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A novel label-free and signal-on electrochemical aptasensor based on the autonomous assembly of hemin/G-quadruplex and direct electron transfer of hemin

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ABSTRACT

In this study, we developed a signal-on and label-free aptasensor based on the direct electron transfer of hemin and signal amplification by target-catalyzed hairpin assembly followed by a hybridization chain reaction (HCR). Upon the addition of a target protein, it facilitates the opening of the hairpin structure of A1 and thus accelerates the hybridization between A1 and A2; the target protein was displaced from hairpin A1 with hairpin A2 through a process similar to DNA branch migration. The released target participates in the next hybridization process with A1. Finally, each target passes through many cycles, confining numerous A1 close to the gold electrode. Subsequently, the single-strand fragment on the electrode surface initiated HCR, resulting in the hybridization reaction to form double-strand DNA concatemers on the electrode surface. Consequently, hemin stacked into the G-quadruplex-forming region, and the hemin/G-quadruplex was formed, generating an amplified electrochemical signal by differential pulse voltammetry. In our sensing approach, the introduction of HCR significantly enhanced the signal of the sensor response. Moreover, the approach is free of any label conjugation step for signal amplification and simple and thus has great potential for the development of robust aptasensors.

1. Introduction

Aptamers are synthetic DNA or RNA sequences that are selected through SELEX (systematic evolution of ligands by exponential enrichment) processes from a random sequence bank. A selected aptamer and its related target molecule usually have a high affinity and specific binding [1,2]. A combination of good binding affinity and excellent selectivity makes aptamers attractive similar as antibodies when used as critical identification materials during protein analysis [3,4]. Aptamers are, however, more stable, easier to store, and inexpensive than antibodies. Furthermore, aptamers can be synthesized without using a host. Therefore, aptamers have been used as alternatives to antibodies in various fields for various applications [5–7]. Because of their specific affinity, the existence of an aptamer technique has a great potential for the development of aptamer-based sensors (aptasensors) as the molecular recognition component because of their relative ease of isolation and modification, high specificity, good stability [8–10]. Extensive efforts have been devoted to the development of aptasensors including colorimetric sensors [11–13], fluorescent sensors [14–17], electrochemical sensors [18,19], chemiluminescence sensors [20], Raman sensors [21,22], and SPR sensors[23]. Among these aptasensors, electrochemical aptasensors are the most attractive because of their advantages such as a fast response, portability, high sensitivity, simple instrumentation, and low cost [24,25].

In the recent years, several electrochemical aptasensors have been developed based on target-induced conformational changes or structural switching of redox-tagged aptamers immobilized on an electrode surface [26,27]. Moreover, 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 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 electrode, thus reducing the

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signaling current [28,29]. 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 condition [30–32]. "Signal-on" sensors, in contrast, can achieve a much improved signaling; as the background current observed in the absence of a target is reduced, the gain of such a sensor, at least in theory, increases without limit [33–35]. However, an additional redox mediator was still needed for producing the electrochemical signal, resulting in the fussy labeling process of ferrocene or methylene blue and high cost. The deactivation of aptamer and high background noise are often encountered. In this study, a label-free and signal-on electrochemical aptasensor was constructed based on the direct electron transfer of hemin.

Hemin consists of an Fe(III) ion held in a heterocyclic ring known as a porphyrin. A porphyrin ring contains four pyrrole molecules cyclically linked together with the Fe(III) ion bound in the center [36,37]. So far, most of the research has focused on the peroxidase activity of hemin and DNA aptamer–hemin complexes such as G-quadruplex-based DNAzymes with significantly higher peroxidase activity than hemin alone [38–41]. For example, Xu et al. constructed an electrochemical aptasensor based on horseradish peroxidase (HRP)-mimicking activity of hemin@GO hybrid nanosheets [42]. However, the abovementioned methods used hemin as an electrocatalyst based on HRP-mimicking activity. The hemin/G-quadruplex DNAzyme not only acts as an electrocatalyst, but also is used as an electron transfer medium based on the reversible redox of Fe (III)/Fe(II) of hemin. Unlike the most commonly used redox mediator, hemin is not covalently modified at the end of the oligonucleotide, but directly works as an electroactive molecule [36,43]. The current of these sensors depends on the amount of immobilized hemin on the electrode. However, one probe only immobilizes one hemin molecule. To improve the sensitivity of this aptasensor, a large number of hemin/G-quadruplex is accumulated on the electrode through DNA recycling amplification methods (target-catalyzed hairpin assembly and hybridization chain reaction (HCR)).

In this study, a novel label-free and signal-on electrochemical aptasensor was developed based on target-catalyzed hairpin assembly, HCR amplification strategy, and hemin/G-quadruplex concatemers as the trace labels, and target recycling and dual-signal amplification were successfully achieved. To demonstrate the utility of our approach, human thrombin (TB) was selected as the target. The DNA hairpin assembly on the electrode is triggered by the target TB, thus accompanying the release of the target TB for the successive assembly process. Then, the initiator capture probe and two hairpin helper DNAs lead to the formation of extended dsDNA polymers consisting of G-quadruplex units through HCR on the electrode surface. The electrochemical signal originated from many hemin/G-quadruplexes. Our approach is free of any label conjugation step for signal amplification and is simple.

2. Experimental

2.1. Oligonucleotides and reagents

Immunoglobulin G (IgG), L-cysteine, TB, tris (2-carboxyethyl) phosphane hydro-chloride (TCEP) and bovine serum albumin (BSA) 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). DNA oligonucleotides used in this work were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China).

A1:5′-AGTCCGTGGTAGGGCAGGTTGGGGTGACTTTTTACCACGG-ACTGGGTAGGGCGGGTTGGGTAGAAGAAGGTGT TTAAGTA-3′,

A2: 5′-AGTCCGTGGTAAAAAGTCACCCCAACCTGCCCTACCACGG-GGTG ACTTTTTACCA-SH-3′,

H1: 5′ −AGG GCG GGT GGG TGT TTA AGT TGG AGA ATT GTA CTTAAA CAC CTT CTT CTT GGG T-3′,

H2: 5′ −TGG GTC AAT TCT CCA ACT TAA ACT AGA AGA AGG TGTTTA AGT TGG GTA GGG CGG G-3′,

2.2. Electrode modification

Prior to modification, the bare gold electrode (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 A2 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 A2 (1 μM) was dropped on the cleaned GE for 2 h at room temperature in the dark. During this process, the A2 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 (MCH) in10 mM Tris–HCl buffer (pH 7.4) for 1 h at room temperature. A1 (350 nM, 10 μL) mixed with different concentrations of human TB (10^{-15}–10^{-8} M) were dropped on the surface of the electrode. After the process was performed for 120 min at 37 °C, it was terminated by washing thoroughly. To amplify the electrochemical signal, a mixture of 10 μL of H1 (10 μM) and 10 μL of H2 (10 μM) was dropped on the surface of the electrode and incubated for 150 min to complete the long-range self-assembly. Finally, the obtained electrode was incubated with 10 μL of 5 mM hemin solution for 30 min to form hemin/G-quadruplex complex. When the assembly finished, the resulting electrode was again thoroughly rinsed and dried before electrochemical characterization. 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 electrode, and an Ag/AgCl reference electrode. Differential pulse voltammograms (DPVs) were registered in the potential interval −0.67 to −0.17 V vs Ag/AgCl under the following conditions: pulse amplitude 0.05 V, pulse width 0.06 s, and sample width 0.02 in 20 mM Tris–HCl buffer (pH 7.4) containing 1.0 M $NaClO_4$ (in all experiments, 1 M $NaClO_4$, which is a weak nucleophile, was used as the electrolyte to avoid the instability of the oxidized form of hemin) [46]. The Electrochemical impedance spectroscopy (EIS) measurement was also carried out with the CHI 660C electrochemical analyzer. Supporting electrolyte solution was 1.0 mmol/L $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 mol/L KCl. The ac voltage amplitude was 5 mV, and the voltage frequencies used for EIS measurements ranged from 100 kHz to 100 mHz.

3. Results and discussion

3.1. Design strategy

The working principle of our dual-signal amplification strategy is shown in Scheme 1. This work first immobilized A2 on the GE through Au–S bonds. In the presence of TB, the TB-binding aptamer (A1) preferred to form a TB/aptamer complex instead of