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A reusable ratiometric electrochemical biosensor on the basis of the binding of methylene blue to DNA with alternating AT base sequence for sensitive detection of adenosine

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ABSTRACT

We develop a reusable ratiometric electrochemical biosensor on the basis of the binding of methylene blue (MB) to DNA with alternating AT base sequence for sensitive detection of adenosine. We design a strand 1 with MB-modified thymine (T) base in the proximal 3′ termini as the capture probe for its immobilization on the gold electrode and a 3′ termini ferrocene (Fc)-modified aptamer for the recognition of adenosine. The hybridization of strand 1 with the aptamer leads to the formation of a double-stranded DNA (dsDNA) and consequently the away of MB from the electrode surface and the close of Fc to the electrode surface, generating a small value of I_{MB}/I_{Fc} (I_{MB} and I_{Fc} are the peak currents of MB and Fc, respectively). In the presence of adenosine, its binding with the aptamer induces the release of Fc from the electrode surface and the close of MB to the electrode surface, generating a large value of I_{MB}/I_{Fc}. As a result, adenosine may be accurately quantified by the measurement of ratiometric signal (I_{MB}/I_{Fc}). This ratiometric electrochemical biosensor can be simply fabricated and exhibits high sensitivity with a limit of detection of as low as 90.8 pM and a large dynamic range from 0.1 nM to 100 μM. Moreover, this biosensor demonstrates good performance with excellent selectivity, regeneration capability, high reliability and good reproducibility, and may become a universal platform for the detection of various biomolecules which can be recognized by aptamers, holding great potential for further applications in biomedical research and clinical diagnosis.

1. Introduction

Electrochemical biosensors have distinct characteristics of easy fabrication, simple signal generation, and convenient miniaturization and integration, and have been widely applied in the design of portable detection devices (Rowe et al., 2011; Wu et al., 2011; Wu and Lai, 2016; Xiao et al., 2009, 2007a; Yang and Zhang, 2010). A number of electrochemical DNA (E-DNA) sensors have been developed on the basis of that the hybridization-induced conformational changes may significantly alter the electron transfer from a redox-tagged electrode-bound DNA probe to the electrode (Abi and Ferapontova, 2012; Dauphin-Ducharme and Plaxco, 2016; Du et al., 2014; Wu and Lai, 2013; Xiao et al., 2007a). Plaxco group demonstrated the development of a series of E-DNA sensors based on the binding-induced conformational changes for the detection of proteins (Lai et al., 2007; Xiao et al., 2005a, 2005b), heavy metal ions (Xiao et al., 2007b) and DNAs (Fan et al., 2003; Xiao et al., 2006, 2007a). A barrier to the wide adoption of these electrochemical biosensors is the issues related to the reproducibility, robustness and reliability, which result from the hard-to-avoid variations in electrode areas, DNA loading densities, and nontarget-induced reagent degradation/dissociation. The idiosyncratic background currents observed in disparate electrodes make it difficult to accurately detect target analyst in spite of the involvement of time-consuming background scans for each new electrode and each new analysis. Therefore, the development of a reusable ratiometric electrochemical biosensor with good reproducibility, robustness and reliability is highly desirable.

The ratiometric measurement has been extensively employed in various analytical techniques such as fluorescence (Dai et al., 2015; Liu et al., 2014), electrochemistry (Chai et al., 2014; Cheng et al., 2015; Jin

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et al., 2017; Ren et al., 2015; Wang et al., 2017; Zhang et al., 2015), electrochemiluminescence (Feng et al., 2015; Zhang et al., 2013) and photoelectrochemistry (Hao et al., 2016). Among them, the ratiometric electrochemical biosensor has attracted increasing attention because of its rapid response, simplicity and cost-effectiveness. The ratiometric measurement has the capability of self-reference by removing the signal fluctuation caused by complex experimental conditions (Cui et al., 2017), and greatly improves the reproducibility and robustness of electrochemical biosensors. The ferrocene (Fc) and methylene blue (MB) are usually used as the internal references for ratiometric measurement of proteins (Deng et al., 2016; Ren et al., 2014; Wang et al., 2017), DNAs (Cui et al., 2017; Du et al., 2014; Xiong et al., 2015), and other analysts (Jia et al., 2016; Xiong et al., 2015). In comparison with the single-signal readout, the ratio calculation of dual-signal readouts may significantly improve the detection sensitivity (Wu et al., 2013). So far, most of the reported ratiometric electrochemical biosensors are developed on the basis of the binding-induced conformational changes of terminal redox-tagged DNA, and the electrochemical response induced by only DNA-mediated electron transfer relies on the tuning of the probe structural flexibility with the limitations of high background and poor sensitivity (Campos et al., 2014; Rowe et al., 2011; Xiao et al., 2009; Yang et al., 2014). Notably, the electron transfer between the redox tag and the electrode is also dependent on the way of the redox tag conjugated to the DNA duplex (e.g., intercalation and groove binding) (Hsieh et al., 2011; Lubin et al., 2009; Rohs et al., 2000; Tuite et al., 1994; Wang et al., 2015). Rohs et al. investigated the binding behavior of methylene blue (MB) to a double-stranded decamer, with a groove binding mode for DNA with AT sequences (Rohs et al., 2000) and an intercalation binding mode for GC alternating DNA (Rohs et al., 2004). Hsieh et al. developed an electrochemical biosensor for the identification of single-nucleotide mismatches based on the principle that the interaction between MB and poly(thymine–adenosine) (T-A) duplexes may effectively slow down MB electron transfer rate (Hsieh et al., 2011). To the best of our knowledge, a reusable ratiometric electrochemical biosensor on the basis of the binding of methylene blue to DNA with alternating AT base sequence has never been reported so far.

Herein, we demonstrate the development of a reusable ratiometric electrochemical biosensor on the basis of the binding of methylene blue to DNA with alternating AT base sequence for sensitive detection of adenosine. We design a strand 1 with MB-modified thymine (T) base in the proximal 3′ termini as the capture probe for its immobilization on the gold (Au) electrode and a 3′ termini ferrocene (Fc)-modified aptamer for the recognition of adenosine (the aptamer binds adenosine with a dissociation constant (K_d) of 6 μM (Huizenga and Szostak, 1995)). In the presence of adenosine, its binding with the aptamer induces the release of Fc from the electrode surface and the close of MB to the electrode surface, generating a large value of I_{MB}/I_{Fc} (I_{MB} and I_{Fc} are the peak currents of MB and Fc, respectively). The measurement of ratiometric signal (I_{MB}/I_{Fc}) can be used for accurate quantification of adenosine. This ratiometric biosensor exhibits high sensitivity with a limit of detection of as low as 90.8 p.M. and a large dynamic range from 0.1 nM to 100 μM, and it performs well in real sample analysis with distinct advantages of excellent selectivity, regeneration capability, high reliability and good reproducibility.

2. Materials and methods

2.1. Materials and reagents

Mercaptohexanol (MCH), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), tris-(hydroxymethyl)aminomethane (Tris), adenosine, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), cytidine, guanosine and uridine were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All solutions were prepared with ultrapure water obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA) with a resistivity of 18.2 MΩ cm. All of the oligonucleotides were synthesized and purified with HPLC by Takara Biotech. Co. Ltd. (Dalian, China) and their sequences were listed in Table S1.

2.2. Fabrication of electrochemical biosensor

Prior to the modification, the Au electrode was polished to a mirror-like surface with 1.0, 0.3 and 0.05 μm α-Al_2O_3 slurry, respectively, followed by successive sonication with ethanol and ultrapure water for 3 min to remove residual alumina powder. The electrode was immersed into fresh piranha solution (3:1 v/v mixture of concentrated H_2SO_4 and 30% H_2O_2) for 10 min, followed by a thorough rinse with ultrapure water and drying by nitrogen. Then the electrode was electrochemically cleaned in 0.1 M H_2SO_4 solution by potential scanning between −0.2 and +1.6 V at a scan rate of 100 mV s^{-1} until a stable reproducible cyclic voltammogram was obtained.

After washing with ultrapure water and drying in a nitrogen stream, the electrode was incubated with 6 μL of solution containing 0.5 μM strand 1 and 25 μM TCEP (TCEP is used to reduce the disulfide bonded oligonucleotides) at room temperature for 12 h to make strand 1 immobilize onto the gold electrode surface via gold-sulfur chemistry. The electrode was then thoroughly rinsed with 10 mM PBS (pH 7.4) and dried under a stream of nitrogen gas. Subsequently, 6 μL of 1 mM MCH was dropped on the electrode for 60 min to block the unmodified sites. After rinsing thoroughly with 10 mM PBS (pH 7.4), the electrode was incubated with aptamer conjugates in 10 mM PBS (pH 7.4) containing 100 mM NaCl at 37 °C for 2 h. At last, the electrode was thoroughly rinsed and stored at 4 °C prior to use.

2.3. Detection of adenosine and regeneration of electrochemical biosensor

The 10 μL of adenosine with different concentrations was added into the electrochemical biosensor and incubated at 37 °C for 1 h. Then the electrode was thoroughly washed three times with PBS buffer. For the measurement of adenosine, alternating-current voltammetry (ACV) responses of the ratiometric probes before and after the addition of adenosine were monitored in10 mM PBS (pH 7.4) (note: 10 mM PBS (pH 7.4) should be completely purged with high purity nitrogen for 30 min to avoid the interference from the reduction of oxygen prior to the measurement). Finally, the aptamers were added onto the modified electrode to regenerate the electrochemical biosensor.

2.4. Electrochemical measurement

All electrochemical measurements were carried out on a CHI 660e electrochemical workstation (CH Instruments Inc., USA) at room temperature with a conventional three-electrode system composed of a platinum wire, a saturated calomel and a gold electrode (d = 2 mm) as the counter, reference and working electrodes, respectively. Cyclic voltammetry (CV) measurements were carried out in scanning potential from −0.2 to 0.6 V with 5 mM $[Fe(CN)_6]^{3-/4-}$ (containing 0.1 M KCl) as the electrolyte. Electrochemical impedance spectroscopic (EIS) measurements were performed in 5 mM $[Fe(CN)_6]^{3-/4-}$ redox couple solution (1:1 M ratio) containing 0.1 M KCl over a frequency range from 10 kHz to 0.1 Hz using an alternative voltage with an amplitude of 10 mV, superimposed on a dc potential of 0.20 V (vs SCE). The ACV was performed using a potential window of −0.5 to +0.6 V with a pulse amplitude of 50 mV and a pulse width of 200 ms in 5 mL of 10 mM PBS (pH 7.4) buffer. The chronocoulometry measurements were carried out over a range from +0.2 to −0.5 V at a pulse period of 250 ms in 10 mM Tris-HCl buffer containing 50 μM $Ru(NH_3)_6^{3+}$ (pH 7.4).