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Short Communication

A novel signal-on electrochemical DNA sensor based on target catalyzed hairpin assembly strategy

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ABSTRACT

We describe a novel signal-on electrochemical DNA (E-DNA) sensing platform based on target-catalyzed hairpin assembly. The thiolated modified molecular beacon 1 (MB1) was first immobilized onto the Au electrode (GE) surface and then target DNA hybridized to the MB1, the opened MB1 assembled with the ferrocene (Fc)-labeled molecular beacon 2 to displace the target DNA, which became available for the next cycle of MB1 – target hybridization. Moreover, Fc was confined close to the GE surface for efficient electron transfer, resulting in a current signal. Eventually, each target strand went through many cycles, resulting in numerous Fcs confining close to the GE, which leaded to the current of Fc dramatically increase. The observed signal gain was sufficient to achieve a demonstrated detection limit of 0.74 fM, with a wide linear dynamic range from 10^{-15} to 10^{-10} M and discriminated mismatched DNA from perfect matched target DNA with a high selectivity. Thus, the proposed E-DNA sensor would have a wide range of sensor applications because it is enzyme-free and simple to perform.

1. Introduction

DNA detection is great demand in gene profiling, drug screening, clinical diagnostics, environmental analysis, and food safety (Drummond et al., 2003; Guo et al., 2009; Chen et al., 2011; Liu et al., 2011). Motivated by this demand, various techniques for DNA detection have been developed, such as electrochemical (Fan et al., 2005; Hu et al., 2012; Tang et al., 2013), fluorescent (Zhao et al., 2012), chemiluminescent (Liu et al., 2013) methods. Up to now, electrochemical biosensors have received great interests because of the simplicity, fast response, relatively cheap cost, high sensitivity, and low power requirement of electrochemical methods (Zhang et al., 2013). Recent years, we have seen the development of a number of electrochemical DNA (E-DNA) sensors that detect hybridization-induced conformational changes in a redox tags (e.g. ferrocene or methylene blue)-modified, electrode-bound probe DNA, such as "signal-on" and "signal-off" E-DNA architectures (Fan et al., 2003; Immoos et al., 2004; Xiao et al., 2007; Wu and Lai, 2013). These E-DNA sensors 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 (Lubin et al., 2006).

In a "signal-off" sensor, the mechanism is the alternation of distance of the labeled redox tags from the electrode by target DNA-induced conformational change of probe (Lubin and Plaxco, 2010; Wang et al., 2012). However, such "signal-off" sensors suffer from limited signal capacity, in which only a maximum of 100% signal suppression can be attained under any experimental conditions (Ricci et al., 2007). Moreover, such "signal-off" assays might cause false-positive results due to the coexistence of environmental stimulus (Anne et al., 2003; Lubin et al., 2006). To circumvent this limitation, "signal-on" E-DNA sensors have been developed in the past years.

In contrast, "signal-on" sensors can achieve much improved signaling, and the background current observed in the absence of target is reduced, the gain of such a sensor, at least in theory, increases without limit. Thus motivated, others have explored a number of "signal-on" E-DNA architectures, such as DNA pseudoknot (Cash et al., 2009), hybridization-based double-stranded (Xiao et al., 2006), triblock structure (Immoos et al., 2004), inverted stem-loop (Rowe et al., 2011), triplex DNA structure (Idili et al., 2014), and traditional E-DNA sensor probed at new frequencies (White and Plaxco, 2010).

Despite these advances, above-mentioned "signal-on" strategy use redox-labeled capture DNA strand to provide a response signal of target. Unfortunately, although the labeled redox tags of capture DNA far away the electrode, they still have a high background

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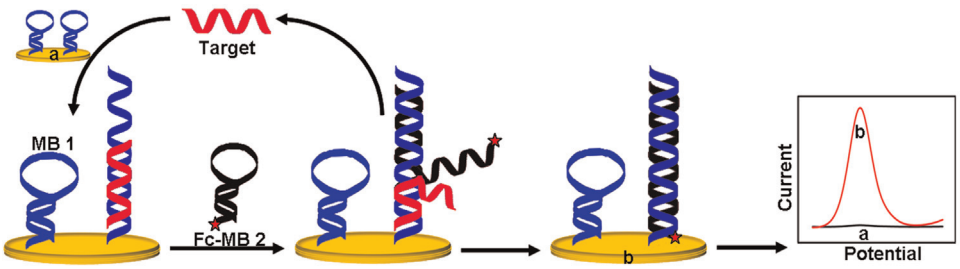

Scheme 1. Schematic of the signal-on E-DNA sensor, which is based on target catalyzing hairpin assembly strategy.

signal. Furmore, above-mentioned "signal-on" sensor is not sensitive enough, because a single target DNA molecule only reacts with a single signaling probe, limiting the total signal gain. In this paper, we reported an efficient "signal-on" electrochemical DNA sensing system based on target catalyzed hairpin assembly, which do not produce a background signal.

The target catalyzed hairpin assembly has recently attracted considerable attention due to enzyme-free DNA detection, in which the signal amplification is achieved by the cycling use of the target (Allen et al., 2012; Huang et al., 2012; Zheng et al., 2012). As shown in Scheme 1, the thiolated modified molecular beacon 1 (MB1) was first immobilized on the Au electrode (GE) surface and then target DNA hybridizes to the MB1, the opened MB1 assembles with the ferrocene (Fc)-labeled molecular beacon 2 (MB2) to displace the target DNA, which becomes available for the next cycle of MB1 – target hybridization. Moreover, Fc is confined close to the electrode surface for efficient electron transfer, resulting in a large current of the redox. Eventually, each target strand can go through many cycles, resulting in many Fc confining close to the GE which leads to the current of Fc dramatically enhancement. By monitoring the change of the current intensity, we can detect the target DNA with high sensitivity. In the absence of target, the MB1 and MB2 can only maintain the sufficiently stable stem-loop structure owing to the binding of the complementary sequences at the ends. Therefore, in addition to the simple and relatively low-cost detection process, the employment of MBs and the recycle of the target make this strategy appealing in amplifying electrochemical signal for selective and sensitive DNA detection.

2. Experimental

2.1. Oligonucleotides and regents

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).

MB1:5′-SH-AAGTAGTGATTGAGCGTGATGAATGTCACTAC
TTCAACTCGCATTCATCACGCTCAATC-3′
MB2:5′-TGATGAATGCGAGTTGAAGTAGTGACATTCA
TCACGCTCAATCACTACTTCAACTCGCA-Fc-3′
Target:5′-GACATTCATCACGCTCAATCACTACTT-3′
Single-base mismatched:5′-GACATTCATCAC
ACTCAATCACTACTT-3′
Three-base mismatched:5′-GACATTCATCACAC
TCAATCACTACTT-3′
Non-complementary:5′-ATGCTGACTGACAAG
CTTAGCAAGGG-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 MB1 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 MB1 (1 μM) was dropped on the cleaned GE for 4 h at room temperature in the dark. During this process, the MB1 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. Finally, 5 μL target DNA with the designed concentration and 5 μL MB2 (300 nM) were dropped on the surface of the electrodes. After the process was performed for 2 h 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. Cyclic voltammetry (CV) results were recorded within a potential range of 0–0.6 V (scan rate = 0.05 V s^{-1}). For all measurements, 4 successive cycles were carried out to ensure signal stabilization and the fourth cycle was kept as the result. Differential pulse voltammograms (DPVs) of Fc tag were registered in the potential interval 0.0 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. 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. The applied potential was 172 mV vs. Ag/AgCl (Zhang et al., 2009). This potential is near the equilibrium of $[Fe(CN)_6]^{3-/4-}$ pair, and makes the redox rates equal. Therefore, the redox species will not be depleted near the electrode surface during the measurement (Pan and Rothberg, 2005).

3. Results and discussion

3.1. Characterization of the E-DNA biosensor

CV measurements were used to monitor the change in surface features of the electrode after each step modification in this work.