which is modified at its 5′ and 3′ ends with a fluorophore and a quencher respectively, consists of the aptamer sequence of human thrombin. Meanwhile, H2 is partially complementary to H1. These two hairpins H1 and H2 interact slowly with each other. Upon the addition of target protein, it can facilitate the opening of the hairpin structure of H1 and thus accelerate the hybridization between H1 and H2, resulting in the significant fluorescence enhancement of the system. By monitoring the change in fluorescence intensity, we could detect the target protein with high sensitivity. The detection limit of this aptasensor is 20 pM, which is more than two orders of magnitude lower than that of reported unamplified aptasensors. Furthermore, this amplified aptasensor shows high selectivity toward its target protein. Thus, the proposed aptasensor could be used as a simple, sensitive and selective platform for target protein detection.

1. Introduction

Proteins are ubiquitous and play critical roles in biology. The detection of disease-related protein is of particular importance in proteomics research, biomedical research and clinical diagnosis. The most commonly used assay for protein is based on antibody, such as ELISA (Lequin, 2005; Schweitzer et al., 2000). However, antibodies are costly and may degrade under unfavorable environmental conditions. Aptamers are single-stranded nucleic acids isolated from random-sequence DNA or RNA libraries by an in vitro selection process termed SELEX (systematic evolution of ligands by exponential enrichment) (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Compared with antibodies, aptamers have many advantages, such as simple synthesis, good stability, high binding affinity and wide applicability, making them suitable candidates for protein detection (Liu et al., 2009; Mayer, 2009; Willner and Zayats, 2007).

In recent years, several aptamer-based methods for protein detection have been proposed (Hamaguchi et al., 2001; Heyduk and Heyduk, 2005; Nutiu and Li, 2004; Wang et al., 2008). Among them, a new class of molecules termed aptamer beacons have been developed for the detection of protein due to their simplicity and rapidness. Similar to molecule beacon (Zhen et al., 2010; Zuo et al., 2010), the aptamer beacon is a hairpin-shaped DNA molecule, which is modified at its 5′ and 3′ ends with a fluorophore and a quencher respectively. The self-complementary stem of aptamer beacon brings the close proximity of the fluorophore to the quencher, resulting in fluorescence quenching by energy transfer. Upon the interaction of aptamer with the corresponding protein, the hairpin structure is open, leading to the fluorescence restoration. However, the sensitivity of these aptamer-based methods including aptamer beacon is not perfect due to the 1:1 binding of target protein and the probe containing aptamer sequence.

Since the disease-related proteins may be present in very small amounts in biological samples, the development of amplification paths for protein aptasensors is essential. Up to now, some highly sensitive aptasensors for protein detection have been developed via different amplification approaches, such as polymerase-based displacement polymerization (Qiu et al., 2011), rolling circle amplification (RCA) (Cheng et al., 2010; Giusto., et al., 2005; Yang et al., 2007), polymerase chain reaction (PCR) (Yang and Ellington, 2008), and nicking endonuclease-based signal amplification (Xue et al., 2010; Zheng et al., 2012). However, all of these amplified

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aptasensors require protein enzymes, which are costly and require special reaction conditions. For example, the polymerase-based methods require the addition of dNTPs and thus a complex polymerase replication process.

Recently, some enzyme-free methods have attracted growing interests for amplification detection of DNA, such as hybridization chain reaction (HCR) (Dirks and Pierce, 2004; Huang et al., 2011; Wang et al., 2011), entropy-driven catalysis (Bi et al., 2011; Zhang et al., 2007), and target-catalyzed hairpin assembly (Li et al., 2011; Yin et al., 2008). However, the study of enzyme-free amplified aptasensors is rare, and the system is complex (Dirks and Pierce, 2004; Li et al., 2011). Herein, we develop a simple and enzyme-free aptasensor for amplified detection of protein via target-catalyzed hairpin assembly. In this aptasensor, the target protein can directly catalyze the assembly of two DNA hairpins for signal amplification, making the system more simple and cost effective.

2. Materials and methods

2.1. Materials

Oligonucleotides designed in this study were synthesized by Shanghai Sangon Biotechnology Co, which were purified by HPLC and confirmed by mass spectrometry. Table 1 shows the sequences of the used oligonucleotides. Each oligonucleotide was heated to 90 °C for 5 min, and cooled on ice for 30 min before use. All proteins were purchased from Sigma-Aldrich Chemical Co., USA. Ultrapure water obtained from a Millipore water purification system was used in all runs. All other reagents were of analytical grade.

2.2. Apparatus

The fluorescence data were all recorded on a Hitachi F-4600 fluorometer (Hitachi Co. Ltd., Japan) under room temperature. In which, the optical path length of a quartz fluorescence cell was 1.0 cm. The excitation was made at 480 nm with a recording emission range of 500–600 nm. The fluorescence intensity at 518 nm was used to evaluate the performance of the proposed aptasensor. The calibration curve was made by using the fluorescence intensity at 518 nm as the ordinate and the concentrations of human thrombin as the abscissa.

2.3. The sensing procedure

H1 and H2 stock solutions were prepared in reaction buffer (20 mM Tris, pH 7.5; 140 mM NaCl; 2 mM $MgCl_2$). Human thrombin stock solution was prepared by first dissolving in glycerol (about 50% of the final volume) and then further diluting with reaction buffer to obtain the desired concentration. Upon optimizing various conditions, the following procedure was used to the fluorescence experiments. H1 (500 nM, 25 μL) and H2 (500 nM, 25 μL) were mixed with different concentrations of human thrombin for 2 h at 37 °C. The final volume of the solution was 250 μL, and the final concentrations of human thrombin in samples varied from 20 pM to 100 nM. Then, the fluorescence intensities were detected.

Table 1
Sequences of the used oligonucleotides (in 5′ to 3′ direction).

Type	Sequence
H1	5′-FAM- **AGT CCG TGG TAG GGC AGG TTG GGG TGA CT**T TTT ACC ACG GAC T- DABCYL- 3′
H2	5′- AGT CCG TGG TAA AAA GTC ACC CCA ACC TGC CCT ACC ACG GGG TGA CTT TTT ACC A -3′

The underlined bold letters of H1 are the aptamer sequences of human thrombin, which is blocked by the hairpin stem region. The *Tm* of H1 is 70.3 °C, while the *Tm* of H2 is 77.9 °C, which were calculated by the software.

2.4. Selectivity of the aptasensor

H1 (50 nM) and H2 (50 nM) were mixed with human thrombin or other non-specific protein (bovine serum albumin (BSA), human serum albumin (HSA), hemoglobin (Hb) or bovine thrombin, each at a concentration of 10 nM) respectively. Successively, the mixture was allowed to incubate for 2 h at 37 °C. Then, the fluorescence intensities were detected.

3. Results and discussion

3.1. The principle of enzyme-free fluorescence aptasensor for amplification detection of human thrombin

To demonstrate the utility of our approach, human thrombin is chosen as the target, which plays an important role in the coagulation cascade, thrombosis and hemostasis. Fig. 1(A) depicts the principles of the analytical process for amplification detection of human thrombin. The system involves two hairpins termed H1 and H2. H1, which is modified at its 5′ and 3′ ends with a fluorophore and a quencher respectively, contains two domains termed as I, II according to their different functions as shown in Fig. 1(A). The region I consists of the aptamer sequence of human thrombin (Tasset et al., 1997), which is blocked by hybridization

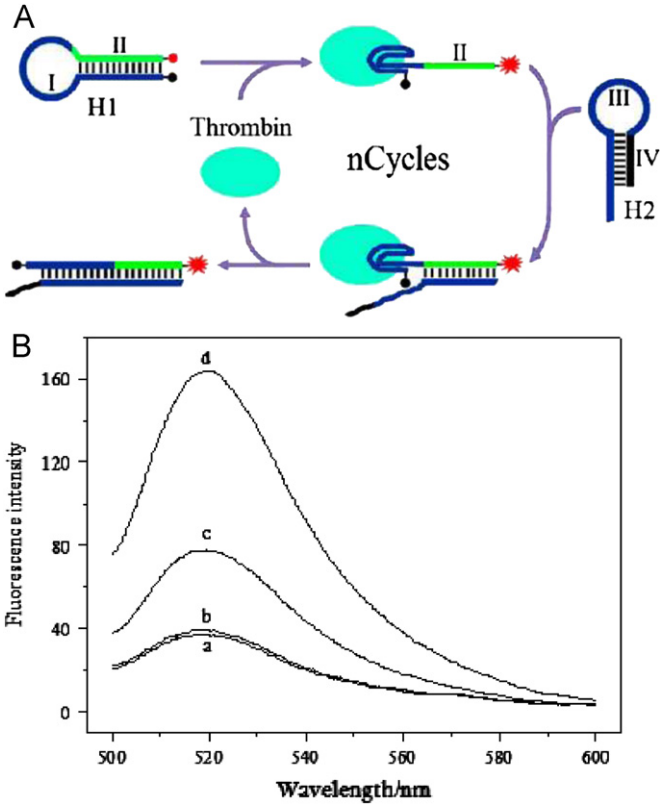

Fig. 1. (A) Schematic illustration of enzyme-free fluorescence aptasensor for amplification detection of human thrombin. (B) Fluorescence emission spectra of H1 (50 nM) at different conditions: (a) H1 itself; (b) H1+human thrombin (20 nM); (c) H1+H2 (50 nM); and (d) H1+H2 (50 nM)+human thrombin (20 nM).