Electrochemical DNA Biosensors Based on Gold Nanoparticles / Cysteamine / Poly(glutamic acid) Modified Electrode

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Abstract

In the present study, an electrochemical DNA sensing strategy for highly sensitivity detection of DNA hybridization was reported based on a gold nanoparticles (AuNPs) / cysteamine (Cys) / poly glutamic acid (PGA) modified electrode. A glassy electrode was first modified with the polymer film by cyclic voltammetry (CV), and then AuNPs were immobilized on the surface of PGA with Cys as a linker, to which probe DNA was covalently linked onto the surface of AuNPs through a 5′-thiol-linker. The DNA hybridization events were monitored by differential pulse voltammetry (DPV) measurement of the intercalated adriamycin. Under optimal conditions, the reduction peak currents were linearly increased with increasing the concentration of complementary target DNA from 9.0×10^{-11} to 4.8×10^{-9} M with a detection limit of 4.2×10^{-11} M. This DNA biosensor exhibited excellent selectivity, reproducibility and stability in DNA hybridization assay.

Keywords: DNA hybridization; Gold nanoparticles; Glutamic acid; DNA biosensor.

1.0 Introduction

In recent years, the detection of specific DNA sequences has attracted considerable attention of chemists and biologists due to its potential applications in disease diagnosis, epidemic prevention and forensic identification. As a result, various techniques have been developed for the detection of specific DNA sequences, including electrochemistry [1, 2], fluorescence [3, 4], radiochemical assays [5, 6], surface plasmon resonance spectroscopy [7, 8] and quartz crystal microbalance [9, 10]. Among these, electrochemical detection assays have the advantage of being simple, reliable, inexpensive, sensitive, and selective for genetic detection. A variety of electrochemical approaches to detecting DNA hybridization assay have been reported. Xia

and his colleagues have fabricated a DNA biosensor based on silica-nanoparticle interface [11-12]. Li and his colleagues have fabricated a controllable DNA hybridization sensor based on magnetic nanoparticles [13]. Some reviews have recently reported electrochemical DNA sensors [14-17].

Biosensors consist of two main parts: a biorecognition interface, which enables the selective detection of the analyte, and a transducer, which converts the recognition event into an electronic signal [18, 19]. For electrochemical DNA sensors, the transducer is an electrode (constructed of gold or glassy carbon) onto which DNA, as the biorecognition species, is immobilized. As is well known, the sensitivity and lifetime of DNA sensors depends on the immobilization of DNA probes onto electrode surfaces. Several methods of immobilizing DNA probes onto electrode surfaces have been reported, including physical adsorption, entrapment in a gel or polymer, covalent binding, cross-linking and electrochemical polymerization. One of these promising approaches is electrochemical polymerization using conducting polymers [19-25] such as polypyrrole [24, 25], and this kind of polymeric DNA biosensor has good sensitivity and stability.

It is reported that sensitive detection of specific DNA sequences on the basis of the hybridization reaction can be improved utilizing target or signal amplification strategies. Most of the recent developments in ultrasensitive detection of DNA have been based on the use of nanomaterials and nanotechnologies. The use of nanomaterials, nanoparticles [26-28], nanotubes [29-31] and nanowires [32,33] as a medium for signal amplification has provided many opportunities for the advance of biomolecule and gene detection.

Previously, we reported a type of electrochemical DNA biosensor based on multilayer films fabricated by LBL covalent attachment of multi-walled carbon nanotubes and GNPs [34, 35]. This strategy improves the stability of multilayer films and provides an efficient way to produce complex layered biosensor structure, however the preparation process is complex. Subsequently, we developed an DNA biosensor based on a silver nanoparticle / poly(3-(3-pyridyl) acrylic acid) / carbon nanotube modified electrode. This DNA sensor also has good selectivity and sensitivity [36]. In this work, we have fabricated an ssDNA /AuNPs / Cys / PGA electrochemical biosensor. Differential pulse voltammetry (DPV) was used to detect DNA hybridization using adriamycin as an electroactive indicator. The experimental results show that the DNA biosensor has good selectivity, sensitivity, stability and reusability. The approach to probing DNA immobilization and hybridization with target oligonucleotides is illustrated in Scheme 1.

2.0 Experimental

2.1 Reagents

Glutamic acid was purchased from Shanghai Biochemical Institute. Hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, 2-mercaptoethylamine hydrochloride (Cysteamine), sodium dodecyl sulfate (SDS), N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Alfa Aesar Co, Ltd. (Tianjing, China). Adriamycin hydrochloride was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). NaOH, HNO_3 and H_3PO_4 were obtained from Nanjing Chemical Reagent Co, Ltd. (Nanjing, China). Potassium ferricyanide / ferrocyanide were purchased from Shanghai Reagent Company (Shanghai, China). All oligonucleotide fragments used in this work were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China), and sequences of all oligonucleotides are listed as follows.
Probe sequence: SH-$(CH_2)_6$-5′-AAG CGG AGG ATT GAC GAC TA-3′
Complementary sequence: 5′-TAG TCG TCA ATC CTC CGC TT-3′
Non-complementary sequence: 5′-AAG CGG AGG ATT GAC GAC TA-3′
Three-base mismatched sequence: 5′-TAG ACG TCA TTC CTC CCC TT-3′

Stock solutions of DNA were prepared in PBS solution (pH 7.0) and stored at −20°C until used. The buffer solutions employed in this study

are as follows: 0.01 M PBS (0.1 M NaCl + 0.01 M sodium phosphate buffer solution, pH 7.0), 0.1 M PBS (0.1 M sodium phosphate buffer solution, pH 7.0). All chemicals were of analytical grade and used without further purification. All solutions were prepared with twice-quartz-distilled water.
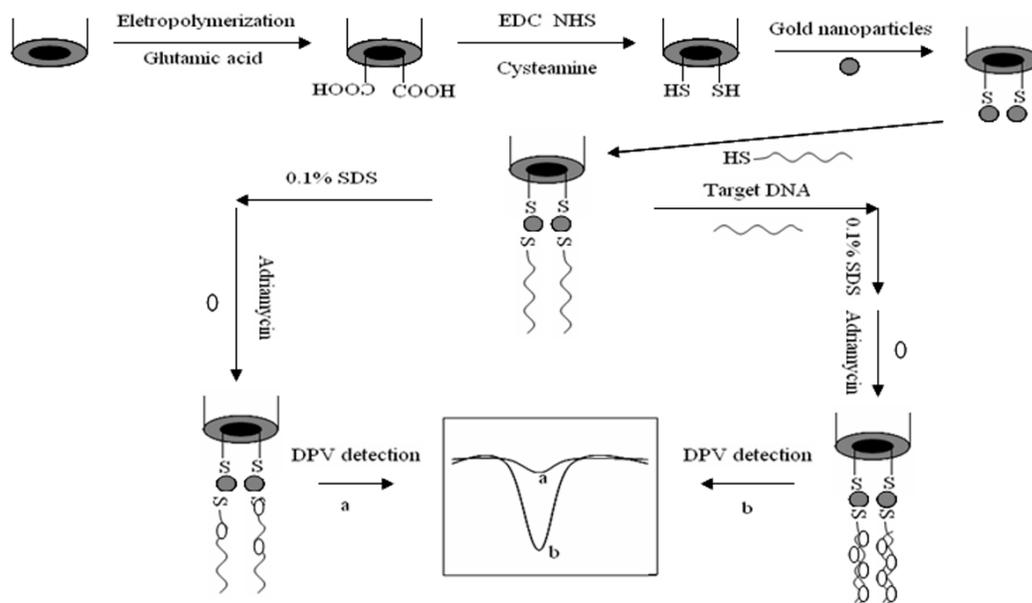
Scheme 1. Schematic representation of the immobilization and hybridization detection of probe DNA.

2.2 Apparatus

All electrochemical measurements were performed on a CHI 650C electrochemical workstation (Shanghai Chenhua Instruments Co., China). The three-electrode system used consisted of the working electrode of interest, a saturated calomel electrode (SCE) reference electrode, and a platinum wire auxiliary electrode. CV and DPV were carried out in a 10 mL electrochemical cell, which was degassed with high-purity nitrogen for 30 minutes. All the potentials given in this paper were referenced to SCE. Electrochemical Impedance Spectra (EIS) was performed in the presence of 5.0 mM $[Fe(CN)_6]^{3-/4-}$ as a redox probe in the frequency range between 1 and 10^5 Hz at the formal potential of 0.199V. The AC voltage amplitude was 5 mV. The morphologies of various modified electrode surfaces were investigated by scanning electron microscopy (SEM) using a JEOLJSM-6700F microscope (Hitachi, Japan).

2.3 Synthesis of Gold Nanoparticles

AuNPs were prepared according to the literature [39] with a little modification. Breifly, 100 mL sample of aqueous HAuCl$_4$ (0.01%) was brought to a boil, and 2.5 mL of 1% trisodium citrate solution was added with vigorous stirring. The mixed solution was stirred for 30 min and allowed to return to room temperature under stirring. The resulting solution of GNPs was examined using a UV-vis spectrometer, which yielded a strong absorption peak at 518 nm, characteristic of monodispersed gold colloid. The diameter of the AuNPs was about 16 nm as measured by transmission electron microscopy (picture not shown). The obtained colloidal gold solution was stored in dark glass bottles at 4 °C for further use.

2.4 Fabrication of ssDNA/ AuNPs /Cys /PGA probe electrode

2.4.1 Preparation of PGA Modified Electrode

Prior to modification, the bare GCE was polished to a mirror-like surface with Gamma alumina suspensions (1.0μm, 0.25μm and 0.05μm, respectively). Then, the electrode was successively ultrasonically washed in ethanol and twice-quartz-distilled water for 3 min, respectively. The PGA modified electrode was prepared according to previous reports [37, 38].

Briefly, a clean glassy electrode was immersed into 0.02 M glutamic acid solution in 0.1M PBS (pH 7.0) and subjected to cyclic potential scans (15 cycles from −1.0 to +2.0 V with a scan rate of 100 mV/s). The free polar carboxylic acid groups layer was deposited on the surface of electrode. Afterwards, the electrode was gently washed with twice-quartz-distilled water and ready for use.

2.4.2 Preparation of ssDNA /AuNPs /Cys /PGA Modified Electrode

The PGA modified electrode was activated for 1 h in 1:1(v/v) EDC / NHS mixture (10 mM EDC and 10 mM NHS, pH 5.0), and allowed to react with a 0.5 M cysteamine solution for 40 min. This process yields thiol groups on self-assembled monolayer film. Next, the Cys/PGA modified electrode was immersed into AuNPs solution for 12 h in a refrigerator. The resulting electrode was carefully washed with twice-quartz-distilled water and allowed to dry at room temperature. Finally the AuNPs /Cys/PGA modified electrode was immersed in a 1.0×10^{-6} M ssDNA probe solution for 6 h, which gives a probe ssDNA self-assembled layer via S-Au binding. The probe-modified electrode was immersed into 0.1% SDS for 10 min and washed with twice-quartz-distilled water and 0.1 M PBS for three times, respectively. The obtained electrode was denoted as a ssDNA / AuNPs /Cys /PGA modified electrode.

2.5 Electrochemical Detection of DNA Hybridization

The hybridization reaction was performed by immersing the probe DNA modified electrode into 0.01 M PBS solution containing varying concentrations of target DNA for 20 min at 40 °C. The hybridized double-stranded DNA electrode was next immersed into 0.1% SDS for 10 min, then it was immersed in a 0.01 M PBS solution containing 1.0×10^{-6} M adriamycin for 10 min, finally it was washed with twice-quartz-distilled water and 0.1 M PBS for three times to remove physically absorbed adriamycin.

The DNA hybridization was assessed with DPV peak current of adriamycin in 0.1 M PBS (pH 7.0). The concentration of target oligonucleotides was quantified by the increase of reduction peak current (ΔI) of adriamycin, which was subtracted from the reduction peak current generated at the ssDNA/ AuNPs /Cys/PGA modified electrode.

3.0 Results and Discussions

### 3.1. Characterization of the different modified electrode

The bare glassy electrode, PGA and AuNPs/Cys/ PGA modified electrode were characterized by SEM, which are shown in Figure 1, from which we can observe that AuNPs were equably coated onto the surface of electrode. The result indicated that AuNPs /Cys/ PGA modified electrode had been successfully fabricated.
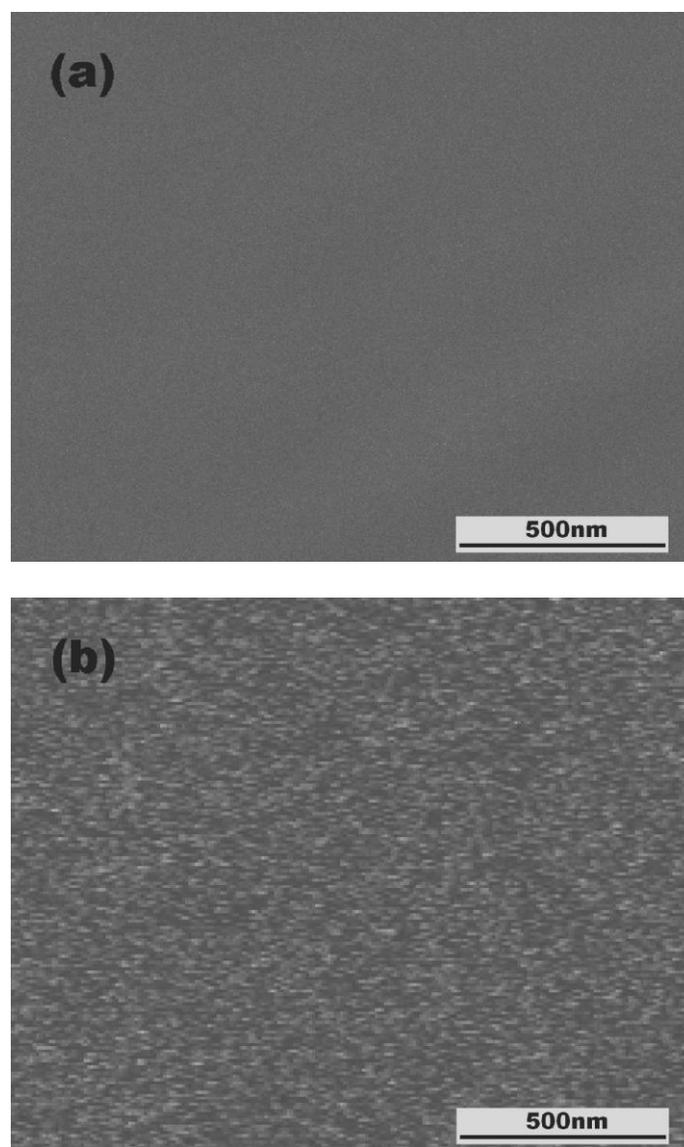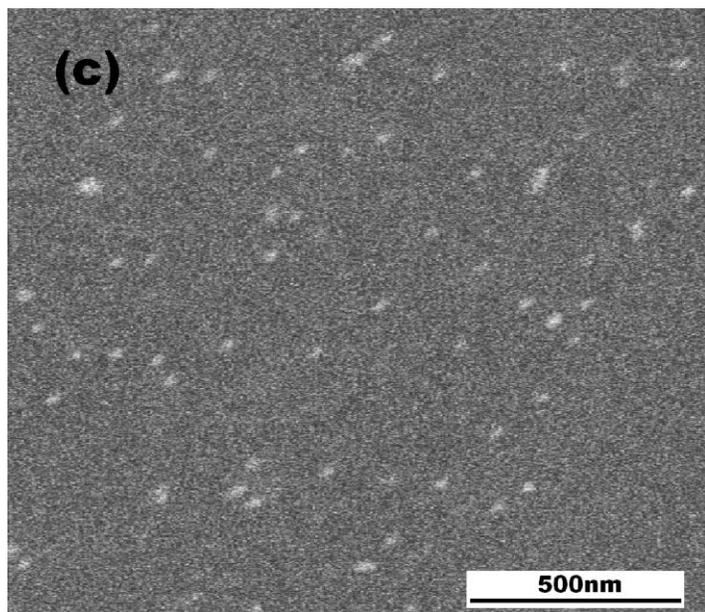
Figure 1. SEM pictures of the bare GCE (a), Cys/PGA (b) and AuNPs /Cys/PGA (c).

Previous studies have revealed that the fabrication of polymeric film and assembly of nucleic acids on electrodes can be indicated by EIS. Figure.2 shows Nyquist plots of impedance for bare GCE (Figure 2a), GCE modified with PGA (Figure 2b), GCE modified with AuNPs /Cys/PGA (Figure 2c) and after immobilization of probe DNA (Figure 2d). In EIS, the semicircle diameter corresponds to the electron transfer resistance (R_{et}). We can observe there is a small R_{et} at the bare GCE or PGA modified electrode. When AuNPs were immobilized on the electrode, the R_{et} is increased. This increase is a result of AuNPs having a negative charge which can stop $[Fe(CN)_6]^{3-/4-}$ from approaching the surface of electrode. When the DNA probe was immobilized onto the modified electrode, the R_{et} is increased greatly, as the negatively charged phosphate skeletons of DNA cause an electrostatic repulsion to $[Fe(CN)_6]^{3-/4}$.
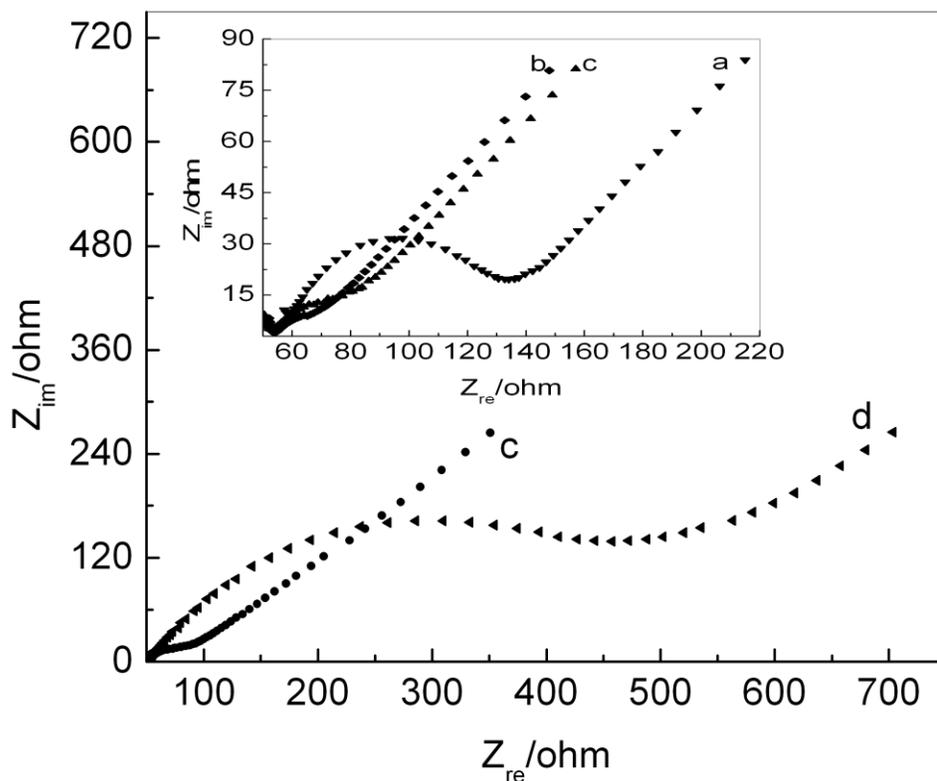

Figure 2. Nyquist plots obtained for different electrodes: the bare GCE (a), the PGA (b), the AuNPs /Cys/PGA (c) and the ssDNA/ AuNPs /Cys/PGA (d).

In this work, CV response of Adriamycin at different electrodes was used to investigate the immobilization of probe DNA. When AuNPs were introduced onto a PGA electrode (data not shown), the peak currents of adriamycin were greatly enhanced with respect to the bare electrode. After probe DNA was introduced to the surface of AuNPs /Cys /PGA, a much larger voltammetric response of adriamycin was observed. This larger voltammetric can be attributed to the electrostatic interaction between the negatively charged phosphate backbone of DNA and adriamycin. This result indicates the AuNPs thin film could provide a good platform for probe DNA immobilization.

3.2 Optimization of Experiment Conditions

3.2.1 Hybridization Time

Figure 3 shows the influence of the DNA hybridization time on the peak current of adriamycin. It is apparent that the reduction current rapidly increased initially with a hybridization time from 0 to 10 min, then increased slightly from 10 to 20 min and remained constant after 20 min. This indicated that the hybridization reaction was dominantly completed after 20 min. Considering the sensitivity and assay time, 20 min was chosen as the hybridization time in this work.
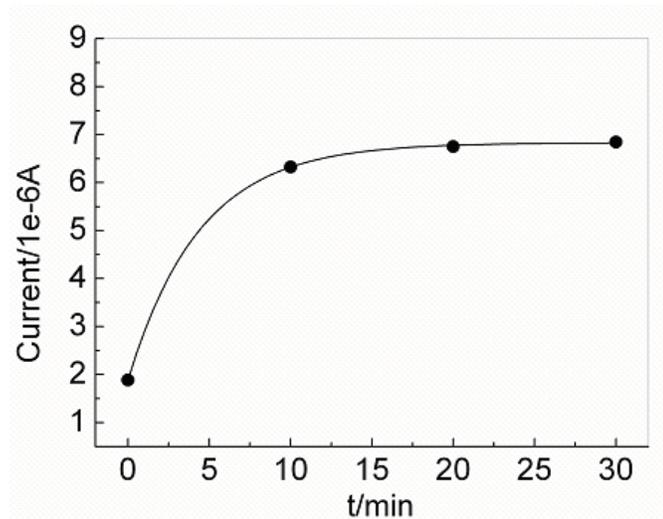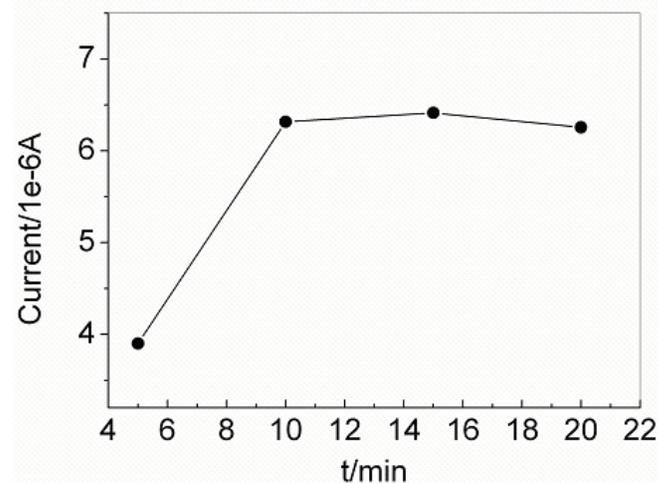

Figure 3. The effect of DNA hybridization time on the reduction peak current of adriamycin

The DNA biosensor was incubated in 0.01 M PBS (pH 7.0) containing 4.8×10^{-8} M cDNA at 40 ℃ for different times. The DPV measurements were completed in 0.1 M pH 7.0 PBS. The DPV parameters: initial potential: 0.7 V; terminal potential: 0 V; pulse amplitude: 50 mV; pulse period: 0.2 s; pulse width: 50 ms.

3.2.2 Accumulation Time of Adriamycin

The effect of accumulation time of adriamycin was investigated by DPV measurements of adriamycin at hybridized electrodes which were incubated in 1.0×10^{-6} M adriamycin for varying times. The results are shown in Figure 4. It can be seen the peak current of adriamycin increased significantly with the increasing accumulation time from 0 to 10 min. When the accumulation time is higher than 10 min, the peak current of adriamycin remains relatively constant, so 10 min was selected as the optimum accumulation time.


Figure 4. The effect of accumulation time on the reduction peak current of adriamycin.

The hybridized electrode was incubated in 0.01 M PBS (pH 7.0) containing 1.0×10^{-6} M adriamycin for various times. The DPV measurements were completed in 0.1 M pH 7.0 PBS. The DPV parameters were the same as in Figure 3.

### 3.3 Analytical performance

Under optimal conditions, the sensitivity of the DNA sensor was investigated using an immobilized DNA probe to hybridize with

different concentrations of the cDNA. Figure 5 displays DPVs recorded of adriamycin as redox intercalator when the immobilized probe hybridized with different concentrations of the cDNA. We can observed the reduction peak current of adriamycin increased with the concentration of the cDNA increased, and the increases of peak current are linear with the concentration of the cDNA in the range from 9.0×10^{-11} to 4.8×10^{-9} M (shown in the inset of Figure. 5) The regression equation was ΔI (μA) = 0.5797 + 0.058C (c: the concentration of complementary sequence (10^{-10} M), i: the peak current of adriamycin, μA), and a regression coefficient (r^2) of 0.9907. A detection limit of 4.2×10^{-11} M was achieved ($S/N = 3$).
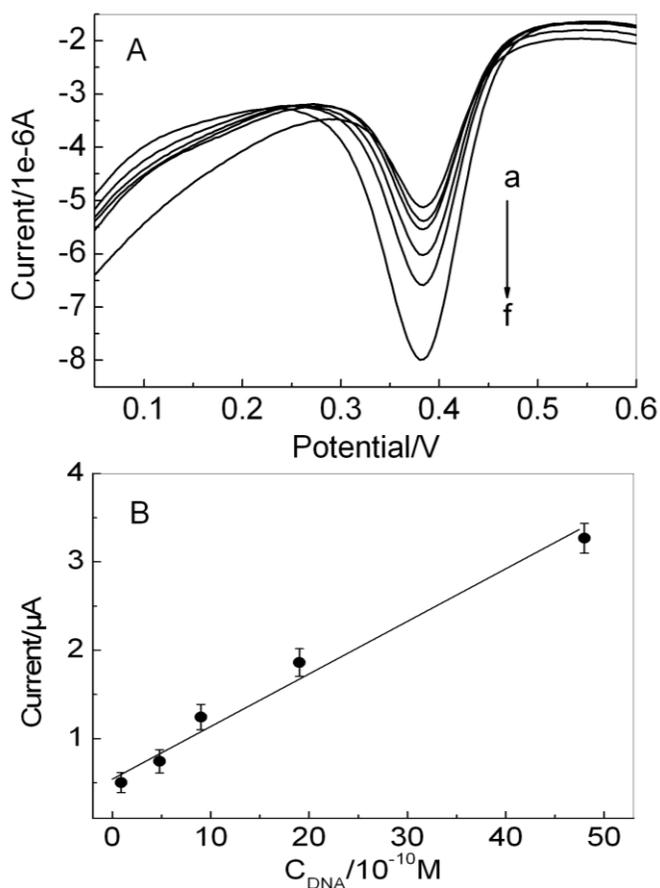
Figure.5. A) DPV responses of the intercalated adriamycin recorded for the ssDNA/ AuNPs /Cys/PGA/GCE which hybridized with different concentrations of cDNA: 0.0 M (a), 9.0×10^{-11} M (b), 4.8×10^{-10} M (c), 9.0×10^{-10} M (d), 1.9×10^{-9} M (e), 4.8×10^{-9} M (f). B): The peak current vs. the

concentration of cDNA. The DPV parameters were the same as in Figure. 3.
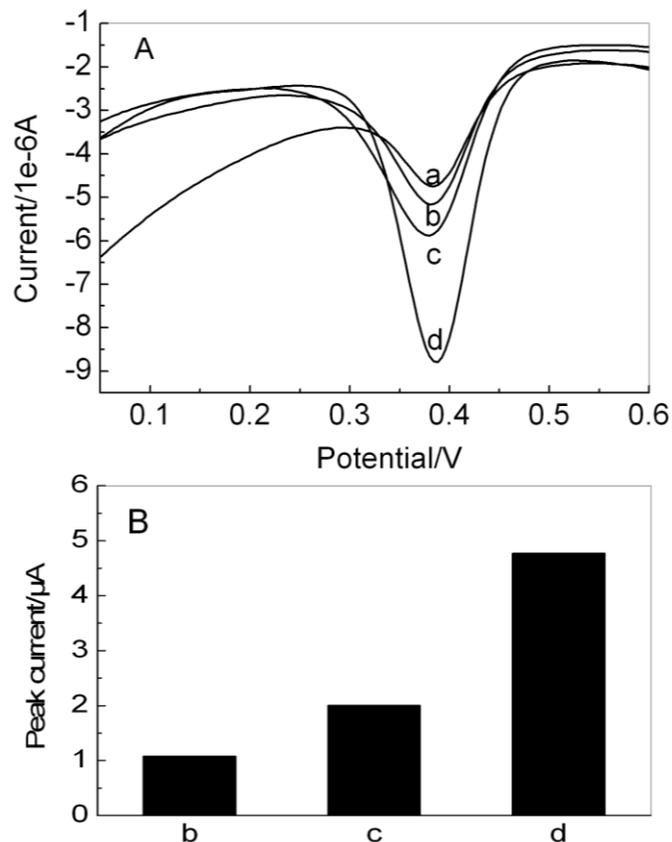
Figure 6. A)The DPV responses of adriamycin recorded for ssDNA/ AuNPs /Cys/PGA without hybridized (a); hybridized with non-complementary DNA (b); hybridized with three-base mismatched DNA (c); hybridized with cDNA (d). B) Corresponding histograms of the reduction peak currents. The concentration of DNA was 4.8×10^{-8} M. The DPV parameters were the same as in Figure 3.

Selectivity is a crucial factor to be considered for a DNA biosensor. In this work, it was also evaluated using its complete complementary, three-base mismatched and non-complementary DNA sequences. As it is shown in Figure 6, when non-complementary and three-base mismatched DNA sequences exist, the signal of adriamycin was lower (curves b and c in Figure 6), when its complete cDNA sequence exists, the signal of adriamycin is obviously increased. So the DNA sensor can distinguish between three-base

mismatched DNA sequences and cDNA sequences. This result suggests that the fabricated DNA sensor has excellent selectively.

We also compare the performances of different electrochemical DNA sensors with this work and results are shown in table 1. From the table 1, we know can observe this DNA sensor reported in this study has shorter hybridization time and lower detection limit. In conclusion, the proposed DNA sensor exhibits good analytical performance for the specific sequences in DNA detection.

The DNA sensor	Detect technique	linear detection range (nM)	limit (nM)	Hybridization time (min)	Reference
PPy/DNA/CNT/GCE	DPV	0.1 –1000	0.05	180	[40]
DNA/PICA/GCE	CV	3.34 – 10.6	1.0	45	[41]
Polypyrrole/DNA/MWCNTs Paste electrode	DPV	0.1 – 10	0.085	30	[42]
DNA/ AuNPs /cys/PGA	DPV	0.09 – 4.8	0.042	20	this work

Table 1 Comparison of analytical performances of several electrochemical DNA sensors

3.4 Reproducibility, Regeneration and Stability of the DNA Sensor

The reproducibility of the DNA sensor was also investigated. Three DNA sensors, which were fabricated independently at the same conditions, were used to detect 4.8×10^{-8} M complementary DNA, respectively. The reduction peak current of adriamycin were 6.846×10^{-6} A, 6.656×10^{-6} A, 6.256×10^{-6} A, respectively, which gave an average value 6.586×10^{-6} A with a relative standard deviation (R.S.D.) of 4.6%. This result suggests that the DNA sensor is reproducible.

The regeneration of the DNA sensor was also investigated by immersing hybridized electrodes in hot water (80 ℃) for 5 – 10 min, which completely removed hybridized DNA via thermal denaturation [43]. The reduction peak current of adriamycin was 6.656×10^{-6} A at the first detection. The DNA sensor was regenerated according to the above description. The reduction peak currents of adriamycin of three times successive DNA sensor regeneration were 6.413×10^{-6} A, 6.316×10^{-6} A, 6.238×10^{-6} A, respectively. The third regenerated electrode has 94.0% response of the initial electrode, indicating the proposed DNA sensor was of good reusability.

When the DNA sensor was stored in the refrigerator at 4 ℃ and measured after ten days, it kept about 86.2% of its original response. This indicates that the proposed DNA sensor has good stability.

4.0 Conclusions

In present work, we introduce an electrochemical DNA sensor, and the DNA sensor displays good selectivity and high sensitivity. The experiment result indicates the DNA sensor has a fast response time, easy fabrication, and is capable of detecting a three-base mismatched DNA sequence assay.

Acknowledgements

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