Biosensors and Bioelectronics 71 (2015) 158–163

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Target catalyzed hairpin assembly for constructing a ratiometric electrochemical aptasensor

Fenglei Gao [a,*], Yong Qian [b], Lei Zhang [b], Shizhen Dai [b], Yanfei Lan [b], Yu Zhang [a], Lili Du [a], Daoquan Tang [a]

[a] Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, School of Pharmacy, Xuzhou Medical College, 221004 Xuzhou, China
[b] Fundamental Science on Radioactive Geology and Exploration Technology Laboratory, East China Institute of Technology, Nanchang, Jiangxi 330013, China

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form
27 March 2015
Accepted 13 April 2015
Available online 14 April 2015

Keywords:
Ratiometric
Thrombin
Electrochemical
Aptasensor

ABSTRACT

In this paper, we develop a novel dual-signaling amplified aptasensor for protein detection via target-catalyzed hairpin assembly. Thrombin was chosen as a model target. This aptasensor contains two DNA hairpins termed as H1 and H2. H1, which is modified at its 3′ ends with a methylene blue (MB), consists of the aptamer sequence of human thrombin. Meanwhile, H2 which is modified at its 3′ ends with a ferrocene (Fc), is partially complementary to H1. 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, the target protein can be displaced from hairpin H1 by hairpin H2 through a process similar to DNA branch migration. The released target found another H1 to trigger the cycle, resulting in the multiplication of the Fc confined near the GE surface and MB away from the GE surface. When I_{Fc}/I_{MB} is used as the response signal for quantitative determination of thrombin, the detection limit (41 fM) is much lower than that by using either MB or Fc alone. This new dual-signaling aptasensor is readily regenerated and shows good response toward the target. Furthermore, this amplified aptasensor shows high selectivity toward its target protein. The clever combination of the functional DNA hairpin and the novel device achieved a ratiometric electrochemical aptasensor, which could be used as a simple, sensitive high repeatability and selective platform for target protein detection.

1. Introduction

Aptamers are molecule recognition elements made of single stranded DNA or RNA oligonucleotides, which are elicited by the systematic evolution of ligands by the exponential enrichment procedure, they can bind a wide range of specific targets (e.g., small molecules, proteins, amino acids, and even cells) with high specificity and affinity (Tang et al., 2008; Liu et al., 2011, Zhao et al., 2012; Ye et al., 2013; Xie et al., 2014). Compared with the conventional binding pair of antigen and antibody, aptamers have been an ideal sensing element in the biochemical analysis for their high selectivity, stability, versatile target binding, easy regeneration capabilities and high resistance against denaturation (Shlyahovsky et al., 2007; Zhang and Johnson, 2009; He et al., 2010; Zhang and Sun, 2011; Zhang et al., 2012; Ma et al., 2014). Various aptasensors have been very popular in the field of analytical chemistry for detecting molecules based on different technologies including electrogenerated chemiluminescence aptasensors (Zhu et al., 2010; Shan et al., 2011; Jin et al., 2015), fluorescent aptasensors (Zhu et al., 2011a, b; Xue et al., 2012; Kong et al., 2013; Chen et al., 2014; Li et al., 2014; Wang et al., 2015; Xu et al., 2015), chemiluminescence aptasensors (Zheng et al., 2011), colorimetric aptasensors (Zhu et al., 2011a, b; Huang et al., 2013a, 2013b; Zheng et al., 2014; Zhang et al., 2014), surface plasmon resonance aptasensors (Kwon et al., 2012; Baek et al., 2014; He et al., 2014), and electrochemical aptasensors (Yi et al., 2013; Zhang et al., 2013a, 2013b; Eissa et al., 2014; Peng et al., 2014; Zhao et al., 2014). Among these aptasensors, electrochemical aptasensors are the most attractive due to their advantages of fast response, portability, high sensitivity, simple instrumentation, low cost (Zhang et al., 2007; Huang et al., 2013a, 2013b; Song et al., 2014). Recent years, we have seen the development of a number of electrochemical aptasensors that were usually based on target-induced conformational changes or structural switching of redox-tagged aptamers immobilized on the electrode surface (Baker et al., 2006; Lai et al., 2007). These aptasensors require no addition of reagents or target labeling, and detection is a rapid single-step process. Additionally, as the signaling mechanism is linked to a specific conformational change, these sensors are capable of functioning in complex, multicomponent samples. Based on the signal change

* Corresponding author. Fax: +86 516 83262138.
E-mail address: jsxzgfl@sina.com (F. Gao).

http://dx.doi.org/10.1016/j.bios.2015.04.040
0956-5663/

induced by the structural transformation, aptasensors are divided into “signal-on” and “signal-off” biosensors.

In a “signal-off” sensor, target binding limits collisions between the redox tag and the electrode thereby reducing the signaling current (Yoshizumi et al., 2008; Han et al., 2009; Liu et al., 2009). This signal-off mechanism significantly limits the gain of the sensor, in which only a maximum of 100% signal suppression can be attained under any experimental conditions. Furmore, a second limitation of signal-off sensors is that probe degradation can be misinterpreted as an authentic response to target (Yu and Lai, 2012; Prieto-Simón and Samitier, 2014). In contrast, “signal-on” sensors can achieve much improved signaling; as the background current observed in the absence of target is reduced, the gain of such a sensor, at least in theory, increases without limit (Zuo et al., 2007; Zuo et al., 2009). Thus motivated, Xiao et al. reported a novel “signal-on” aptasensor based on target induced association of the two fragments thus increases the concentration of methylene blue at the electrode surface, which can be readily monitored via voltammetry (Lu et al., 2008). Despite recent advances, the aptasensors still only use one DNA strand of the DNA duplex to provide a response signal of target based on "signal-on" or "signal-off" mechanism alone; the another DNA strand does not have any contribution to the response signal. It is no doubt that both DNA strands of the DNA duplex with redox labels which could provide the multiple response signals at various redox potentials would have obvious advantages. In this work, we construct a ratiometric electrochemical aptasensor based on utilizing two electrochemical labels as an amplification strategy.

Ratiometric detection has extensively been developed in fluorescence and electrochemiluminescence analysis of biomolecules (Cheng et al., 2014; Liu et al., 2014; Yu et al., 2014; Zhang et al., 2014). Recently this technology has been employed in electrochemical detection of ATP which was structured using a methylene blue (MB)-labeled DNA modified gold electrode to hybridize ferrocene (Fc)-labeled aptamer probe for detection of ATP with the sum of peak current changes (Wu et al. 2013a, 2013b). However, above-mentioned method is not sensitive enough, because a single target only opens a single signaling capture probe and makes a Fc-labeled probe away from the GE surface, limiting the total signal gain and corresponding sensitivity. In this paper, thrombin was used as the model and *a* novel ratiometric electrochemical aptasensor for thrombin detection based on target catalyzed hairpin assembly amplification strategy has been developed, in which target recycling and dual-signaling amplification are successfully achieved. The proposed strategy exhibits high sensitivity and superior selectivity towards target thrombin, which may provide *a* universal sensing platform for protein-based molecular diagnostics.

2. Experimental

2.1. Oligonucleotides and regents

Thrombin, immunoglobulin G (IgG), adenosine-triphosphate (ATP), tris (2-carboxyethyl) phosphane hydro-chloride (TCEP) and human serum albumin (HSA) were obtained from Sigma-Aldrich Chem. Co. Water was purified with a Milli-Q purification system (Branstead, USA) and used throughout the work. All chemicals used in this work were of analytical grade. The buffers used in the study were HEPES buffer (10 mM HEPES, 150 mM NaCl, pH 7.4) for target binding. The washing buffer was PBS (50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 1 M NaCl, pH 7.5). To avoid the instability of ferrocenium (the oxidized form of the ferrocene), 1.0 M $NaClO_4$ solution was used as the supporting electrolyte when electrochemical behavior of the working electrode was investigated. DNA oligonucleotides used in this work were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China).

H1:5′-AGTCCGTGGTAGGGCAGGTTGGGGTGACTTTTTACCACG-GACT-Fc- 3′

H2: 5′-SH-AGTCCGTGGTAAAAAGTCACCCCAACCTGCCCTACCACG GGGTG

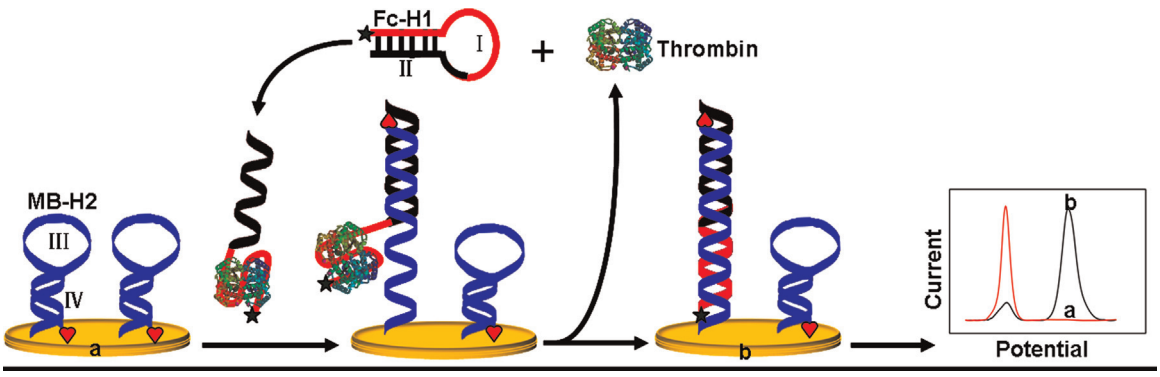
ACTTTTTACCA-MB-3′

2.2. Electrode modification

Prior to modification, the bare GE (3 mm in diameter) was polished to a mirror-like surface with alumina suspensions and then sequentially cleaned ultrasonically in 95% ethanol and twice-quartz-distilled water for 5 min. Prior to attachment to the GE surface, 100 μL of 100 μM thiolated hairpin loop H2 was incubated with 0.1 μL of 100 mM TCEP for 1 h to reduce disulfide bonds and subsequently diluted to 1.0 μM with phosphate buffer. 10 μL of thiolated H2 (1 μM) was dropped on the cleaned GE for 2 h at room temperature in the dark. During this process, the H2 was conjugated onto the GE via the Au–S bond. After rinsing with distilled water, the modified GE was incubated with 1.0 mM 6-mercaptohexanol in10 mM Tris–HCl buffer (pH7.4) for 1 h at room temperature. H1 (300 nM, 25 μL) mixed with different concentrations of human thrombin were dropped on the surface of the electrode. After the process was performed for 50 min at 37 °C, it was terminated by washing thoroughly. The whole procedure was shown in Scheme 1.

2.3. Measurement procedure

Electrochemical experiments were carried out using the CHI 660C electrochemical analyzer. All electrochemical experiments were performed with a conventional three-electrode system comprising a gold working electrode, *a* platinum wire auxiliary



Scheme 1. Schematic illustration of a novel ratiometric electrochemical aptasensor for thrombin detection based on target catalyzed hairpin assembly strategy.