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A new dual-signalling electrochemical aptasensor with the integration of "signal on/off" and "labeling/label-free" strategies

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ABSTRACT

The integration of "signal on/off" and "labeling/label-free" strategies was applied to the fabrication of a sensitive and selective dual-signalling electrochemical aptasensor. Ferrocene (Fc), being labeled on single stranded DNA as one signal indicator, could provide "turn on" signal through the DNA conformational changes resulted from target binding with aptamer. $[Ru(NH_3)_6]^{3+}$ (RuHex), as another signal indicator, could provide "turn off" signal through the electrostatic interaction between the anionic DNA phosphate backbones and the cationic RuHex, which is a label-free signal. Cyclic voltammetry (CV) and electrochemical impedance spectrum (EIS) were employed for monitoring the stepwise fabrication process of the biosensor. Under the optimized conditions, the dual signal changes were quantified using square wave voltammetry (SWV). Based on the superposition of the dual signal changes ($|DI_{RuHex}| + \Delta I_{Fc}$), the sensitivity of this aptasensor for lysozyme detection was improved compared to individual signal. The results indicated that lysozyme could be detected in a wide linear range (1.0×10^{-11}–1.0×10^{-7} M) with a low detection limit down to 0.8 pM. Moreover, the resulting biosensor exhibited good specificity, stability and reproducibility, indicating that the present strategy was promising for broad potential application in clinic assay.

1. Introduction

The development of rapid, simple, sensitive and precise analytical method has an important significance in clinical diagnosis, environmental monitoring and food analysis. Over the past few years, electrochemical aptasensor has attracted great attention due to the high sensitivity, rapid response, low-cost measurement systems and convenient operation. It has been used for the detection of many kinds of targets, including biomarkers [1–4], drug molecules [5,6] and so on [7–10]. Generally, the targets are measured directly by using target-induced conformational changes or structural switching of redox-tagged aptamers, which can regulate the redox species on electrode surface. "Signal off" mode, in which target binding leads to a reduction of the electrochemical signal, is the usual strategy for target detection at the beginning [11]. But it suffered from limited signalling capacity, in which a maximum of 100% signal suppression can be attained under any experimental conditions. To circumvent this limitation, several "signal-on" electrochemical aptasensors, in which target binding leads to an increase of the electrochemical signal, have been developed in the past years [12–14]. However, aptasensors that employ single signalling mechanismare still having limitations due to the false-positive results.

Recently, several researches on dual-signalling electrochemical aptasensor coupling "signal-on" and "signal-off" modes have been reported [15,16], in which the analytical performances can be improved. For example, the dual-signalling ratiometric sensors can improve the robustness and reproducibility of electrochemical DNA sensors by importing an internal control redox probe into the sensing platform [17]. Moreover, the dual-signalling changes can improve the sensitivity of aptasensor [18]. Wu et al. developed a simple electrochemical aptasensor for the determination of adenosine triphosphate on the basis of dual-signalling, in which the DNA was labelled with methylene blue (MB) and ferrocene (Fc) as the signal indicators. The lower detection limit was obtained with the superposition of the dual signal changes [19].

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Despite the recent advances, most of dual-signalling aptasensors involve dual-labeling, which is too complicated and high-cost to be a suitable method for practical application. In order to avoid tedious labeling processes, our proposed dual-signalling aptasensor is based on the combination of labeling and label-free strategies. Up to now, there has been little dual-signalling aptasensor that utilized labeling and label-free strategies simultaneously for robust detection of one specific aptamer-binding target. To achieve label-free detection, many kinds of strategies were proposed, including hemin/G-quadruplex [20], nanomaterials [21,22], the interaction between specific DNA structure and redox species [23,24]. Among these strategies, using the interaction between DNA and redox species is an effective way. Chen et al. have reported a label-free DNA sensor using $[Ru(NH_3)_6]^{3+}$ (RuHex) as the signal indicator, which is intercalated into the DNA polymers via electrostatic interaction between the cationic RuHex and the anionic DNA phosphate backbones. In their protocol, target-induced DNA self-assemblies can electrostatically adsorb massive RuHex indicators, leading to the increase of electrochemical response [25,26].

Herein, lysozyme (Lys) was used as the model target and a novel dual-signalling electrochemical aptasensor for the sensitive and selective determination of Lys was proposed with the integration of "signal on/off" and "labeling/label-free" strategies. Both Fc and RuHex were chosen as the signal indicators. Fc was labeled on single stranded DNA to provide "turn on" signal, which were the "signal on" and "labeling" strategies. RuHex could intercalate into the DNA duplex through the electrostatic interaction between the anionic DNA phosphate backbones and the cationic RuHex, which were the "signal off and 'labeling/label-free' strategies. Then the dual-signal superposition method was used to improve the sensitivity. The resulting dual-signalling electrochemical aptasensor could be applied to the clinical detection.

2. Experimental section

2.1. Reagents and materials

Immunoglobulin G (IgG) and 6-mercaptohexanol (MCH) were obtained from Aladdin. Lysozymes (Lys), bovine serum albumin (BSA), thrombin (Thrb), hemoglobin (Hb) and Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Tris(hydroxymethyl) aminomethane (Tris) and NaCl were purchased from Shanghai AIBI Chemistry Preparation Co., Ltd. RuHex was obtained from J&K Chemical Ltd. (Shanghai, China). All other reagents were of analytical grade and used without further purification.

The sequence of lysozymes aptamer (A-Lys) was 5′-ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′ [27,28]. The sequence of Fc-modified signaling probe (P-Fc) was 5′-SH-C6-GTGCAGAGCTAAGTAACTCTGCAC-Fc-3′. The underlined 16 bases at both its 5′ and 3′ ends were complementary to form a stem-loop structure. And the 8-bp-long loop was long enough to ensure that DNA duplexhybridization affinity would be stronger than the signaling probe self-hybridization and would not restrict the aptamer/target binding in the presence of a target. All oligonucleotides were obtained from SangonBiotechnol. Co. Ltd. (Shanghai, China), and were HPLC-purified and freeze-dried.

2.2. Apparatus

All electrochemical measurements were performed on a CHI-660C Electrochemical Workstation (Chenhua Instrument Company of Shanghai, China) and the temperature condition was room temperature. A planar gold electrode (2 mm in diameter) modified with DNA probe as the working electrode, a platinum wire as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode were used to form a conventional three-electrode configuration.

2.3. Electrochemical aptasensor preparation

Au electrode was first polished to a mirror-like surface with 0.3 and 0.05 μm wet alumina slurries and then ultrasonicated in anhydrous alcohol and double-distilled deionized water for 30 s in sequence, followed by repeatedly rinsed with double-distilled deionized water and dried under N_2 blowing. The well-polished electrode was immersed into 0.5 M H_2SO_4 aqueous solution and voltammetrically cycled with the potential of 0.2–1.65 V at the scan rate of 100 mV/s until the cyclic voltammogram characteristic for clean gold electrode was obtained. After that, the electrode was washed thoroughly with plenty double-distilled deionized water and dried by nitrogen gas for subsequent DNA immobilizing.

In order to form the DNA duplex, the single strand ferrocene (Fc)-modified signalling probe (P-Fc) was firstly dissolved in 100 mMTris–HCl buffer (containing 100 mMNaCl and 10 mM TCEP, pH 7.4) and incubated at room temperature for 1 h, which can reduce disulfide bonds modified on the 5′ ends. Then, 20 μM of P-Fc solution was added to 20 μM of lysozyme aptamer (A-Lys) solution (100 mM Tris–HCl buffer, containing 100 mM NaCl, pH 7.4). The solution was then diluted to ensure that the concentrations of both P-Fc and A-Lys are 2 μM. Afterward, the mixture was heated at 90 °C for 5 min and gradually cooled to room temperature. Finally, the DNA duplex structure (P-Fc-A-Lys) was formed by the incubation of cooled mixture at 37 °C for 2 h. To obtain the aptasensor (P-Fc-A-Lys/Au electrode), the pre-prepared Au electrode was incubated in DNA duplex solution at room temperature for 20 h. And then the electrodes were rinsed with washing buffer solution (10 mMTris–HCl, pH 7.4) to wash off DNA strands, which were not immobilized on the electrodes surface. Subsequently, the electrode was passivated with 1 mM MCH solution (in 10 mM Tris–HCl) for 1 h to block the uncovered Au electrodes surface. After all steps, the electrodes were rinsed with double-distilled deionized water and washing buffer in sequence.

The Cyclic voltammetry (CV) and electrochemical impedance spectrum (EIS) methods were used for characterization of the process of immobilization in 0.1 M of KCl aqueous solution containing 5 mM of $[Fe(CN)_6]^{3-/4-}$ as the probe. EIS was performed in the frequency range of 0.01–100 kHz, together with 5 mV as the amplitude and 0.22 V as the biasing potential. CV measurements were recorded with a step potential of 1 mV in the potential range of −0.6 to 0.8 V at a scan rate of 100 mV/s.

2.4. Electrochemical detection of Lys

The P-Fc-A-Lys/Au electrode interacted with different concentrations of Lys through the incubation in 50 μL of Lys solution (100 mM of Tris–HCl containing 100 mM NaCl, pH 7.4) at 37 °C for 70 min and further rinsed with washing buffer to rinse out unbinding targets. The electrode was then immersed into the solution with high ionic strength (100 mM Tris-HCl containing 140 mM NaCl and 5 mM $MgCl_2$, pH 7.4) at room temperature for 30 min. And then, the resulted electrode was immersed into the 10 mL detection solution (100 mM Tris–HCl, containing 10 μM RuHex, 140 mM NaCl and 5 mM $MgCl_2$, pH 7.4) for 6 min. Its electrochemical performance was investigated by SWV under a step potential of 4 mV, a frequency of 25 Hz, and an amplitude of 25 mV by scanning the potential from −0.5 to 0.5 V in that detection solution. The concentrations of Lys were quantified by calculating the change of oxidation peak currents (ΔI), which was the combination of the RuHex signal changes (ΔI_{Ru}) and Fc signal changes (ΔI_{Fc}). ($\Delta I = \Delta I_{Fc} + |\Delta I_{Ru}|$, $\Delta I_{Fc} = I_{Fc} - I^0_{Fc}$, $|\Delta I_{Ru}| = I^0_{RuHex} - I_{RuHex}$, where I_{Fc},