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Ultrasensitive electrical biosensing of syphilis DNA using target-guided formation of polyaniline based on enzyme-catalyzed polymerization

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ABSTRACT

An ultrasensitive assay for electrical biosensing of syphilis DNA was proposed by using target-guided formation of polyaniline (PANI) based on an enzymatically catalyzed method. The sensitive biodetection relies on the DNA hybridization and biotin–streptavidin interaction. After coupling of biotinylated catalase with streptavidin-modified hybrids, a head-to-tail structure of PANI templated by hybridized DNA was formed. The current response of PANI was linearly related to target DNA concentration between 1.0 pM and 1.0 nM with a correlation coefficient of 0.998. The detection limit was determined to be 0.5 pM with the signal-to-noise ratio of 3. The synergistic performances of DNA hybridization, strong biotin–streptavidin binding ability, and highly efficient polymerization provide a general platform for simple, highly sensitive and selective biosensor for the detection of the specific *polA* gene fragment of *T. pallidum*. It is expected that the proposed biosensor holds great promise for diagnosing disease in practice.

1. Introduction

Sexually transmitted diseases (STDs) constitute the most common infectious diseases around the world and bear significant consequences for both the individual and public health of the community (Bruisten et al., 2001). Control of STDs has recently become a greater priority in global public health campaigns, due to the association between STDs (particularly genital ulcers) and the acquisition of HIV infection (O'Farrell, 1999; Grosskurt et al., 2000; Kamali et al., 2003).

The traditional strategy for managing STDs has relied on individual patient diagnosis and treatment. However, due to scarce laboratory facilities for diagnosing STDs in resource-poor settings, different laboratory methods, such as direct antigen detection, virus isolation by cell culture and polymerase chain reaction (PCR), are commonly used for the diagnosis of genital herpes. PCR has markedly improved the laboratory diagnosis of genital herpes (Scoular et al., 2002; Totten et al., 2000) as well as of *T. pallidum* and *H. ducreyi* (Liu et al., 2001; Marfin et al., 2001). However, PCR has its own drawbacks, such as reproducing the relative concentrations of nucleic acid sequences from complex mixtures unfaithfully, time consuming and not free of error (Wabuyele and Soper, 2001; Johnson, 2000). Moreover, the presence of false positive products, which can result from non-specific amplification of approximately the same size of the expected target fragment, would potentially confuse the authenticity and validity of the assays (Strerath and Marx, 2005; Alexander et al., 2007). Thus, simple, inexpensive, and sensitive sequence-specific DNA detection is needed urgently for diagnosing disease.

Biosensors based on electrochemical transduction are useful for DNA detection due to their high sensitivity, low cost, rapid response, small dimensions, low power requirements, and compatibility with micro-fabrication technology (Bard and Rodriguez, 1990; Drummond et al., 2003; Kavanagh and Leech, 2006; Macanovic et al., 2004). At present, many new electrochemical procedures are under intense investigation to get a fast, sensitive and selective method for nucleic acids quantification and meet the new challenges associated with the application of DNA biosensors in various fields. Among them, amplified transduction of biological recognition events remains a major challenge to electrical bioassays. New schemes based on coupling the biocatalytic amplification of enzyme tags with additional amplification units and processes are highly desired for meeting the high sensitivity demands of electrochemical detection of proteins and nucleic acids (Wang et al., 2004; Fan et al., 2007; Patolsky et al., 1999). A variety of modified enzymatic polymerizations using biological based materials as templates have been proposed and investigated (Nagarijan et al., 2001b; Gao et al., 2007; Ma et al., 2004; Miranda-Castro et al., 2007). Polyaniline (PANI), which is an intrinsically and versatilely conductive polymer, has been studied extensively due to its environmental

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stability, redox properties and reversible nature of electrical conductivity (Fan et al., 2007; Gao et al., 2007). It was demonstrated that H_2O_2 that was electrogenerated by an intercalator bound to DNA linked to an electrode could oxidize aniline units associated with the DNA in the presence of horseradish peroxidase (HRP) (Fan et al., 2007). An intramolecular complex was formed between PANI and DNA, where the phosphate groups in nucleic acid served as templates to guide the deposition of electroactive PANI.

In the present work, a simple fabricated, sensitive and selective biosensor for the detection of the specific *polA* gene fragment was achieved by connecting the biocatalytic amplification with biotin–streptavidin binding ability. *PolA* gene is a specific gene for DNA polymerase I of *T. Pallidum* that have two rich-cysteine domains, which is a unique feature of the enzyme from other spirochaetes. Significantly, greatly amplified sensitivity was achieved by the association of the strong biotin–streptavidin binding affinity and biocatalytic precipitation of electroactive PANI. It is expected that the proposed biosensor holds great promise for diagnosing disease in practice.

2. Experimental

2.1. Reagents and probes

Dithiothreitol (DTT) and 6-mercapto-1-hexanol (MCH) were obtained from Sangon Biotech (Shanghai, China). HRP (EC 1.11.1.7, type VI), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl), *N*–hydroxysuccinimide (NHS) and aniline (99.5%) were purchased from Sigma (Aldrich). Oligonucleotides used in this experiment were synthesized and purified by Sangon Biotech (Shanghai, China). Immobilized capture probe, signaling probe and other oligonucleotides were designed with software OLIGO-6. Streptavidin and biotin were purchased from Sigma (USA). *Taq* polymerase was obtained from Takara (Dalian, China). All solutions were prepared with ultrapure water (>18 MΩ cm) obtained from a Millipore Milli-Q water purification system.

PCR Primer design: According to the published *polA* gene sequence (Genbank accession number TPU 57757), one pair of primers was designed on the two unique sequences of *polA*. Sequences of primers were as follows (corresponding amino acids in the bracket).

Forward primer: 5′-TGCGCGTGTGCGAATGGTGTGGTC-3′ (CACANGVV)

Reverse primer: 5′-TGCACATGTACACTGAGTTGACTCGG-3′ (TESTQCTCA)

Synthetic complementary target sequence:

5′-TGCACATGTACACTGAGTTGACTCGGTATGAAGAGAAACGTC-CGGAACAATAAGAGGAACATAAAAAGCCTCTGCTTCCTGAAAGCAG-ATCGAAAATCCGACCAGACGTGTATCGTGCGGATGCAATCCATCCG-break TTTCACAATCAAACGCGACCACACCATTCGCACACGCGCA-3′

Synthetic non-complementary target sequence:

5′-TGCACATGTACACTGAGTTGACTCGGATACTTCTCTTTGCAGG-CCTTGGACCACACCATTCGCACACGCGCA-3′

Capture probe: 5′-SH-$(CH_2)_6$-TGGTGTGGTCGCGTTTGATTG-3′

Signal probe: 5′-CTCTTCATACCGAGTCAACTCA-Biotin 3′.

All oligonucleotides were dissolved with 0.01 M Tris-buffer into stock solutions and stored at −20 °C. They were diluted with 0.1 M phosphate buffer solution (PBS) to suitable concentrations prior to use. Thiol-modified capture probe was treated with 0.05 M DTT at room temperature overnight in the dark to reduce the S–S bonds prior to use.

Genomic DNA from Nichols strain *T. pallidum* was provided by Dr. Jianfang Zhang (Department of Laboratory of Xijing Hospital, the Fourth Military Medical University), PCR procedure included a denaturation at 94 °C 5 min, 30 cycles of 30 s at 94 °C, 30 s at 65 °C, 30 s at 72 °C, and final step of extension 10 min. Two negative controls were set up: (1) a parallel reaction without adding *Taq* polymerase, and (2) a parallel reaction without template. Sizes of PCR products were determined by running 10 μL of PCR mixture in 2% agarose gel for 30 min and observed under ultraviolet light.

2.2. Apparatus

A Model CHI660A Electrochemistry Workstation (Shanghai Chenhua Instruments Co. Ltd., China) was employed for all electrochemical experiments. A conventional three-electrode system was used. A saturated calomel electrode (SCE) and a platinum wire electrode were used as the reference electrode and the auxiliary electrode, respectively. A gold electrode (2 mm in diameter) was used as a working electrode. The atomic force microscope (AFM) was a digital Instruments Multimode SPM-9500J3 (Shimadzu Corporation, Japan), operating in ex-situ Tapping Model.

2.3. Fabrication of DNA biosensors

2.3.1. Biomodification of the gold electrode

A schematic illustration of the sensing procedure was depicted in Scheme 1. Prior to use, the bare gold electrode was polished with 0.3 and 0.05 μm alumina slurries and rinsed with double-distilled water. Then, the bare gold electrode was immersed in piranha solution (70% concentrated H_2SO_4 and 30% H_2O_2; *Caution: piranha solution is strongly oxidizing and should be handled with care!*) for 5 min to eliminate other substances, and then ultrasonically treated in absolute ethanol and ultrapure water, successively. Subsequently, the electrode was rinsed with liquid nitrogen.

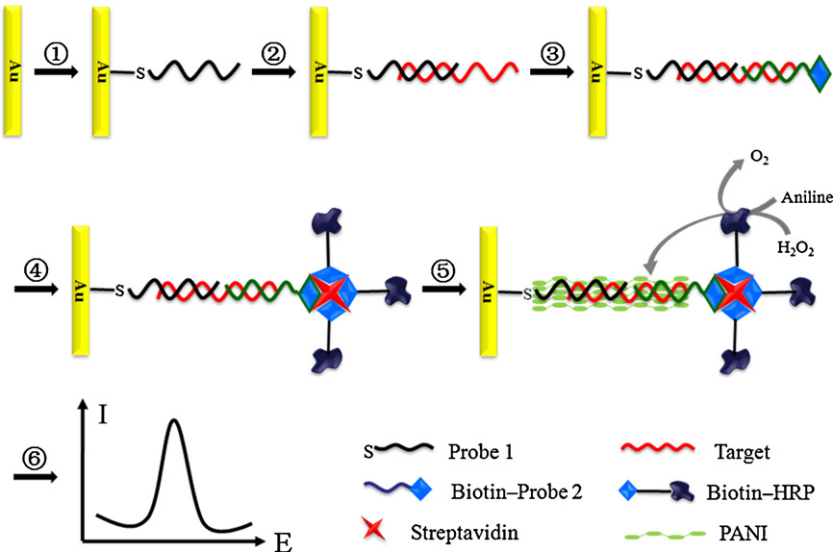
In order to obtain well-aligned monolayer of thiol-modified capture probe on gold surface, the gold electrode was first immersed in 0.5 μM thiol-modified capture probe solution diluted with 0.1 M PBS and incubated for 3 h at 16 °C in the dark to avoid oxidation of SH-group (Scheme 1, step 1). After that, the electrode was further treated with 0.1 μM MCH for 2 h to block the uncovered gold electrode surface and finally rinsed with 0.01 M Tris-buffer.

2.3.2. Hybridization on the gold electrode

The hybridization process on the gold electrode consists of two steps. First, the modified electrode was immersed into a 50.0 μL 0.01 M Tris-buffer solution containing certain concentration of target DNA and incubated for 20 min (Scheme 1, step 2). After rinsed with 0.1 M PBS, the electrode was transferred into another hybridization solution containing 2.0 μM biotinylated signaling probe (Scheme 1, step 3). The hybridization solution was heated at 75 °C for 5 min and annealed by gradual cooling to 37 °C for 1 h to avoid formation of hairpins between or inside ssDNAs. After hybridization, the electrodes were rinsed with 0.1 M PBS thoroughly.

2.3.3. Ligation of streptavidin and biotin-labeled HRP

The hybridized electrode was immersed into 500 μL of 0.5 μM streptavidin solution diluted with 0.1 M PBS for 30 min (Scheme 1, step 4). Streptavidin could specifically ligate to biotins labeled at the 3′ end of the signaling probe. After that, the electrode was thoroughly rinsed with PBS to remove unligated streptavidin and was incubated in biotin-labeled HRP solution for 30 min. Because of strong biotin–streptavidin binding interaction (dissociation constant, $K_d = 10^{-13}$ M) (Klumb et al., 1998), the biotin-labeled HRP binds to the streptavidin-conjugated of the detection probe on the electrode surface. This incubation step was followed by thoroughly rinsing of the surface with 0.1 M PBS to eliminate detergents.



Scheme 1. Scheme diagram of the proposed biosensor for the amplified detection of the target DNA.

2.3.4. Enzymatic deposition of PANI and electrochemical measurements

To perform the deposition of PANI templated by DNA under enzymatic condition and the voltammetric detection, the resulting modified electrode was immersed into a 0.1 M HAc–NaAc solution (pH 4.3) containing 1.0 mM aniline and 5.0 mM H_2O_2 for a desirable accumulation time (about 15 min) (Scheme 1, step 5). After that, the electrode was removed from the above solution and transferred into a 0.1 M HAc–NaAc solution (pH 4.3), after which cyclic voltammerty (CV) and square wave voltammetry (SWV) were conducted (Scheme 1, step 6).

Electrochemical impedance spectra (EIS) were performed in 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.2 M KCl. The impedance measurements were recorded at a bias potential of +195 mV (*vs.* SCE) within the frequency range of 10^5 to 10^{-2} Hz.

3. Results and discussion

3.1. Feasibility study

In this new biosensing protocol (see Scheme 1), the hybridization of target DNA with capture and signal probe and the biotin–streptavidin binding interaction play important roles in the recognition and transduction events for amplified DNA biosensing. To demonstrate this concept, we first evaluated the response of the biosensor with 2.0 pM target DNA perfectly complementary to the DNA capture probes in 0.1 M HAc–NaAc solution (pH 4.3) containing 1.0 mM aniline and 5.0 mM H_2O_2 for 15 min. For comparison, a control sample was also tested by applying a non-complementary DNA for hybridization and then followed with the same procedure as that for the complementary DNA. After accumulation in the H_2O_2/aniline solution for 15 min, the modified electrodes were washed and transferred into blank 0.1 M HAc–NaAc solution (pH 4.3). CVs of the modified electrodes were illustrated in Fig. 1(A). As shown in Fig. 1(A), one pair of well-defined redox peaks with the reduction and oxidation peak potentials at 0.202 V and 0.281 V (*vs.* SCE) was observed (curve a), ascribing to the first redox process of PANI (leucoemeraldine/emeraldine transition) (Liu et al., 1999; Thiyagarajan et al., 2003). This has been further proved by recording the SWV curves of the modified electrodes and the results were shown in Fig. 1(B). As can be seen, almost symmetric SWV curves were obtained and no other peaks were observed. Curve b was the CV of PANI using non-complementary oligonucleotide as target DNA, redox peaks cannot be observed in potential range of −0.2 to 0.7 V, indicating that no PANI was formed on the gold electrode surface owing to failure in hybridization. It has been demonstrated

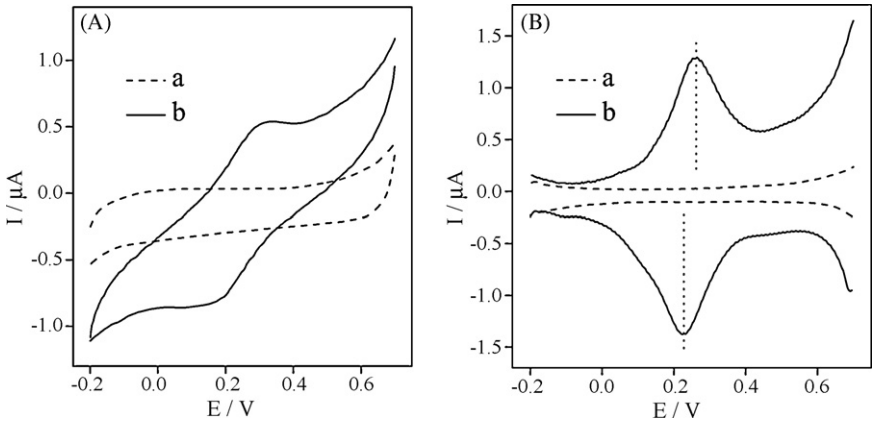


Fig. 1. (A) CVs of the modified electrodes in 0.1 M HAc–NaAc solution (pH 4.3) after hybridization with (a) non-complementary oligonucleotide and (b) complementary oligonucleotide. (B) SWVs of the modified electrodes in 0.1 M HAc–NaAc solution (pH 4.3) after hybridization with (a) non-complementary oligonucleotide and (b) complementary oligonucleotide.

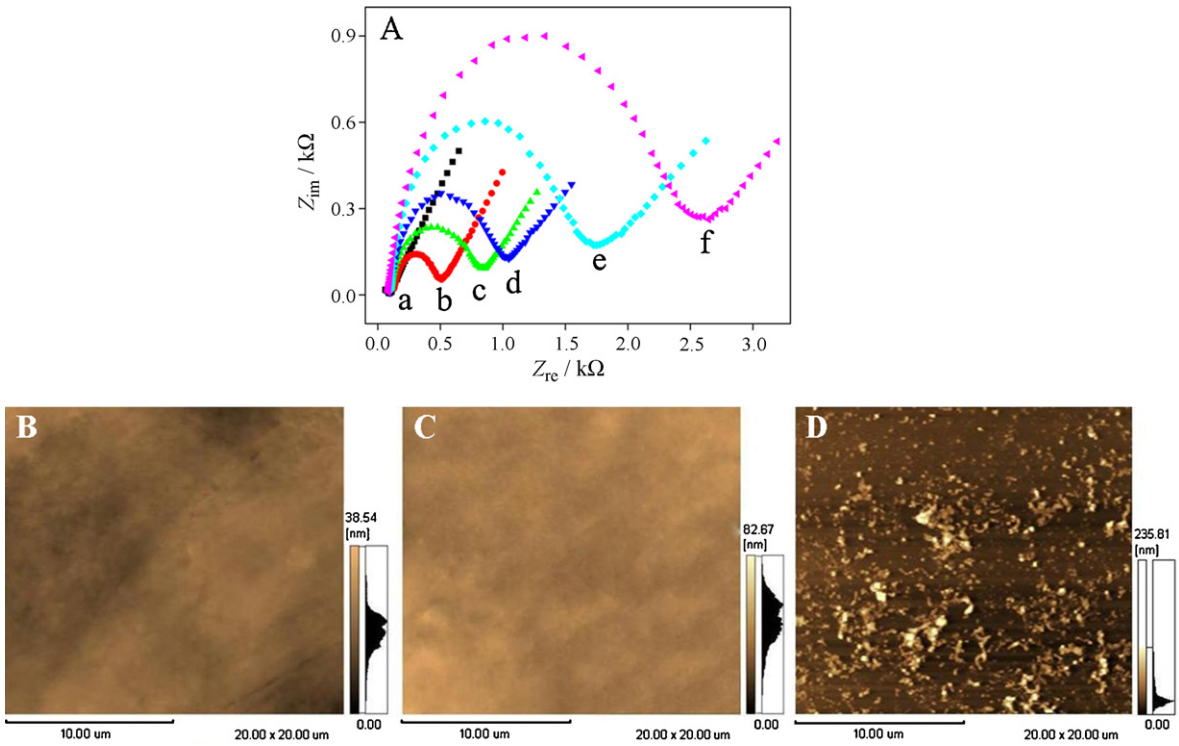


Fig. 2. (A) EIS plots obtained from different modified electrodes in 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.2 M KCl. (a) Bare gold electrode; the (b)–(d) show the EIS after the sandwich format reaction of the corresponding biosensor. Applied potential: +0.195 V (*vs.* SCE). Frequency range: 10 mHz to 10 kHz. Amplitude: 5 mV. (B) AFM images of gold surfaces after the assembling steps of ①, (C) ②, and (D) ⑤.

previously that two sets of redox peaks can be observed with electrochemically grown and chemically prepared PANI (Wei et al., 1989). The reason that only one pair of redox peaks appears in CV is believed to be caused by the high stability of PANI or the inability of PANI to be further oxidized (Gao et al., 2007). PANI deposition was performed using the hybridized DNA strands as templates. It has been known that the hybridization of DNA resulted in a negatively charged surface originated from the phosphate groups on the DNA backbone. This high proton concentration around the DNA provides a local environment of high acidity that permits polymerization of aniline in a much less acidic medium than that in conventional electrochemical and chemical approaches, and facilitates a predominantly head-to-tail coupling and deters parasitic branching during the polymerization, offering the much desired structure for high conductance. Additional experiments showed that no obvious changes occurred after extensive washing and potential cycling once the electroactive PANI was formed around the hybridized DNA strands. The resulting PANI/DNA complex was utilized for DNA sensing.

EIS is an effective method for probing the features of surface-modified electrodes, which provides useful information on the impedance changes of the electrode surface during the fabrication process (Ehret et al., 1997). Fig. 2(A) showed the results of EIS during the fabrication process. Significant increases in the electron-transfer resistances (R_{et}) were observed upon the stepwise formation of the modified electrode. It can be seen that the bare gold electrode exhibited no semicircle but an almost straight line (Fig. 2(A), curve a) that is characteristic of a diffusional limiting step of the electrochemical process. After the assembling of capture probe, target DNA sequence and the biotinylated signaling probe, the repulsion of the redox label was anticipated to inhibit the interfacial electron transfer and thus leads to enhanced R_{et}. As can be seen from Fig. 2(A), the R_{et} increased upon the buildup of the assembling and the hybridization process (curves b–d). Upon the association of the streptavidin and biotin–HRP biocatalytic conjugate to the electrode, a considerable increase in the R_{et} was observed due to the partial insulation of the electrode by the DNA and HRP molecules (curve e). After the biocatalytic precipitation of PANI onto the electrode, a very high increase in the R_{et} was observed (curve f), suggesting the formation of PANI further inhibited the interfacial electron transfer.

AFM images (Fig. 2(B–D)) were taken to investigate changes of the surface morphology of the gold electrode during the assembling process of the biosensor construction (Scheme 1, step 6). Fig. 2(B) showed a relatively clean and smooth surface of the bare gold substrate. After the assembling of capture probe, a uniform distribution of capture probe in the gold substrate was obtained (Fig. 2(C)). For the gold electrode after treatment of hybridization and the biocatalytic precipitation of PANI, an obvious change on the electrode surface can be clearly observed. The filose particles can be seen clearly when PANI was formed on the gold electrode (Fig. 2(D)). These AFM images gave us a clear impression about the morphology of the surface. On the basis of the EIS and AFM results, we can conclude that the assembling, hybridization and the biocatalytic precipitation process were successfully achieved on the gold electrode surface.

3.2. Optimization

Previous works have proved that the efficient and interfacial bio-architecture of probe on the gold electrode surface was the first factor that influences the analytical signal of the biosensor (Herne and Tarlov, 1997; Levicky et al., 1998). Thus the influences of probe concentration followed by a post-treatment with MCH were investigated. In this step, 0.1 μM MCH was used for post-treatment and 0.5 μM capture probe was selected to reveal the influence of probe immobilization time. Studies showed that a treatment time of 2 h with 0.1 μM MCH was enough to obtain well-ordered capture probe on the gold electrode surface. Then, the effect of immobilization time for increasingly amounts of time was examined. In this

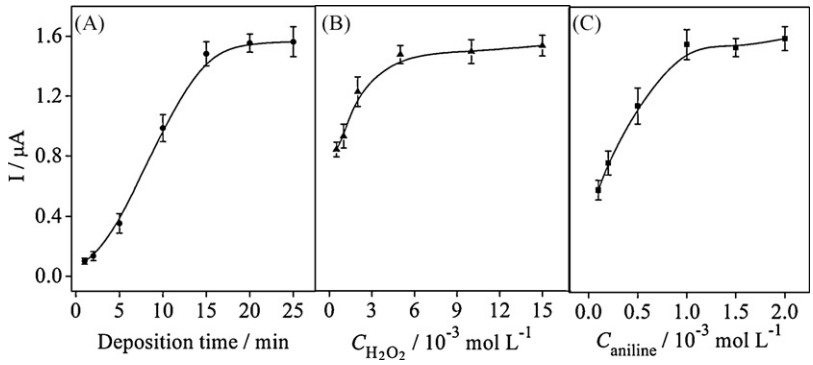


Fig. 3. Influence of (A) deposition time, (B) H_2O_2 and (C) aniline concentrations on the response of the biosensor. (A) The electrodes modified with 0.5 μM capture probe were immersed into a 50.0 μL 0.01 M Tris-buffer solution containing 0.1 μM synthetic complementary oligonucleotides for hybridization and incubated in 0.1 M HAc–NaAc solution (pH 4.3) contains 5.0 mM aniline and 1.0 mM H_2O_2 for different times; (B) deposition solution: 0.1 M HAc–NaAc solution (pH 4.3) contains 5.0 mM aniline and different concentrations of H_2O_2 for 15 min; (C) deposition solution: 0.1 M HAc–NaAc solution (pH 4.3) contains 1.0 mM H_2O_2 and different concentrations of aniline for 15 min. Other conditions were the same to those described in (A).

step, 0.1 μM synthetic complementary and non-complementary oligonucleotides were used as target sequences. Results showed that the current responses measured at sensors exposed to the complementary sequence increased with the increase of immobilization time and reaching the maximum value after a 3 h exposure. As for non-complementary sequence, the current responses were nearly the same. Therefore, the non-specific binding of the non-complementary sequence was assumed to be negligible.

The pH of the incubation solution is critical in controlling the alignment of the aniline molecules for the target-guided formation of PANI. Previous results showed that the enzymatic polymerization of aniline is strongly pH dependent (Liu et al., 1999). A lower pH (4.0–4.5) is required to produce the conductive PANI, whereas a pH of 6.0 or higher results in a more branched, insulating form of PANI. Therefore, an acidic buffer solution at pH 4.3 was selected for PANI deposition because it is the most optimal acidity to provide adequate protonation of aniline and thus strong enough electrostatic interaction between aniline and the phosphate groups and also maintains sufficient activity of HRP (Gao et al., 2007; Liu et al., 1999).

It is expected that the performance of the biosensor would mainly be affected by the incubation time, the H_2O_2 concentration, and the aniline concentration for polymerization. The effect of PANI deposition time on the biosensor was first investigated. As expected in Fig. 3(A), the longer was the deposition time, the higher was the peak current, with the maximum signal achieved after 15 min. Therefore, a deposition time of 15 min was selected to ensure the best performance of the biosensor. The optimization of H_2O_2 concentration was carried out in the range of 0.5–15.0 mM. The results were depicted in Fig. 3(B). The peak current of the PANI increased with increasing H_2O_2 concentration in the range of 0.5–5.0 mM, and further increase in H_2O_2 concentration did not show appreciable changes in peak current. Therefore, 5.0 mM of H_2O_2 was used for subsequent experiments. The effect of aniline was then studied by varying the aniline concentration in the mixture solution from 0.1 to 2.0 mM while keeping the H_2O_2 concentration and the incubation time unchanged. As illustrated in Fig. 3(C), with the aniline concentration increased from 0.1 to 2.0 mM, the current response of PANI around DNA first increased and then attained a steady value at 1.0 mM. It is known that the rate of enzyme-catalyzed reaction increases with increasing substrate concentration until it is high enough to saturate the enzyme. 1.0 mM of aniline was therefore selected to perform PANI deposition, which is high enough to facilitate the longer polymer chain growth and avoid short chain segments formation (Nagarajan et al., 2001a).

Such biodetection relies on hybridization and biotin–streptavidin interactions to the achievement of ultrasensitive measurements. It is well known that streptavidin is a tetrameric protein with each of the four identical subunits capable of binding one biotin molecule. Thus, the integration of biotin–streptavidin interaction with DNA assays could resulting more enzyme molecules bind to the electrode surface and leading more PANI accumulated around DNA backbone. To clarify the important aspects of the ultrasensitive mechanism, we have evaluated the ability of the biotin–streptavidin interaction in the electrochemical assays of DNA process. We studied the direct ligation of HRP to the biotinylated DNA probe instead of the biotin–streptavidin interaction. Studies showed the biocatalytic precipitative accumulation of PANI can also be observed. However, the current response of the resulting biosensor was much smaller than that of using the biotin–streptavidin interaction in the electrochemical assays of equal amount of target DNA. Another major limitation inherent to the biosensor without the use of biotin–streptavidin is that a narrow linear range response (5.0–200.0 pM) of concentration occurs.

3.3. Amplification of target sequence and identification of PCR products by Southern-blotting

In order to examine the selectivity of the biosensors for hybridization with target DNA sequences, a 174-bp fragment of the primers of *polA* gene was PCR-amplified from genomic DNA of three individual samples. As shown in Fig. 4(A), non-specific bands were observed together with the target fragment. Interestingly, a non-specific band of almost the same size as the target fragment was amplified from *polA* gene (lane 4), indicating that a PCR approach without subsequent hybridization assays would be inconclusive.

The specificity of the biosensor is a critical factor for a successful assay. In PCR, amplification of unwanted DNA fragments is consistently unavoidable. Although purification of target bands from gel electrophoresis could remove most non-specific fragments, non-specific PCR products of the same size as a specific sequence might not be completely eliminated. In order to verify the results obtained by the electrochemical method, Southern blot hybridization of PCR product was carried out based on the procedure described by Ryschkewitsch et al. (Ryschkewitsch et al., 2004). As shown in Fig. 4(B), single bands of the correct size were observed in lanes containing PCR products. No bands were observed in lanes corresponding to PCR blanks (lanes 4, 6 respectively). The results were in agreement with electrochemical results detected by our biosensors, indicating that the biosensors generated in this study bear the same specificity as Southern blot analyses.

To elucidate the analytical performance of the assay, a calibration experiment was designed. Because of the superior sensitivity,

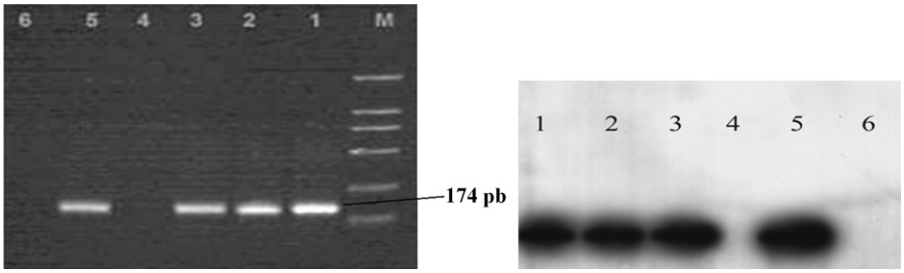


Fig. 4. (A) Confirmation of the quality of PCR products. Lanes 1–3—PCR products of different samples respectively with the primers of *polA* gene, lane 4—PCR blank (without template), lane 5—PCR product of synthetic complementary target sequence used as template (positive control) with the primers of *polA* gene, lane 6—PCR blank (without adding *Taq* polymerase); (B) Southern hybridization of PCR products using 174 bp probe of *polA* gene labeled by Digoxin Southern-blotting of PCR products. Lanes 1–3—PCR products of different samples respectively with the primers of *polA* gene, lane 4—PCR blank (without template), lane 5—PCR product of synthetic complementary target sequence as template (positive control) with the primers of *polA* gene, lane 6—PCR blank (without adding *Taq* polymerase).

SWV was used in quantification experiments. As can be seen in Fig. 5, the peak current originated from oxidation of PANI increased as the concentration of target DNA increased, suggesting that the amount of deposited PANI around DNA skeleton was related with the target DNA concentrations. The calibration plot corresponding to amperometric responses (inset of Fig. 5) was linear *vs.* the concentrations of target DNA ranging from 1.0 pM to 1.0 nM. The linear regression equation was I_p (nA) = 12.9 + 0.97 C (pM) with the correlation coefficient of 0.998. The error bars shown in inset of Fig. 5 represent standard deviations. The detection limit was determined to be 0.5 pM with the signal-to-noise ratio of 3. This value was much lower than HIV specific DNA determination based on the measurements of light scattering signals of gold nanorods (80 pM) (He et al., 2008) and using the electroactive self-assembled monolayer itself as a signal-on transducer of DNA hybridization (0.3 nM) (March et al., 2008).

In order to test the applicability of the present method, we applied it to the detection of target DNA sequence in genomic DNA sequence obtained from real samples. PCR reaction was made in order to make the final real sample of DNA sequence for detection. The PCR amplification products from the *polA* gene PCR reaction were diluted with buffer solution for different concentrations and used directly as the hybridization mixture. Hybridization of probes to PCR-amplified fragment of *polA* gene was performed in the same way as for synthetic DNA sequence. *SWV* curves of the electrode using PCR products of *polA* gene were recorded and three repetitive measurements yielded reproducible results. Results showed that the peak current increased as the concentration of the PCR amplification products increased, indicating the successful detection of the hybridization between the targets DNA and the probe DNA. By the way, a control experiment of PCR blanks indicated that the PCR blanks did not interfere with the detection. The above results were also consisted with the results obtained from Southern blot hybridization of PCR product. Therefore, the present method for detecting target DNA in real samples related to the HIV gene may be applied in practice.

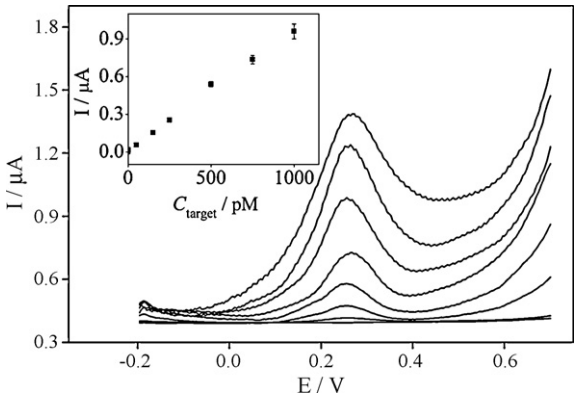


Fig. 5. SWVs obtained at the biosensor after hybridization with synthetic complementary oligonucleotide. Incubation solution: 0.1 M HAc–NaAc solution (pH 4.3) contains 1.0 mM H_2O_2 and 5.0 mM aniline for 15 min. Inset showed the calibration plot of peak current *vs.* different concentrations of synthetic complementary oligonucleotide in the range of 1.0 pM to 1.0 nM.

4. Conclusions

In this work, a new DNA electrochemical biosensor was designed and characterized for detection of primers of specific *polA* gene. Specificity of the biosensor was significantly improved upon the design of thiol-modified capture probe and biotinylated signaling probe that are complementary to target regions flanked by PCR primers, respectively. The optimization results showed that the proposed method by using the biocatalytic amplification and biotin–streptavidin binding ability for the deposition of PANI template by hybrized DNA is fixable and holds great promise for the construction of novel electrochemical biosensors with other bio-systems and could provide a platform for establishment of a reliable, sensitive and selective system for diagnosing in the field of emerging infectious diseases transmission.

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