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Short communication

A new photoelectrochemical biosensors based on DNA conformational changes and isothermal circular strand-displacement polymerization reaction

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ABSTRACT

We report a strategy for the transduction of DNA hybridization into a readily detectable photoelectrochemical signal by means of a conformational change analogous to electrochemical DNA (E-DNA) approach. To demonstrate the effect of distance change for photosensitizer to the surface of electrode on the change of photocurrent, photosensitizer $Ru(bpy)_2(dcbpy)^{2+}$ tagged DNA stem–loop structures were self-assembled onto a nanogold modified ITO electrode. Hybridization induced a large conformational change in DNA structure, which in turn significantly altered the electron-transfer tunneling distance between the electrode and photosensitizer. The resulting change in photocurrent was proportional to the concentration of DNA in the range of 1.0×10^{-10}–8.0×10^{-9} M. In order to improve the sensitivity of the photoelectrochemical biosensor, an amplified detection method based on isothermal strand displacement polymerization reaction was employed. With multiple rounds of isothermal strand replication, which led to strand displacement and constituted consecutive signal amplification, a detection limit of 9.4×10^{-14} M target DNA was achieved.

1. Introduction

Rapid, specific detection of nucleic acid sequences has attracted significant attention due to possible applications in fields ranging from pathogen detection to the diagnosis of genetic diseases. Among various detection techniques, photoelectrochemical detection has attracted significant interest (Liang et al., 2006, 2008). Firstly, this method is very sensitive due to the separate forms of signal for excitation and detection. Secondly, compared to optical detection methods such as fluorescence, CL and ECL, the instrument of photoelectrochemistry is much simpler and low cost inherent to electronic detection (Qian et al., 2010; Zhang et al., 2011). Strategies for the photoelectrochemical DNA analysis have focused on the intercalation of rutheniumbipyridine derivatives into DNA duplexes (Zhang et al., 2010; Gao and Tansil, 2005) and semiconductor nanoparticle assembled on the electrode (Hojeij et al., 2008; Wang et al., 2009; Sheeney-Haj-Ichia et al., 2005). Although possessing remarkable advantages, photoelectrochemistry is mainly used in the field of solar cell and its usage in biosensor is much limited compared with other techniques. So it is still necessary to improve the utilization of photoelectrochemistry in the aspect of analytical detection.

Recently, many electrochemical DNA (E-DNA) sensors have been developed based on conformational changes in the dynamics of the probe DNA (Xiao et al., 2007, 2009). A redox-labeled linear (Ricci et al., 2007; Xiao et al., 2006) or stem–loop (Lubin et al., 2006; Fan et al., 2003) DNA probe was chemisorbed onto a gold electrode *via* gold–thiol bond. After binding with target, the conformation of probe DNA changed, thus significantly affected the rate of electron transfer between the redox moiety and the electrode. The associated change in redox current produced a signal indicative of the target. Such single-step, reagentless electrochemical sensors have been widely used for detection of target DNA (Yang and Zhang, 2010), enzyme (thrombin) (Xiao et al., 2005), ion (potassium ion) (Radi and O'Sullivan, 2006), or small organic molecules (cocaine (Baker et al., 2006), adenosine triphosphate (Zuo et al., 2007), and theophylline (Ferapontova et al., 2008)), etc.

Inspired by this simple and easy to use strategy of E-DNA sensors, we designed a photoelectrochemical DNA sensor based on target induced conformational changes of photosensitizer modified DNA probe for the first time. $Ru(bpy)_2(dcbpy)^{2+}$ (bpy=2,2′-bipyridine; dcbpy=2,2′-bipyridine-4,4′-dicarboxylic acid) was selected as photoelectrochemically active species. It was tethered to DNA through amide bound. Triethanolamine (TEOA) was used as an electron donor to produce sustained photocurrents. After addition of targets, the distance change for the photosensitizer from the electrode surface might cause the change of photocurrent, and targets could be quantified accordingly.

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However, the sensitivity of such DNA sensor was restricted when DNA detection was based on simple hybridization recognition, in which one target strand was utilized just once. The ability to sense and detect ultralow concentration of specific DNA sequences by using simple and inexpensive assays is important in clinical diagnostics. Recently, Guo et al. (2009) reported a method for amplified detection of DNA based on the inherent signal-transduction mechanism of the hairpin fluorescence probe and strand-displacement property of polymerase. To enhance the sensitivity of our photoelectrochemical sensor further, the isothermal amplification based on DNA polymerization was investigated here for the detection of DNA. As a result, the sensitivity enhancement was realized in a concise manner and as low as 4.0×10^{-13} M target DNA could be detected. Moreover, the application scope as well as detection methods of photoelectrochemical biosensor can be expanded in this way.

2. Experimental

2.1. Materials and chemicals

2.1.1. Materials

$HAuCl_4\cdot4H_2O$ were purchased from Shanghai Chemical Reagent Inc. (Shanghai, China). Fifteen percent tin (IV) oxide, as a colloidal dispersion of 15-nm particles in water, was obtained from Alfa Aesar. Ruthenium(III) chloride hydrate was purchased from ACROS Organics (Japan). Triethanolamine (TEOA), N-hydroxysuccinimide (NHS), N,N′-dicyclohexyl carbodiimide (DCC), 3-mercaptopropyl-triethoxysilane (MPTES), 2,2′-bipyridyl-4,4′-dicarboxylic acid (dcbpy) and sodium hexafluorophosphate were purchased from Sigma (USA). The polymerase Klenow Fragment (2–5 U/μL) was purchased from TaKaRa Bio Inc. (Dalian, China). The oligonucleotides used in this study were purchased from SBS Genetech Co., Ltd. (China) with the following sequences:

P1: 5′–SH-TCT TGG ACA CAG TAA AGA GAG GTG CGC CCA TTG TGT CCA AGA-NH_2-3′;
T1: 5′–TCT TGG ACA CAA TGG GCG CAC CTC TCT TTA CTG TGT CCA AGA-3′.
T2: 5′–ATG GGC GCA CCT CTC TTT ACT GTG TCT TT-3′;
Primer: 5′–TCTTGGAC-3′;
Mismatched sequence: 5′–ATG GGG GCA CCT CTC TTT ACT GTG TCT TT-3′.
Random DNA: 5′–TGCAAGGTGTCAGTATAATCCGACGT TTT-3′;

The preparation of AuNPs and $Ru(bpy)_2(dcbpy)$NHS labeled ss-DNA were described in supplementary material.

2.1.2. Apparatus

The photocurrent was measured on an electrochemical workstation (Zahner Zennium, Germany). A three-electrode system was employed with Pt wire as an auxiliary electrode, Ag/AgCl as a reference electrode and ITO conductive glass supplied by Weiguang Corp. (Shenzhen, People's Republic of China, ITO coating 180 ± 25 nm, sheet resistance $\leq10\,\Omega$/square) as a working electrode. The preparation of SnO_2 modified ITO electrode was described in supplementary material.

3. Experiment

3.1. Fabrication of the biosensor

The SnO_2 modified ITO electrode was prepared in supporting information and then silanized in ethanol solvent containing 1 mM MPTES through sonication for 2 h at room temperature. After this time, the electrode was rinsed with double distilled water, dried under N_2 atmosphere and then cured at 110 °C for 15 min in an oven.

Sulfhydryl-derivated ITO electrode was immersed into the solution of AuNPs, and reacted in the dark for 24 h at room temperature. After rinsed with double distilled water, 10 μL 2.0×10^{-6} M $Ru(bpy)_2(dcbpy)^{2+}$ modified ssDNA (**P1**) was dipped on the electrode surface, and the electrode was incubated in the dark at room temperature for 4 h. Then the modified electrode was washed three times with Tris–HCl (pH 7.4) to remove the unbound probe. The modified surface was subsequently passivated with excess 2-mercaptoethanol at 1 mM in 0.10 M Tris–HCl and washed three times with buffer solution.

The **P1** modified electrode was immersed into 0.1 M Tris–HCl (pH 7.4) solution containing 0.1 M NaCl and **T1**-DNA for 2 h at 37 °C. After that, dsDNA-modified electrode was washed three times with Tris–HCl (pH 7.4) and photocurrents were recorded.

3.2. Amplified detection based on isothermal circular strand-displacement polymerization reaction.

The ssDNA probe (**P1**) modified electrode was immersed into 0.1 M Tris–HCl (pH 7.4) containing 2.0 μM primer, 5U polymerase Klenow Fragment dissolved in Klenow buffer (0.1 M Tris–HCl, pH 7.5, 15 mM $MgCl_2$ and 1 mM dithiolthreitol) and 100 μM dNTPs. Then a series of targets **T2** at different concentrations were added to the mixture solution and incubated for 2 h at 37 °C. Then photocurrents were recorded.

3.3. Photoelectrochemical detection.

Photoelectrochemical detection was carried out in 0.1 M Tris–HCl (pH = 7.4) containing 40 mM TEOA, which was served as a sacrificial electron donor during the photocurrent measurement. Light excitation of 430 nm was switched every 20 s. The applied potential was 0.1 V.

4. Results and discussion

4.1. The feasibility for photoelectrochemical detection based on the conformation change of DNA.

To determine whether the change of conformation for probe DNA can lead to the change of photocurrent as it does in E-DNA sensor, following experiment was carried out firstly. As shown in Fig. 1. A SnO_2 nanoparticle-modified ITO electrode was silanized with MPTES and then reacted with AuNPs. Probe DNA (**P1**) with sulfhydryl group at one end and photosensitizer ($Ru(bpy)_2(dcbpy)^{2+}$) at the other end was assembled on ITO/SnO_2/AuNPs electrode through Au–S bond. In the absence of target DNA, the DNA probe was in hairpin form and the photosensitizer $Ru(bpy)_2(dcbpy)^{2+}$ was close to the surface of the electrode, which led to strong photocurrent. After binding with target, the hairpin opened to the extended rigid duplex form, the increased distance between the photosensitizer and the electrode was manifested as the depression photocurrent.

4.2. Sensitivity without amplification

The detection conditions for the photoelectrochemical biosensor mentioned above were optimized in Figs. S1 and S2 (supplementary material). The result showed higher concentration of the probe DNA (**P1**) produced bigger depression of photocurrents at the same concentration of target DNA. So, 2.0×10^{-6} M **P1** was used in the following study and the assemble time was 4 h. While

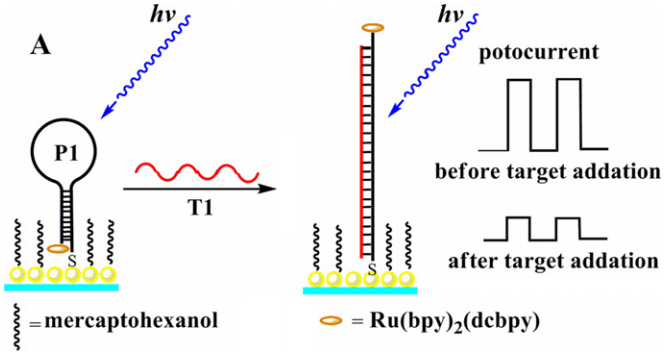

Fig. 1. Principle of the photoelectrochemical DNA biosensor based on the conformation change of DNA.

the equilibration time for hybridization was 2 h. Under optimal condition, different target DNA (**T1**) was introduced. As shown in Fig. S3A (supplementary material), the photocurrent intensity decreased upon the increasing concentration of **T1**. Hence the quantitative behavior of the photoelectrochemical hybridization assay could be assessed by measuring the dependence of the decreased photocurrent intensity (ΔI) before and after incubated with **T1**. From the result of Fig. S3B (supplementary material), a linear range from 1.0×10^{-10} to 8.0×10^{-9} M was achieved with an equation of $\Delta I=0.99147+6.7116C_{DNA}$ (10^{-10} M) ($n=11$, $R=0.9985$) and a detection limit of 3.7×10^{-11} M was obtained. This sensitivity is identical to the ECL DNA biosensor based on hairpin DNA probe labeled with ruthenium complex (9.0×10^{-11} M)[13].

4.3. The principle for amplified detection

To improve the sensitivity of the developed photoelectrochemical biosensor, isothermal circular strand-displacement reaction was applied. The amplified principle based on the conformation change of the probe DNA was schematically shown in Fig. 2A. This DNA detection system consisted of a hairpin probe **P1**, a short primer and polymerase. Without the target DNA (**T2**), the hairpin could not be opened because the stem of hairpin was 11 bases long, and the primer was 8 bases. This led to a strong photocurrent as described above. While in the presence of **T2**, the target sequences hybridized with hairpin probes and opened their loop parts, enabling primers to attach on the stem part. **P1** acted as a template to form complementary DNA duplex. Under the action of polymerase Klenow Fragment and dNTP, polymerization reaction was initiated and the target was displaced. The released target **T2** then opened another hairpin probe **P1** and triggered another polymerization reaction circularly. Thus, even minute amounts of targets could produce obvious photocurrent decrease based on the circular polymerization reaction and conformational changes. Therefore, by monitoring the decrease of photocurrent intensities, target DNA could be detected easily and sensitively.

In order to verify the amplification effect of isothermal circular strand-displacement, here, we compared the photoelectrochemical performance of the sensor under different conditions. As shown in Fig. 2B, in the absence of target DNA, the hairpin could not open, and a bigger photocurrent (550 nA) was obtained (curve a). When 8×10^{-12} M **T1**-DNA (or **T2**-DNA) was added, photocurrent was almost the same (curve b) because of low concentration. When the concentration of **T1**-DNA was increased to 6×10^{-9} M, which lay in the linear range for the detection **T1**-DNA, the photocurrent decreased to 223 nA (curve c). While with the amplification of polymerization reaction (the polymerization reaction time was 2 h, Fig. S4, see supplementary material), the decrease of photocurrent could be easily detected when the concentration of **T2**-DNA was as low as 8×10^{-12} M (205 nA, curve d). The results meant that the polymerization reaction could amplify the signal dramatically. So the present design had the capability to detect the target DNA with high sensitivity.

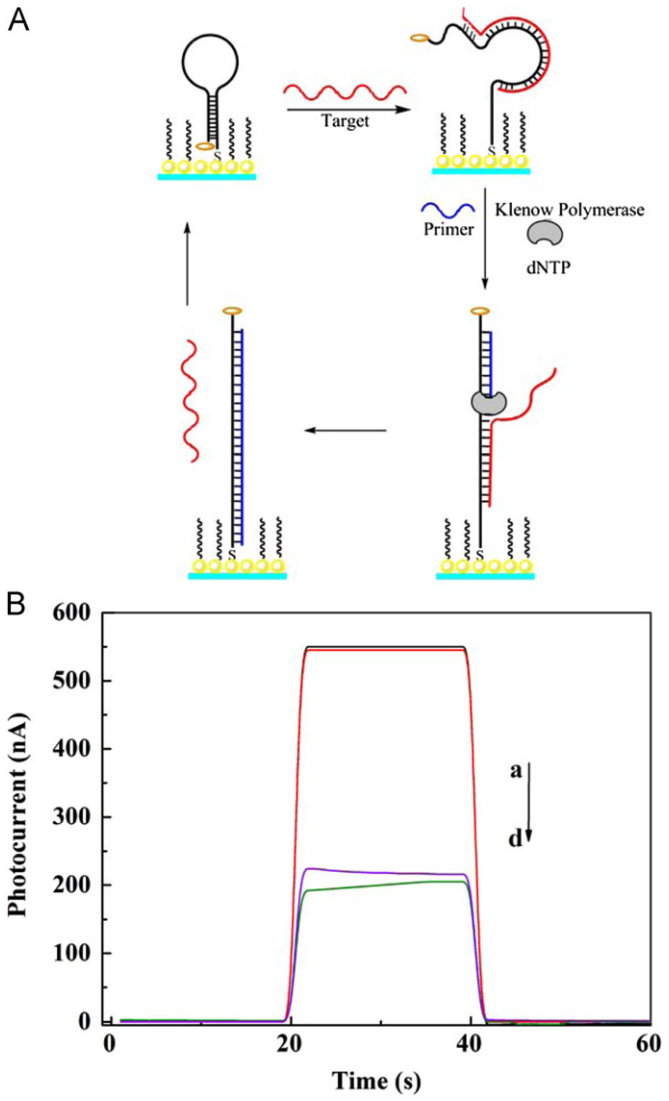

Fig. 2. (A) Principle of the photoelectrochemical DNA biosensor based on isothermal circular strand-displacement polymerization reaction. (B) Photocurrent responses for (a) **P1** modified electrode, (b)–(d) **P1** modified electrode with different concentration of target DNA (b) 8×10^{-12} M **T1** (or **T2**), and (c) 6×10^{-9} M **T1**; (d) 8.0×10^{-12} M **T2** with polymerase Klenow Fragment and dNTP.

4.4. Sensitivity and selectivity

. Fig. 3A showed the photocurent signals that were responsive to the changing concentrations of **T2** (a–j). The photocurrent intensity gradually decreased with increasing **T2** concentrations due to the distance change of probe DNA. As shown in Fig. 3B, the decrease of photocurrent intensity was linearly related to the concentration of **T2** in the range from 4.0×10^{-13} to 1.0×10^{-11} M. The regression equation was $\Delta I\,(nA)=0.218+4.152C_{DNA}$ (10^{-13} M) with a regression coefficient of 0.9990 ($n=10$). A detection limit of 9.4×10^{-14} M could be estimated using 3σ. With the amplification by polymerization reaction, the sensitivity of the proposed method was enhanced about 390-fold compared to a simple photoelectrochemical detection based on conformational changes.

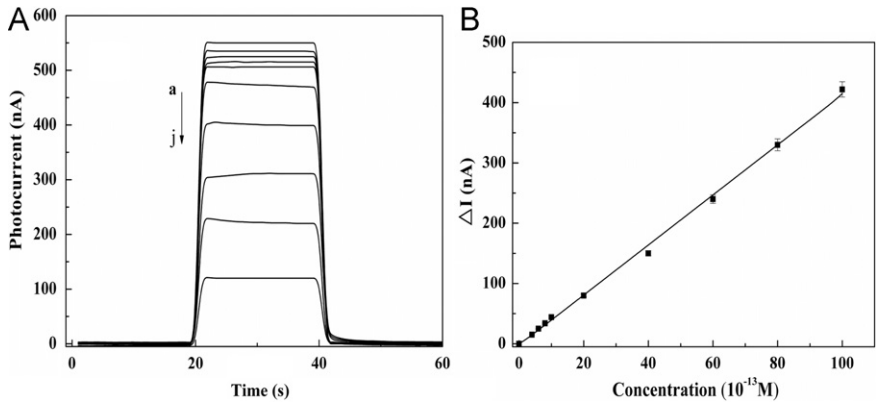

Fig. 3. (A) Photocurrent responses with increasing levels of **T2**: (a) 0, (b) 4.0×10^{-13} M, (c) 6.0×10^{-13} M, (d) 8.0×10^{-13} M, (e)1.0×10^{-12} M, (f) 2.0×10^{-12} M, (g) 4.0×10^{-12} M, (h) 6.0×10^{-12} M, (i) 8.0×10^{-12} M and (j) 1.0×10^{-11} M. (B) The calibration plot of peak current decrease versus the concentration of **T2** from 4×10^{-13} to 1.0×10^{-11} M. The excitation light was 430 nm and the light was turned on and off every 20 s.

The ability to discriminate the complementary sequence from single-base mismatch DNA is crucial to gene detection in early stage diagnosis. Selectivity of the present biosensor in discriminating completely complementary target DNA from single-base mismatched and noncomplementary sequences was investigated under the concentration of 8.0×10^{-12} M **T2**. As shown in Fig. S5 (see supplementary material), after hybridization, the photocurrent for random DNA (curve b) was identical to that of blank (curve a). The photocurrent was suppressed by only 9% for 1-base mismatches DNA, whereas the signal was suppressed by 61% for complementary sequences **T2**. This result indicates that the proposed photoelectrochemical assay for DNA exhibited a good specificity and such amplified photoelectrochemical biosensors have potential use for the detection of single-base mismatch DNA.

5. Conclusions

In summary, inspired by E-DNA sensors, a novel photoelectrochemical DNA biosensor based on target-induced conformational change of DNA was developed with ruthenium complex as the signal-producing compound. At the same time, circular strand-displacement polymerization reaction was employed in the photoelectrochemical assays for the first time, which significantly improved the sensitivity. With its simplicity, selectivity, sensitivity, this strategy shows great promise in the photoelectrochemical detection not only for DNA, but also generalizable to other sensor designs in which significant protein or aptamer conformational changes occur on target binding.

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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.2012.07.057.

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