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Molecular beacon mediated circular strand displacement strategy for constructing a ratiometric electrochemical deoxyribonucleic acid sensor

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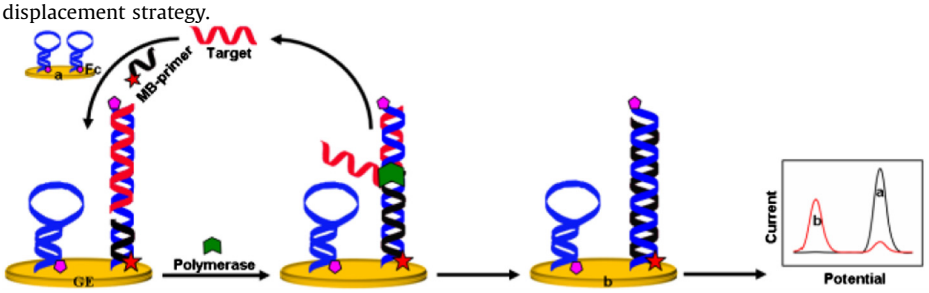
HIGHLIGHTS

- A novel ratiometric electrochemical method is developed for detection of target DNA.
- The signal amplification is based on molecular beacon mediated circular strand displacement strategy.
- The biosensor can detect DNA down to 28 fM level with a dynamic range spanning 5 orders of magnitude.
- The special structures of the hairpin probes promote the detection selectivity.

GRAPHICAL ABSTRACT

A novel ratiometric electrochemical DNA-sensor has been developed based on circular strand displacement strategy.



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ABSTRACT

A novel ratiometric electrochemical sensor for sensitive and selective determination of deoxyribonucleic acid (DNA) had been developed based on signal-on and signal-off strategy. The target DNA hybridized with the loop portion of ferrocene (Fc) labeled hairpin probe immobilized on the gold electrode (GE), the Fc away from the surface of GE and the methylene blue (MB) was attached to an electrode surface by hybridization between hairpin probe and MB labeled primer. Such conformational changes resulted in the oxidation peak current of Fc decreased and that of MB increased, and the changes of dual signals are linear with the concentration of DNA. Furthermore, with the help of strand-displacement polymerization, polymerase catalyzed the extension of the primer and the sequential displacement of the target DNA, which led to the release of target and another polymerization cycle. Thus the circular strand displacement produced the multiplication of the MB confined near the GE surface and Fc got away from the GE surface. Therefore, the recognition of target DNA resulted in both the "signal-off" of Fc and the "signal-on" of MB for dual-signal electrochemical ratiometric readout. The dual signal strategy offered a dramatic enhancement of the stripping response. The dynamic range of the target DNA detection was from 10^{-13} to 10^{-8} mol L^{-1} with a detection limit down to 28 fM level. Compared with the single signaling electrochemical sensor, the dual-signaling electrochemical sensing strategy developed in this paper was more selective. It would have important applications in the sensitive and selective electrochemical determination of other small molecules and proteins.

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1. Introduction

Sensitive and selective detection of DNA sequences plays a significant role in clinical diagnosis and biomedical studies. DNA plays essential roles in clinical diagnosis and therapy, pathogen detection and environmental monitoring [1–4]. In recent years, various biosensors, including fluorescent [5–7], chemiluminescent [8,9], electric signals [10,11], Raman spectroscopic [12,13], colorimetric [14,15], and electrochemical methodologies [16–18] have been developed. Among these protocols, the electrochemical DNA sensors (E-DNA) have a variety of intrinsic advantages, including high sensitivity, relatively low cost, and amenability to miniaturisation and multiplexing. These devices are composed of a redox-reporter-modified DNA probe immobilized on a gold electrode (GE). Hybridization-linked changes in the flexibility of the redox-reporter-modified DNA probe, which include "signal-on" and "signal-off" E-DNA sensors [19,20]. In a "signal-off" sensor target binding limits collisions between the redox tag and the electrode thereby reducing the signaling current [21–24]. 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 conditions [25–27]. "Signal-on" sensors, in contrast, can achieve much improved signaling [28–30]; as the background current observed in the absence of target is reduced, the gain of such a sensor, at least in theory, increases without limit [31–33]. By this incentive, others have explored a number of signal-on E-DNA architectures, including a DNA pseudoknot, a hybridization-based double-stranded sensor, a triblock structure, an inverted stem-loop, and a traditional E-DNA sensor probed at new frequencies [34–39]. Despite recent advances, the E-DNA biosensors still only use one DNA strand of the DNA duplex to provide a response signal of target based on "signal-on" or "signal-off" mechanism alone; the another DNA strand does not have any contribution to the response signal. It is no doubt that both DNA strands of the DNA duplex with redox labels which could provide the multiple response signals at various redox potentials would have obvious advantages. In this paper, we construct a ratiometric electrochemical DNA sensor based on utilizing two electrochemical labels as an amplification strategy.

Ratiometric detection has been developed extensively in fluorescence and electrochemiluminescence analysis of biomolecules [40–42]. Recently, there was only one paper reported that E-DNA sensors utilize two electrochemical labels as an amplification strategy simultaneously for robust detection of DNA [43]. However, above-mentioned method is not sensitive enough, because a single target DNA only opens a single signaling methylene blue (MB) and ferrocene (Fc) (Fc as an internal control)-labeled probe, limiting the total signal gain and corresponding sensitivity. This paper construct a ratiometric electrochemical DNA sensor based on molecular beacon mediated circular strand displacement for target DNA recycling strategy.

This work first immobilized Fc-labeled hairpin probe (Fc-HP) on the GE through Au—S bond, in which Fc is confined near the electrode surface, giving an intense electrochemical signal. In the presence of target DNA, the loop of HP was opened, and the Fc away from the surface of GE, significant reduction of the Fc signal. At the same time, MB-labeled primers (MB-primer) are introduced onto the electrode surface by hybridizing with the stem of HP, Fc is confined near the electrode surface, resulting in the generation of electrochemical signals. MB got away from the electrode surface and Fc approached to the electrode, which led to "signal-off" and "signal-on" elements for dual-signal electrochemical ratiometric readout (Scheme 1). However, above-mentioned method is not sensitive enough, because a single target DNA only opened a single signaling Fc-HP probe and introduced a MB-labeled primer on the GE surface, limiting the total signal gain and corresponding sensitivity. To circumvent this limitation, we introduced polymerase-based circular strand-replacement polymerization (CSRP) for target DNA recycling amplification. The polymerase catalyzed the extension of the primer and the sequential displacement of the target DNA. The released target found another HP to trigger the polymerization cycle, resulting in the multiplication of the Fc confined near the GE surface and MB got away from the GE surface. The dual-peak current ratiometry provided precise and sensitive measurement. It would have important applications in the sensitive and selective electrochemical determination of other small molecules and proteins.

2. Experimental

2.1. Oligonucleotides and reagents

DNA polymerase and the mixture of deoxyribonucleotides (dNTPs) were obtained from Fermentas Biotechnology Co. Ltd. (Canada). Human serum samples were kindly provided by the affiliated hospital of Xuzhou Medical College (Xuzhou, China). 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).

Fc-HP: 3′-SH-ATCGATTACCGCGTTCGGTGGCTGTTCTACGTAATC-GAT-Fc-5′

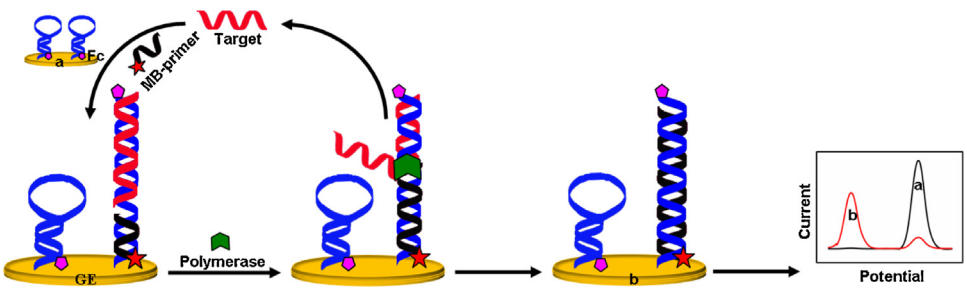
Target: 5′-GGCGCAAGCCACCGACAAGATGCATTAGCTA-3′

MB-primer: 5′-MB-TAGCTAAT-3′

Single-base mismatched: 5′-GGCGCAAGCCACCGGCAAGATG-CATTAGCTA-3′

Three-base mismatched: 5′-GGCTCAAGCCACCAACAAGATG-CATTAACTA-3′

Non-complementary: 5′-TAGCTAGCCGAATGGCAATCCATTA-GATGCT-3′



Scheme 1. Schematic of the ratiometric electrochemical DNA sensor based molecular beacon mediated circular strand displacement strategy.