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An ultrasensitive signal-on electrochemical aptasensor via target-induced conjunction of split aptamer fragments

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ABSTRACT

In this paper, we introduced a signal-on electrochemical sensor based on target-induced split aptamer fragments conjunction. To construct the aptasensor, the sequence of the 15-base anti-thrombin DNA aptamer was split into two fragments, one of which was attached to a gold electrode via thiol self-assembled monolayer chemistry and the second of which was modified with the redox moiety ferrocene. Thrombin-induced association of the two fragments thus increased the concentration of ferrocene at the electrode surface, which could be readily monitored via voltammetry. The sensitivity of the proposed electrochemical aptasensor was investigated by differential pulse voltammogram. The results indicated that, in pH 7.1 Tris–HCl buffer solution, the peak current was linear with the concentration of thrombin in the range of 0.8–15 nM with a detection limit of 0.2 nM. The proposed aptasensor has the advantages of higher sensitivity and lower background current. Given the simplicity in design of the proposed electrochemical aptasensor, it is fairly easy to generalize this strategy to detect a spectrum of targets by splitting the aptamers into two suitable segments. Furthermore, this design could be also used to construct novel optical aptasensors.

1. Introduction

Aptamers are single-stranded oligonucleotides that have been designed through an in vitro selection process called SELEX (systematic evolution of ligands by exponential enrichment) (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Aptamers are able to bind to selected molecules ranging in size from small organic molecules, proteins, even to cells (Shamah et al., 2008; Mayer, 2009; Shangguan et al., 2006). Moreover, aptamers exhibit a number of advantages over antibodies such as simple synthesis, easy labeling and good stability. Accordingly, extensive activities have been directed to the application of aptamers as recognition elements for the design of biosensors (Mok and Li, 2008), especially electrochemical biosensor (Willner and Zayats, 2007).

Most aptamers are able to fold into unique three-dimensional conformations upon target binding. This offers great flexibility in the design of electrochemical aptasensors (Xiao et al., 2005a,b; Radi et al., 2006; Ferapontova et al., 2008; Baker et al., 2006; Zuo et al., 2007). In these contexts, the aptamers were normally labeled with redox tags such as ferrocene or methylene blue, and then immobilized on an electrode. In the presence of targets, the conformational switches of the aptamers modulate the distance of redox tags from the electrode, altering the redox current. Although this kind of electrochemical aptasensors has so far been employed for selective and sensitive detection of thrombin (Xiao et al., 2005a,b; Radi et al., 2006), cocaine (Baker et al., 2006), ATP (Zuo et al., 2007) and theophylline (Ferapontova et al., 2008), they required a large-scale conformational change of the aptamer induced by the specific target binding. Moreover, these aptasensors generally suffer from the problem of substantial background current. To circumvent these drawbacks, here we report the proof-of-principle of a novel electrochemical aptasensor based on target-induced split aptamer fragments conjunction. Recently, conjunction of split aptamer fragments has been used to develop a series of DNAzymes for sensing, nanotechnology and logic gate applications (Willner et al., 2008).

To construct the aptasensor, the sequence of the 15-base anti-thrombin DNA aptamer was split into two different fragments (capture probe and detection probe). It was reported that human thrombin has two positively charged sites termed Exosite I and II on the opposite sides of the protein (Cao and Tan, 2005). The 15-base anti-thrombin DNA aptamer used in this work binds with the Exosite I of thrombin (Tasset et al., 1997) with the dissociation constant (K_d) of 25 nM (Bock et al., 1992). The capture probe was functionalized with a thiol group at one end and then covalently attached to a gold electrode (GE) through S–Au bonding. The electrode was then blocked with 2-mercaptoethanol (MCH) to form a

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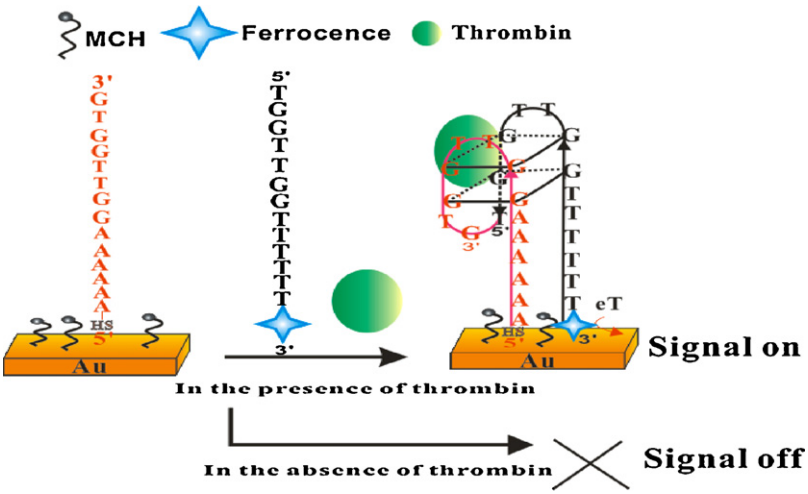

Fig. 1. Fabrication of electrochemical aptasensor based on target-induced split aptamer fragments conjunction. Capture probe sequence is 5′-HS-AAA AAA GGT TGG TG-3′. Detection probe sequence is 5′-TGG TTG GTT TTT T-3′- Ferrocene.

mixed monolayer. It is worth mentioning that the surface coverage by the MCH can effectively prevent the non-specific adsorption of thrombin on the electrode surface and displace the weaker adsorption contacts between aptamer and the substrates. Meanwhile, the detection probe was functionalized ferrocene tag at one end. In the absence of thrombin, the capture probe and detection probe exit predominantly in the dissociated form (at certain concentrations and buffer conditions), so the detection probe can be easily removed by washing and resulting in weak electronic signal. In the presence of thrombin, the detection probe will combine with capture probe to form G-quadruplex structure to bind thrombin. This, in turn, allows the ferrocene tag to collide with the electrode surface, producing a readily detectable Faradaic current (see Fig. 1). By employing this strategy, we demonstrated that this prototype aptasensor has high sensitivity and selectivity. Compared to existing aptasensors based on the conformation change of redox-labeled aptamers, the proposed strategy offers the substantial advantage because the background current is eliminated as the ferrocene-tagged detection probe is specifically ligated to capture probe only in the presence of thrombin.

2. Experimental

2.1. Chemicals

The labeled oligonucleotides were synthesized by TaKaRa Biotechnology (Dalian) Co., Ltd. (China) and purified via C18 HPLC, and confirmed by mass spectroscopy. Their concentrations were quantified by OD260 based on their individual absorption coefficients. their base sequences are as follows: capture probe: 5′-HS-AAA AAA GGT TGG TG-3′; detection probe: 5′-TGG TTG GTT TTT T-3′- Ferrocene; human thrombin, bovine thrombin, human serum albumin (HSA), immunoglobulin G (IgG), and immunoglobulin A (IgA) used in this study was purchased from Sigma–Aldrich, and diluted with sterile water as appropriate. Myoglobin and thrombin from bovine plasma were also purchased from Sigma–Aldrich, Inc. (USA) and used without further processing.

Tris-(hydroxymethyl)aminomethane was from Cxbio Biotechnology Ltd. Ethylenediaminetetraacetic acid (EDTA), mercaptohexanol (MCH), tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and hexaammineruthenium (III) chloride (98%) were purchased from Sigma–Aldrich (St. Louis, MO, USA), The buffer solutions involved in this study are as follows: buffers for electrochemical quantification was 10 mM Tris–HCl solutions (pH 7.1 with 100 mM NaCl, 10 mM $MgCl_2$ and 20 mM KCl). DNA immobilization buffer: 10 mM TE buffer, 10 mM TCEP (pH 7.4) and 1 M NaCl. Washing buffer was 0.1 M NaCl and 10 mM PB buffer (pH 7.4). All solutions were prepared with MilliQ water (18 MΩ cm resistivity) from a Millipore system.

2.2. Electrochemical measurements

All electrochemical measurements were performed by using CHI 660A Electrochemical Workstation (CH Instrument, USA). The electrochemical system consisted of gold working electrode, a platinum wire as the auxiliary electrode, and the reference electrode (Ag/AgCl). Cyclic voltammetry (CV) was carried out at a scan rate of 100 mV/s. Differential pulse voltammograms (DPVs) were registered in the potential interval +0.1 to +0.6 V vs. Ag–AgCl, under the following conditions: modulation amplitude 0.05 V, pulse width 0.06 s, and sample width 0.02 s. Electrochemical impedance experiments were performed in the presence of $[Fe(CN)_6]^{3-/4-}$, the biased potential was 0.18 V (vs. Ag/AgCl) and the amplitude was 5.0 mV, and the electrochemical impedance spectra were recorded in the frequency range of 100 kHz to 0.1 Hz with a sampling rate of 12 points per decade. A Nyquist plot (Z_{re} vs. Z_{im}) was drawn to analyze the impedance results.

2.3. Preparation of aptamer-based electrochemical sensor

Gold electrodes (2 mm in diameter, CH Instruments Inc.) were cleaned following the reported protocol (Zhang et al., 2007). The gold electrodes were polished with 1.0, 0.3 and 0.05 μM alumina powder, respectively, and then ultrasonically rinsed with water, and absolute alcohol for 5 min, respectively. Then electrodes were electrochemically cleaned in fresh 0.5 M H_2SO_4 solution (Fan et al., 2003). The pre-treated electrodes were then rinsed with water again. After being dried with nitrogen, electrodes were immediately used for DNA immobilization. The cleaned electrodes were incubated in the immobilization buffer which contained capture probes modified with thiolate at appropriate concentrations for 2 h at room temperature. After that, the SH-DNA modified electrodes were treated with either 1 mM MCH for 1 h to obtain well-aligned DNA monolayers. The electrode surface was then washed with water to remove the unbound oligonucleotides. The detection probe was mixed with thrombin at 4 °C for 30 min in 10 mM Tris–HCl buffer, and then 5 μL of the solution was dropped on the gold electrodes with DNA self-assembly monolayers (SAMs) film