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Short communication

Electrochemical molecular beacon biosensor for sequence-specific recognition of double-stranded DNA

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ABSTRACT

Direct recognition of double-stranded DNA (dsDNA) was crucial to disease diagnosis and gene therapy, because DNA in its natural state is double stranded. Here, a novel sensor for the sequence-specific recognition of dsDNA was developed based on the structure change of ferrocene (Fc) redox probe modified molecular beacon (MB). For constructing such a sensor, gold nanoparticles (AuNPs) were initially electrochemical-deposited onto glass carbon electrode (GCE) surface to immobilize thiolated MB in their folded states with Au–S bond. Hybridization of MB with target dsDNA induced the formation of parallel triplex DNA and opened the stem-loop structure of it, which resulted in the redox probe (Fc) away from the electrode and triggered the decrease of current signals. Under optimal conditions, dsDNA detection could be realized in the range from 350 pM to 25 nM, with a detection limit of 275 pM. Moreover, the proposed method has good sequence-specificity for target dsDNA compared with single base pair mismatch and two base pairs mismatches.

1. Introduction

Design of simple and sensitive sensor for sequence-specific recognition of double-stranded DNA (dsDNA) was essential in disease diagnosis and human gene therapy, because DNA in its natural state is double stranded (Dylla-Spears et al., 2009; Rucker et al., 2003; Vasquez et al., 2000; McKenzie et al., 2008). Routine protocols for sequence-specific recognition of dsDNA are generally performed by using polyamides (Park et al., 2010; Singh et al., 2013), DNA binding proteins (Thompson, 2006; Roberts et al., 2009) and triplex-forming oligonucleotides (Miao et al., 2011; Patterson et al., 2010; Xiao et al., 2013; Sau et al., 2009). Among which, triplex-forming oligonucleotides that possess the properties of could bind to the major groove of dsDNA and exhibit high sequence specifity, have been increasingly empolyed for the analysis of dsDNA. These triplex-former based methods, however, commonly require the protonation of cytosine for the formation of C·G°C (· denotes Watson–Crick bond, ° denotes Hoogsteen bond) in parallel triplex DNA structure, and acidic pH environment is essential for the protonation of cytosine (Ihara et al., 2008). Thus, a number of researches were mainly performed under acidic pH environment, which are not suitable in real DNA samples detection. In contrast, Ihara group found that C·G°C could form under neutral pH environment upon addition of Ag^{+} recently (Ihara et al., 2009). Inspired by such advance, our laboratory has reported several studies about dsDNA detection in neutral pH environment (Xiao et al., 2013; Xiong et al., 2011).

Molecular beacon (MB), a hairpin-shaped single-stranded oligonucleotide that was initially reported by Tyagi and Kramer (1996), can undergo a structural change from stem-loop to open-chain form based on the hybridization of it with target DNA. Due to the unique structural properties of MB, great efforts have been made worldwide to develop MB-based sensors for DNA (Lin and Tseng, 2012; Xuan et al., 2012; Rai et al., 2012; Li et al., 2011), RNA (Piao et al., 2012), protein (Tang et al., 2011; Wang et al., 2011), metal ions (Kim et al., 2012; Zhuang et al., 2013; Huang et al., 2013; Zhang et al., 2010) and small molecules detection (Wang et al., 2009; Xu and Hepel, 2011; Tang et al., 2011). Up to now, most of MB sensing methods are quencher-fluorophore pair based fluorescence (Yang et al., 2005; Hwang et al., 2004). Compared to such fluorescent methods, MB-based electrochemical sensors have attracted considered attention due to their intrinsic advantages, including good portability, lower cost, simple instrumentation and high sensitivity. For example, Tang groups reported an electrochemical assay for mercury(Π) based on the T–Hg^{2+}–T coordination chemistry (Tang et al., 2011). Cheng et al., developed MB-based electrochemical DNA biosensors for *Legionella sp.* (Rai et al., 2012). However, to the best of our knowledge, no study has been reported about the sequence-specific recognition of dsDNA

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by using MB-based electrochemical methods. Herein we reported a simple and sensitive electrochemical sensor for sequence-specific recognition of dsDNA through triplex DNA formation for the first time.

2. Experimental

2.1. Apparatus

All electrochemical measurements were carried out on a CHI 650E electrochemical workstation (Chenhua, Shanghai, China) with a conventional three-electrode system consisted of a modified working electrode, a platnum wire counter electrode and an Ag/AgCl reference electrode. The circular dichroism (CD) spectroscopy was obtained from a J-810-150S spectropolarimeter (JASCO International Co. Ltd., Japan).

2.2. Fabrication of molecular beacon modified electrode

First, the glass carbon electrodes (GCE, ¢=3 mm, CHI) were polished successively with 1.0, 0.3, 0.05 μm alumina slurry to obtain a mirror surface and ultrasonicated in an ethanol/water bath for 5 min. Then, gold nanoparticles (AuNPs) were electrochemically deposited onto GCE surface according to literature (Xiang et al., 2011). After that, AuNPs modified GCE was immersed into 3.0 μM of thiolated molecular beacon (MB) and incubated overnight at room temperature (before modification, the disulfide bond at the 3′ end of MB was cleaved by using Tri-(2-carboxyethyl) phosphine (TCEP)). The DNA modified electrodes were treated for 60 min with 2 mM of 6-mercapto-1-hexanol (MCH) in phosphate buffer solution (PBS). Finally, the as-prepared DNA sensors were suspended over pH 7.4 PBS at 4 °C for further use.

3. Results and discussion

3.1. Principle of the sensor

The scheme for sequence-specific recognition of dsDNA was illustrated in Fig. 1, ferrocene (Fc) modified MB was assembled on the surface of AuNPs-deposited electrode with Au–S bond. The MB probes were designed as follows: each MB probe has six complementary bases at its 5′ and 3′ ends, which forms the double-stranded stem and brings Fc to proximity of the electrode surface (Wang et al., 2009). The underlined portion of MB is the loop region that can recognized with target dsDNA. Hybridization of MB with target dsDNA induced the formation of triplex DNA and the unfold of its stem-loop structure, which directly induced the redox-lag (Fc) away from electrode and resulted in the decrease of current signals.

3.2. Characterization of modified electrode

The characterization of modified electrode was monitored by differential pulse experiments (DPV). As shown in Fig. 2, compared with that of AuNPs modified electrode (a), after the assembly of MB on the surface of AuNPs deposited electrode, an obvious redox peak was observed (b), which mainly due to the high conductivity of Fc. Then, both the cathodic and anodic peak currents decreased obviously (c) when the hybridization occurred between MB and target dsDNA, indicating that the hybridization of them effectively opened the stem-loop structure of MB, accordingly induced the redox-lag away from the electrode, and resulted in a lower

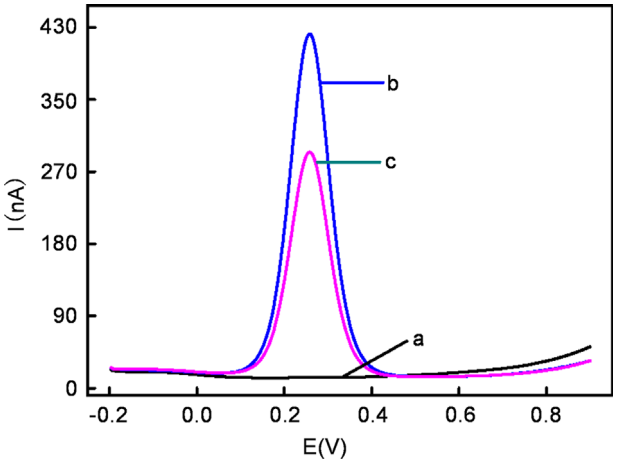

Fig. 2. DPV of the different modified electrodes in 20 mM PBS solution (pH=7.4). (a) AuNPs/GCE; (b) MB/AuNPs/GCE; (c) target dsDNA/MB/AuNPs/GCE. Scan rate: 50 mV/s, scan range: −200–900 mV.

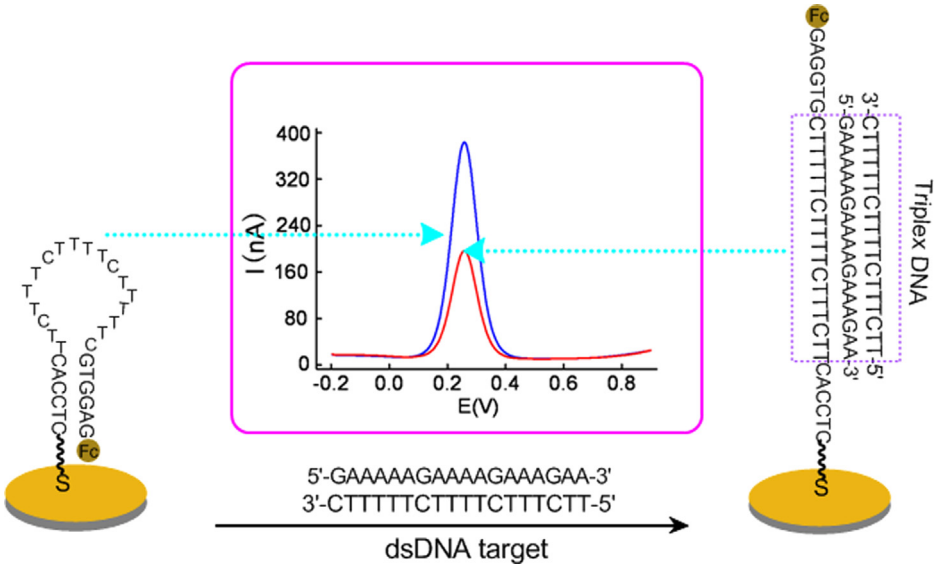

Fig. 1. Scheme of the electrochemical sensor for target dsDNA detection.

efficiency of electron transfer (ET) between the electrode and redox-lag.

3.3. Circular dichroism spectroscopy for DNA strands

To further explore the formation of triplex DNA, circular dichroism (CD) spectroscopy of different DNA strands was investigated in Fig. S1. Compared with MB by itself (a), an obvious negative cotton effect at 210 nm could be observed for the mixture of MB and target dsDNA without (b) or with the presence of spermine (c), which indicated the formation of triplex DNA. Then, upon addition of Ag^+, the negative peak around 210 nm increased dramatically (d), such result obviously revealed that Ag^+ could enhance the stability of triplex DNA effectively, which was in good accordance with the reports of literatures (Xiao et al., 2013; Xiong et al., 2011).

3.4. Optimization of experimental conditions

Parallel triplex DNA that contained C·G°C triads can be formed in neutral pH environments upon addition of Ag^+ (Ihara et al., 2009), Thereby, the concentration of Ag^+ plays an important role in the stability of triplex DNA. Here, the effect of Ag^+ concentration was investigated in Fig. S2A, and the change of current intensities (ΔI, $I=-(I_{\text{target dsDNA}}-I_{\text{no target dsDNA}})$) increased along with the increase of Ag^+ concentration over the range from 0.5 to 25 μM, then it decreased gradually when the concentration of Ag^+ was higher than 25 μM (relative standard deviations (RSD) was 4.16% ($n=5$)). Therefore, 25 μM of Ag^+ was selected for the recognition of dsDNA.

Multivalent cations such as Mg^{2+} and polyamines were usually used to neutralize the negative charges of DNA for triplex formation (Yan et al., 2009). Here, spermine was selected to reduce the electrostatic repulsion between dsDNA and MB. The change of current intensities was found to be proportional to the spermine concentration over the range from 0 to 10 μM. However, it decreased gradually if the concentration of spermine was higher than 10 μM (Fig. S2B, RSD was 3.89% ($n=5$)), which might be due to the reason that excess spermine could induce DNA condensation and precipitation (Saminathan et al., 1999). Thus, 10 μM of spermine was selected for the research.

Hybridization time between MB and dsDNA may affect the current intensities. As shown in Fig. S2C, the ΔI increased with the increase of hybridization time from 0 to 30 min, and then reached a platform after that (RSD was 5.16% ($n=5$). Thus, 30 min was selected for all of the experiments.

3.5. Electrochemical responses and sequence selectivity of the sensor to dsDNA

Under optimal conditions, the relationship between electrochemical response and the concentration of dsDNA was evaluated in Fig. 3A, and the current intensities decreased linearly along with the increase of dsDNA concentration over a range from 350 pM to 25 nM, with a detection limit of 275 pM (3σ/slope). The linear regression equation was $I=-12.75C+403.48$ (C: nM, $R^2=0.9952$) with an acceptable relative standard deviations (RSD) of 4.36% ($n=5$). Such high sensitivity of the assay was mainly due to the magnification properties of deposited AuNPs. Meantime, the characteristics of the proposed method were compared with other methods in Table S2. The sensitivity of the proposed method was much lower than other fluorescent methods. In addition, the proposed method was much more suitable for dsDNA recognition, because it could be realized in neutral pH environment while other electrochemical and dynamic light scattering (DLS) methods were constructed in acidic pH environment. Thereby, the assay was very promising for the recognition of dsDNA.

Moreover, two control dsDNA were designed to estimate the sequence specificity of the assay. Compared with that of target dsDNA, one CG base pair was replaced by TA base pair in control dsDNA 1 while two CG base pairs at two sides were replaced by two TA base pairs in control dsDNA 2. As shown in Fig. 3A, the current intensities was decreased along with the increasing of control-dsDNA 1 concentration in 1.0–20 nM, while control dsDNA 2 concentration was 5.0–14 nM. Meantime, the change of current intensities was manifest upon addition of 10 nM target dsDNA while it was weak for control dsDNA 1 and control dsDNA 2 (Fig. 3B). Such results indicated that the sensor has high sequence specificity for dsDNA detection.

4. Conclusion

In conclusion, highly electrochemical sequence-specific recognition of dsDNA was constructed based on the structure change of ferrocene (Fc) redox probe modified molecular beacon (MB). The sensor has excellent properties such as low detection limit (275 pM) because of the magnification properties of deposited AuNPs. Moreover, target dsDNA detection could be realized under

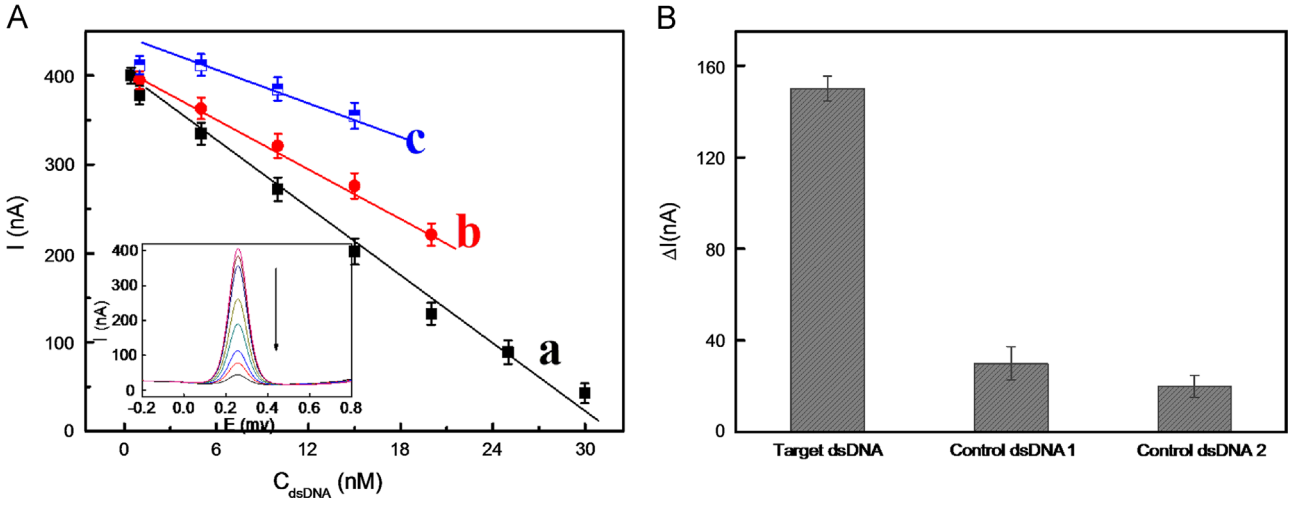

Fig. 3. (A) Calibration curve of target dsDNA detection in 20 mM PBS solution (pH=7.4) by using MB/AuNPs/GCE (Inset: DPV curve). (B) Selectivity of the sensor for dsDNA detection.

neutral pH environment in the presence of Ag^{+}, which accordingly breakthrough the limit that triplex DNA containing C·G°C triads only could be formed only under acidic pH environment, and made the sensor suitable in real DNA samples detection. Meantime, the strategy has the merit of high sequence-specificity.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.03.009.

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