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Short communication

DNA-based amplified electrical bio-barcode assay for one-pot detection of two target DNAs

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ABSTRACT

A sensitive label-free bio-barcode assay provided a PCR-free method for quantitative detection of two nucleic acid targets (HTLV-I and HTLV-II) simultaneously. This DNA biosensor was fabricated with two-component oligonucleotide-modified gold nanoparticles (AuNPs) and two-component oligonucleotide-modified magnetic beads (MBs), which can sandwich a specific target. After liberating the adsorbed thiolated barcode DNA strands (poly A and poly G) from the AuNPs surface with dithiothreitol (DTT) and acidic dipurinization, the electrochemical measurements were directly performed based on the redox activity of guanine (G) and adenine (A) nucleobases. Under the optimal assembling and detection conditions, a good linearity for simultaneous detection was obtained in the range from 4.4×10^{-11} to 2.0×10^{-9} M, and the detection limit (3σ) was estimated to be 1.71×10^{-12} M for T_1-DNA and 1.55×10^{-12} M for T_2-DNA.

1. Introduction

Human T-lymphotropic virus types I and II (HTLV-I and HTLV-II) are associated with the clinical diagnoses of adult T cell lymphoma/leukemia (Poiesz et al., 1980), rare lymphocytic neoplasms (Kalyanaraman et al., 1982), and progressive neurodegenerative disease (Bhagavati et al., 1988). However, there can be as little as a single infected cell in a blood sample (Ho et al., 1989), so it is necessary to develop a powerful amplification technique, such as polymerase chain reaction (PCR), for the reliable detection of retroviral DNA (Vet et al., 1999). Due to the complicated, expensive, time-consuming procedures and nonspecific amplification of PCR, various PCR-free methods have been developed for nucleic acid detection in the past decade (Dubus et al., 2006; Park et al., 2002). Among them, the bio-barcode amplification assay is the only biodetection method that has the PCR-like sensitivity for both protein and nucleic acid targets without a need for enzymatic amplification (Chang et al., 2007; Nam et al., 2003; Liu et al., 2008). This method relies on an oligonucleotide-modified gold nanoparticle (AuNP) for signal amplification and magnetic microparticle for easy separation from unreacted elements. The barcode strands released from the nanoparticle surfaces can be used as a means of amplification to quantitatively detect the target. Mirkin and co-workers have done many works in this field. For example, they have achieved the highly selective detection of four DNA targets (Stoeva et al., 2006b) and three protein cancer markers (Stoeva et al., 2006a) based on the bio-barcoded nanoparticle probes by scanometric analysis.

Until now, electrochemical detection of DNA has attracted considerable interest due to its simplicity, low cost and high sensitivity (Wang, 1999; Palecek and Fojta, 2001). To further increase the sensitivity, several strategies have been developed by employing enzyme labels (Hwang et al., 2005; Alfonta et al., 2001), nanoparticles (Baron et al., 2007) and electroactive tags (Ihara et al., 1997; Kertez et al., 2000). Although these modification techniques could achieve low detection limit, they are often encountered the risk of contamination, human error, and mechanical damage of the probe. It is well-known that G and A nucleobases possess the redox activity and can produce electrochemical responses (Wang et al., 1995). Based on the redox activity of purine nucleobases, several label-free bioelectrochemical detections have been performed. For example, He et al. (2007) realized the detection of thrombin by the highly sensitive redox activity of A nucleobases. Wang and Kawde (2002) developed a route for detection of DNA segments related to the BRCA1 breast cancer gene (mainly composed of A and G nucleobases). Besides, Wang et al. (2004) also reported a bioelectrochemical strategy for the measurement of proteins using nucleic acid tracers.

In this study, a new bio-barcode assay has been explored based on the intrinsic DNA electroactivity at electrochemically modified glassy carbon electrode (GCE). A platform for one-pot simultaneous detection of two target DNAs (HTLV-I and HTLV-II) was achieved by integrating the easy magnetic separation of MBs with the amplifi-

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cation feature of AuNPs and ploy A/G. Under the optimum assembly and detection conditions, the detection limits of both DNA targets were estimated to be pM, which were two orders of magnitude lower than that reported by Wang (Wang and Kawde, 2002). In Wang's work, oligonucleotide probes (captured on magnetic beads) were hybridized to the DNA target, and the measurements of the free nucleobases were carried out after dissociation of the hybrid DNA from the beads and an acidic dipurinization. Compared with this work, we used dG_{25} and dA_{25} tags as barcode DNA, and Au nanoparticles as the amplification carriers which could load hundreds of DNA probes (Zhang et al., 2008). Both of these methods used in our study could amplify the concentration of purine for later electrochemical detection.

2. Experiment performance

2.1. Materials and apparatus

2.1.1. Materials

All the reagents were analytical grade and used without further purification. Doubly distilled water (DDW) was used throughout this work. Carboxyl-modified magnetic beads (diameter 1.0 μm, 20 mg/mL) were ordered from Tianjin BaseLine Chromtech Research Centre. DTT, tri-(2-carboxyethl) phosphine hydrochloride (TCEP) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma. $HAuCl_4$ was obtained from Tianjin Yingda Sparseness & Noble Reagent Chemical Factory. Synthesis and modification of AuNPs were described in supplementary material, and the TEM of MBs and AuNPs were shown in Fig. S1 (supplementary material). The oligonucleotides were acquired from Sangong Inc. (Shanghai, China) with the following sequences:

capture DNA-1, 5′-NH_2-TCC TCC AGG-3′; capture DNA-2, 5′-NH_2-CTC CCC CGA-3′; target DNA-1, 5′-GAG TAT TTG CGC ATG GCC TGG AGGA-3′ (HTLV-I); target DNA-2, 5′-CGA AGG TGG AAA TTG GGT CGG GGAG-3′ (HTLV-II); target binding DNA-1, 5′-CGC AAA TAC TCA AAA AAA AAA AAA AAAA-SH-3′; target binding DNA-2, 5′-TTC CAC CTT CGG GGG GGG GGG GGG GGGG-SH-3′; barcode code DNA-1, 5′-AAA AAA AAA AAA AAA AAA AAA AAAA-SH-3′; barcode code DNA-2, 5′-GGG GGG GGG GGG GGG GGG GGG GGGG-SH-3′

2.1.2. Apparatus

Differential pulse voltammetry (DPV) was performed with a CHI 832B electrochemical analyzer (Shanghai CH Instrument Company, China). A three-electrode system was employed with Pt wire as auxiliary electrode, saturated calomel electrode (SCE) as reference electrode and modified GCE as the working electrode.

2.2. Immobilization and hybridization

The oligomer-coated MBs were prepared according to the modification of the literature (Miao et al., 2008). Briefly, 100 μL suspension of carboxyl-modified MBs were washed three times with 100 μL of 0.1 M imidazole buffer (pH 6.0), and then activated in the same buffer solution (containing 0.2 M EDC) with gentle shaking for 60 min. 70 μL of 4.41×10^{-5} M capture DNA-1 and capture DNA-2 (mixed in an appropriate ratio) was added into the activated MBs and incubated for 1 h at 37 °C with gentle mixing. The resulting probes were washed three times with 100 μL PBS buffer A (0.05 mM PBS, pH 7.4, 0.5 M NaCl) and resuspended in 20 μL PBS buffer A before use.

The sandwich assay involved a dual hybridization event. Corresponding amounts of target DNA-1 and target DNA-2 were added into the hybridization buffer containing two probe-coated MBs. After hybridization for 1 h at 37 °C, the resulting MBs were separated magnetically from the solution and washed twice with PBS buffer A. And then the MBs were resuspended in 100 μL of DNA–AuNPs with gentle shaking for 1 h at 37 °C. The resulting particle-linked DNA assembly was removed from the mixture using the magnetic separator and washed twice with 100 μL PBS buffer A.

2.3. Modification of the glassy carbon electrode

The modification of GCE was carried out as described in the literature (Wang et al., 2002). Firstly, GCE was oxidized at +1.8 V under stirring in 0.1 M PBS (pH 5.0) for 300 s, and then reduced by cyclic sweep between 0.3 and 1.30 V for 20 cycles until a steady-state curve was obtained in the same electrolyte solution.

2.4. Detection of two target DNAs

The MBs were dispersed in DTT (0.05 M, 50 mL) solution for 60 min with gentle shaking to release barcode DNAs (Thaxton et al., 2005). The supernatant was transferred into a 1.5 mL glass cell, followed by addition of 3 M H_2SO_4 (10 μL), and then heated to dryness. An acetate buffer solution (1 mL, 0.5 M, pH 5.9) containing 0.4 mM Cu^{2+} was used to transfer the digested DNA into the electrochemical cell. The accumulation of purine bases at modified GCE was done in a stirred solution at −0.1 V for 4 min. After a 30 s quiet period, DPV measurements were performed from 0.7 to 1.6 V with the pulse amplitude of 50 mV and the pulse width of 50 ms.

3. Results and discussion

3.1. The fabrication of the biosensor and the detection process

The principle of the protocol presented in this work was shown in Fig. 1. A sandwich-type DNA hybridization strategy involved NH_2-functionalized capture DNA coupled to carboxyl-modified MBs and thiol-functionalized target binding DNA self-assembled on AuNPs, both of which flank the DNA target sequence. Barcode DNA dG_{25}-SH and dA_{25}-SH tags were also self-assembled on AuNPs. After magnetic separation from unwanted DNA, the sandwich-type sensor was fabricated. Then the solution was treated with DTT, a common disulfide reducing agent, to liberate the covalently attached barcode sequences from the AuNP probes, followed by acidic dipurinization of the released barcode DNA, accumulation at modified GCE, and DPV measurement of free nucleobases. By this method, one target signal had been transformed into multiple redox signal of purine, hence amplifying the target signal.

In this work, two kinds of capture DNAs were assembled on MBs, and two barcode DNAs dA_{25} and dG_{25} tags were employed, two target DNAs (HTLV-I and HTLV-II) could be detected simultaneously.

3.2. Optimization of assembling condition

In order to establish optimal assembling conditions for simultaneous detection, several parameters were investigated systematically. First, the amounts of capture DNA (given capture DNA-1 as example) on 100 μL of MBs were optimized (see Fig. S2 (supplementary material)). The DPV peak current increased with the increase of capture DNA-1 in the range of 0.4–3.08 nmol indicating that the increased levels of capture DNAs were coupled on the surface of MBs. Above 3.08 nmol of capture DNA-1, the DPV peak current decreased. It could be speculated that the density of capture DNA on each magnetic bead was possibly too high and the hybridization efficiency was thus decreased. Consequently, 3.08 nmol (4.41×10^{-5} M, 70 μL) of capture DNA-1 was employed in the subsequent work.

Since the barcode DNA rather than the target binding DNA provides maximum signals amplification by electrochemical detection (Zhu et al., 2008), the number of barcode DNA conjugated to the surface of a nanoparticle should reach saturation while the

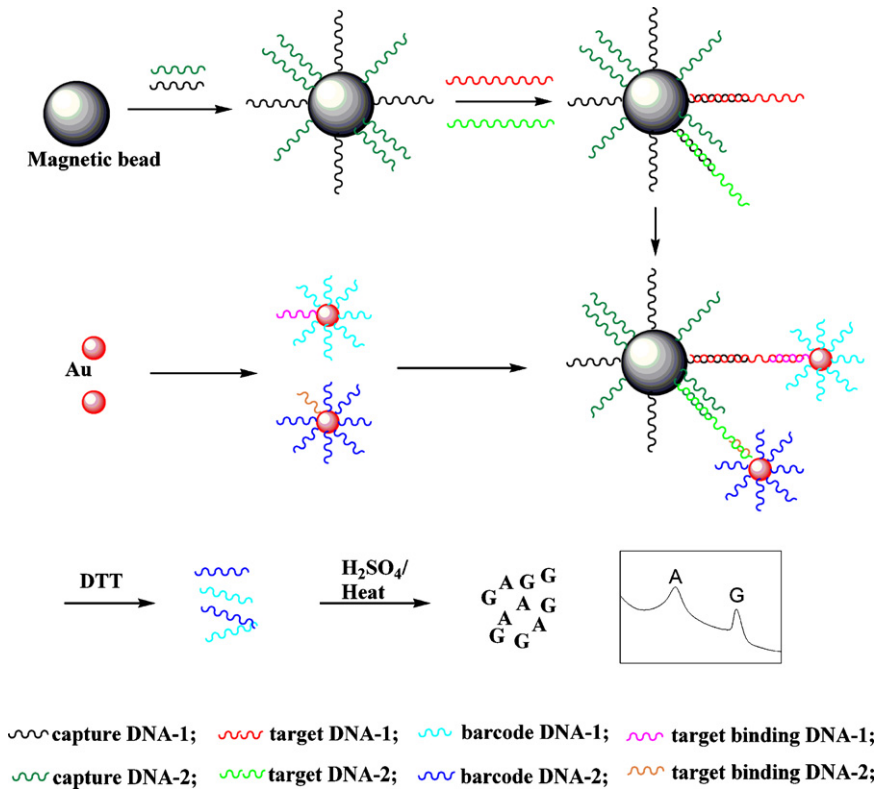

Fig. 1. Schematic diagram for the biosensor fabrication.

number of target binding DNA should keep a minimum. However, low concentration of the target binding DNA may influence the hybridization effect and thus interfere with the formation of sandwich structure. So the ratio of thiolated barcode DNA to thiolated target binding DNA should be optimized. Fig. S3 (supplementary material) shows the effect of barcode DNA-1 to target binding DNA-1 ratio on peak currents. The optimal ratio of barcode DNA-1 to target binding DNA-1 was 7:3, and the result was the same as that of barcode DNA-2 to target binding DNA-2 (figure not shown).

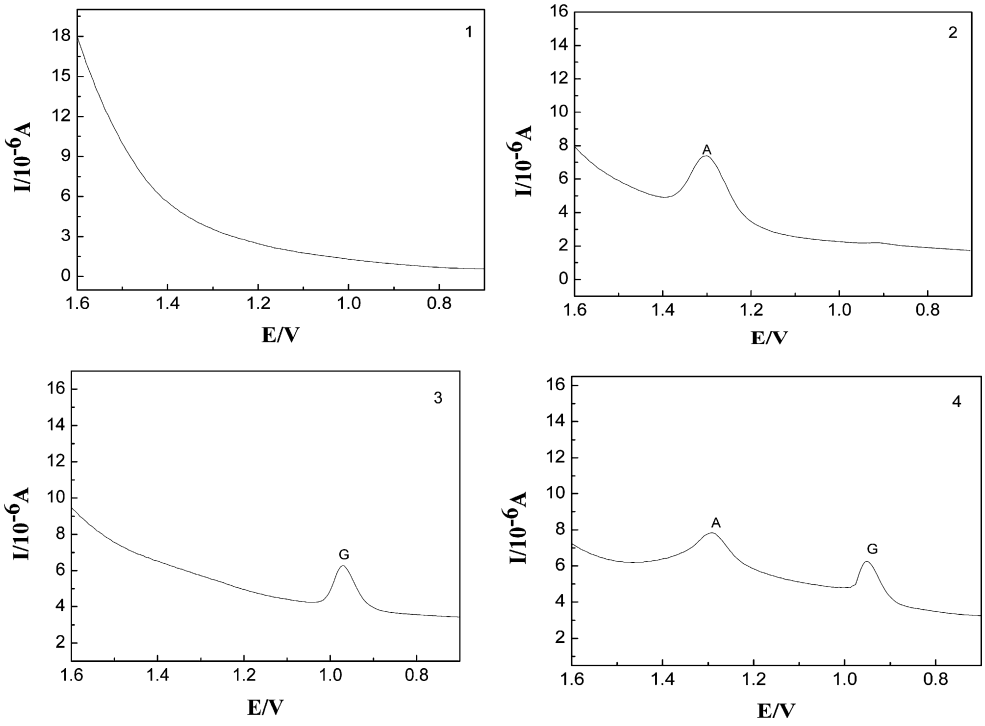

Fig. 2. DPV responses for (a) no target DNA; (b) with 1.5 nM target DNA-1 (70 μL of 4.41×10^{-5} M capture DNA-1); (c) with 1.5 nM target DNA-2 (70 μL of 4.41×10^{-5} M capture DNA-2); (d) with 0.75 nM target DNA-1 and 0.75 nM target DNA-2 (28 μL of capture DNA-1 and 42 μL of capture DNA-2, both in the concentration of 4.41×10^{-5} M). The procedure was carried out under optimized assemble and detection conditions.

3.3. Optimization of detection condition

Chen has demonstrated that electrochemical modified GCE showed more than 100-fold current response of guanine residues compared with that at an unmodified GCE (Miao et al., 2008). Shiraishi and Takahashi (1993) demonstrated that the anodic response of free A and G could be greatly enhanced in a copper solution. In this study, by using modified GCE in the presence of 0.4 mM Cu (II), favorable electrochemical signals could be gained (shown in Fig. S4 (supplementary material)).

To further improve the detection sensitivity, other parameters such as the accumulation time (Fig. S5 (supplementary material)) and accumulation potential (Fig. S6 (supplementary material)), have also been examined. As illustrated here, accumulation at −0.1 V for 4 min is the optimal detection condition (in combination with 3 M sulfuric acid and heating to facilitate the dipurinazation) shown by our modified GCE, which is identical to Wang's pencil graphite electrode (Wang and Kawde, 2002).

3.4. Detection of two kinds of targets

The electrochemical response of purine was used for the detection of DNA segments related to HTLV-I and HTLV-II gene. While DNA acted as an ideal molecular label, the DPV responses of the A and G nucleobases indicated HTLV-I and HTLV-II gene, respectively. As shown in Fig. 2, there was no DPV response signal for the sample without any target DNA (Fig. 2(a)). When there was 1.5 nM target DNA-1 in the sample, a DPV peak at +1.30 V was observed which can be identified as the oxidation of A (Fig. 2(b)), and the oxidation peak of G appeared at +0.97 V for the same concentration of target DNA-2 (Fig. 2(c)). For both 0.75 nM target DNA-1 and 0.75 nM target DNA-2 in the sample, there were two DPV response peaks appearing at +1.30 V and +0.97 V, respectively (Fig. 2(d)). Comparing Fig. 2(d) with Fig. 2(b) and Fig. 2(c), it can be found that the signals at +1.30 and +0.97 V should be corresponding to the oxidation of A and G, respectively, based on the potential.

In addition, the result showed that with the fixed concentration of target DNAs (1.5 nM), when capture DNA-1 was used, the electrochemical signal response was about 2.85×10^{-6} A at potential of +1.30 V (Fig. 2(b)), while signal response of 1.86×10^{-6} A at potential of +0.97 V was obtained by using capture DNA-2 (Fig. 2(c)). The ratio of the two peak current was about 3:2, which was caused by higher sensitivity of the A nucleobase. To obtain similar peak current signals with equal concentration of two target DNAs in one-pot, the ratio of the two capture DNAs on MBs was chosen as 2:3 (capture DNA-1:capture DNA-2) in later simultaneous detection (Fig. 2(d)). The surface coverages of two capture DNAs on MBs could be determined according to UV absorbance and fluorescence. The ratios of MBs, capture DNA-1, and capture DNA-2 were approximately $1/1.57 \times 10^5/2.08 \times 10^5$ (see supporting material for detail).

3.5. Sensitivity of the DNA biosensor

The sensitivity of electrochemical hybridization assay was investigated by varying the concentrations of two target DNAs. As shown in Fig. 3., the average peak current has a good linear relation versus concentration of target DNA-1 and target DNA-2 in the range of 4.4×10^{-11} to 2.0×10^{-9} M. The regression equations were $y_1 = 1.5448x_1 + 0.05788$ and $y_2 = 1.398x_2 + 0.05616$ (x_1 represented the concentration of target DNA-1, and x_2 represented the concentration of target DNA-2, 10^{-10} M; y_1 and y_2 were the peak currents for the oxidation of A and G, respectively), with the correlation coefficient of 0.9955 and 0.9975 for target DNA-1 and target DNA-2, respectively. The detection limit for target DNA-1 estimated using 3σ (where σ is the standard deviation of the blank solution, $n = 11$) was 1.55×10^{-12} M, and the detection limit for target DNA-2 was 1.71×10^{-12} M.

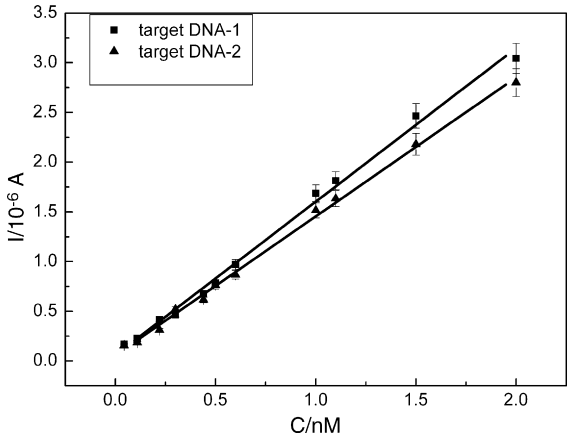

Fig. 3. Calibration curves of peak current versus the mixture of target DNA-1 and target DNA-2 (28 μL of capture DNA-1 and 42 μL of capture DNA-2, both in the concentration of 4.41×10^{-5} M), the procedure was carried out under optimized assemble and detection conditions.

4. Conclusions

In conclusion, an electrochemical bio-barcode assay for one-pot simultaneous detection of two target DNAs through the oxidation of purine nucleobases was developed. Based on the sandwich-type hybridization, this method integrated the magnetic separation of MBs and the amplification feature of AuNPs. The sensitivity of the sensor was further improved by preconcentration of dissolved G and A nucleobases before DPV measurements. The detection limits of both DNA targets were achieved at pM level. This nanoparticle-based bio-barcode electrochemical assay possesses the lable-free detection, high sensitivity and convenience for DNA diagnosis, and might be a good alternative for other dual-analyte sensing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.12.032.

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