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A Label-free Electrochemical Biosensor for Acrylamide Based on DNA Immobilized on Graphene Oxide-Modified Glassy Carbon Electrode

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Acrylamide (AA) is a widely used chemical compound, which has strong neurotoxicity, and potential carcinogenicity and genotoxicity. In the present work, the interaction between AA and DNA was confirmed by UV-visible spectroscopy. According to such an interaction, a label-free DNA biosensor for electrochemical determination of AA was proposed. To fabricate the sensor, graphene oxide (GO) was firstly coated on glassy carbon electrode (GCE) surface. Then DNA was immobilized on GO/GCE by electroadsorption. Due to large surface area of GO, DNA was effectively immobilized on the electrode surface. Moreover, the unique nanostructure and excellent electron transfer ability of GO significantly promoted the direct electron transfer of DNA. Thus, DNA showed two strong oxidation peaks on GO/GCE, which could be used as the electrochemical signal for AA sensing. The Influences of adsorption potential and adsorption time on the immobilized DNA and pH for AA sensing were systematically investigated. Under optimum conditions, the response of DNA/GO/GCE was linear to the concentration of AA from 5.0×10^{-8} to 1.0×10^{-3} mol/L. Moreover, the sensor showed good reproducibility and high stability.

Keywords: Acrylamide, DNA, Graphene oxide, Differential pulse voltammetry, Electrochemical biosensor

1. INTRODUCTION

Acrylamide (AA) is a chemical raw material used for synthesis of polyacrylamides, a polymer widely utilized in wastewater treatment, gel electrophoresis, papermaking, ore processing, tertiary oil recovery, and the manufacture of permanent press fabrics [1]. Some AA is used in the manufacture of dyes and the manufacture of other monomers. AA is known to have strong neurotoxicity, and it has potential carcinogenicity, genotoxicity, reproductive and developmental toxicity [2,3]. In 2002, high level of AA was detected in some fried and grilled starchy foods, which may be produced by the reaction between asparagine and reducing sugars or reactive carbonyls at temperatures above 120 °C [4]. The discovery of AA in cooked starchy foods prompted concerns about the carcinogenicity of those foods. Genetic toxicology studies have found that AA has mutagenic effects both in vivo and in vitro experiments, and it can cause gene mutation and chromosomal abnormalities of somatic and germ cells of mammalian, such as the formation of micronuclei, sister chromatid conversion and so on [5]. Moreover, it has been reported that epoxypropionamide, a metabolites of AA, is the main material of mutagenic activity, which is easy combined with guanine of DNA then forming an adduct. International Agency for Research on Cancer (IARC) have evaluated the carcinogenicity of AA and listed it as a category 2 carcinogen (2A) [6].

Toxicity evaluation of pollutants is mainly carried out through the biological tests which spend a long time and high cost. What is more, it is difficult to test various amounts of contaminants in water individually. In comparison, DNA biosensors providing a cheap and fast approach for the study of toxicity of pollutants have attracted much research interest [7,8]. As we all know, DNA is a macromolecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses. The bases in the double helix structure of DNA have been revealed to be electrochemically active, which is very useful to provide the direct electrochemical signal for fabrication of DNA-based electrochemical biosensors without addition of electrochemical labels [9]. When genotoxic substances interact with DNA, the electrochemical signals of DNA bases would change quickly. Accordingly, DNA-based electrochemical biosensor have be developed and used to detect genotoxic pollutants, analyze the binding constant between pollutants and DNA, and investigate the mechanism of damage of pollutants on DNA [10-14].

In the present work, we demonstrated a label-free DNA-based electrochemical biosensor for AA based on the interaction between AA with DNA. Graphene oxide (GO), a single-atomic-layered nanomaterial was employed to modify the electrode for fabricating the sensor. The unique physicochemical properties such as high surface area, high electrical conductivity, and excellent electron mobility not only provide an excellent platform for DNA immobilization, but also significantly promoted the electrochemical response of DNA bases [15-17]. In the presence of AA, the electrochemical signal of DNA bases immobilized on the electrode surface was declined. Under optimum conditions, the signal of the biosensor was linear to the concentration of AA in the range of 5.0×10^{-8} to 1.0×10^{-3} mol/L.

2. EXPERIMENTAL

2.1 Reagents and apparatuses

Herring sperm DNA was purchased from Sigma. Acrylamide (AA), KCl, $K_3[Fe(CN)_6]$, $K_2[Fe(CN)_6]$, NaH_2PO_4 and Na_2HPO_4 were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All the reagents were of analytical grade and used without further purification. Ultra-pure water was used for all experiments.

Electrochemical experiments were performed on an EC550 electrochemical workstation (Wuhan Gaoss Union Instrument Company, China). All electrochemical measurements were carried out in a conventional three-electrode system. A modified glassy carbon electrode (GCE), a platinum electrode, and a saturated calomel electrode were employed as the working, auxiliary and reference electrodes, respectively. For differential pulse voltammetry (DPV) the parameters were: amplitude: 50 mV, step potential: 5 mV, pulse width: 0.05 s.

UV-visible spectra were recorded using a TU-1901 double beam UV-Vis spectrophotometer (Beijing Spectral Analysis of General Instrument Co., Ltd.).

2.2 Fabrication of DNA/GO/GCE-based sensor

The working surface of GCE was polished with metallographic sandpapers, washed with ultra pure water. After polished with 0.05 μm Al_2O_3 powder for 10min, GCE was cleaned in ultra pure water and ethanol for 20 min in an ultrasonic bath. After being dried with nitrogen gas, the GCE surface was coated with 5 μL 1 mg/mL GO suspension to obtain a GO-modified GCE (GO/GCE). GO was prepared from graphite powder according to a modified Hummers method. The GO suspension was prepared by dispersing 3 mg GO power in 3 mL water with the aid of 30-min ultrasonic agitation.

To prepare DNA/GO/GCE, the GO modified electrode was immersed in DNA solution (dissolved in 0.1 mol/L phosphate buffer at pH 7.0), followed by applying a potential of 0.1 V for some time. After that, the electrodes were rinsed with double distilled water to remove weakly absorbed DNA.

3. RESULTS AND DISCUSSION

3.1 Interaction of DNA with AA

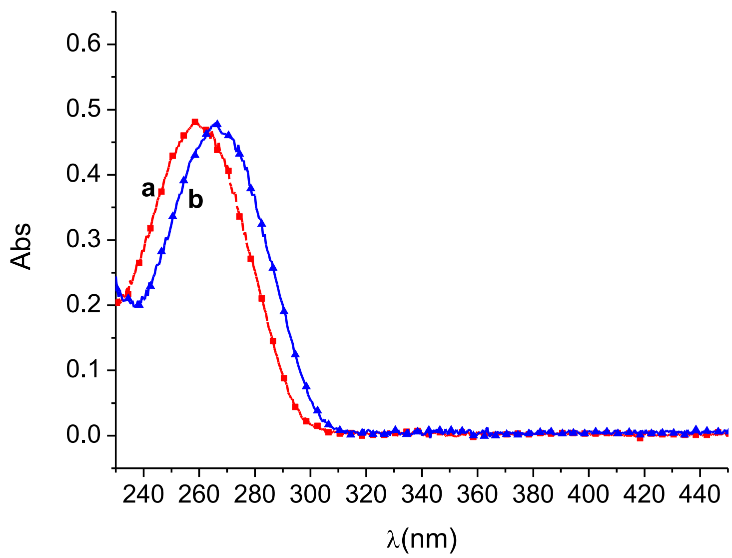


Figure 1. UV spectra of DNA in 0.1 M PBS (pH 7.00) (a) before and (b) after adding AA.

As a biological macromolecule, DNA has a strong UV absorption around 260 nm due to its purine and pyrimidine bases [18]. The UV absorption performance of DNA interact with AA was studied by UV spectroscopy in 0.1 M PBS (pH 7.00). As shown in Fig. 1, the maximum absorption wavelength of DNA exhibits obvious redshift after the addition of 1×10^{-3} mol/L AA, meaning the interaction between DNA and AA. The density functional theory (DFT) analysis [19] shows that AA is not only a good hydrogen bond acceptor, but also a good hydrogen bond donor to the high electronegativity atoms such as O and N. Thus AA can form stable hydrogen bonds with purine and pyrimidine bases of DNA, which leads to the formation of a stable DNA-AA adduct.

3.2 Electrochemical behavior of DNA immobilized on GO-modified electrode

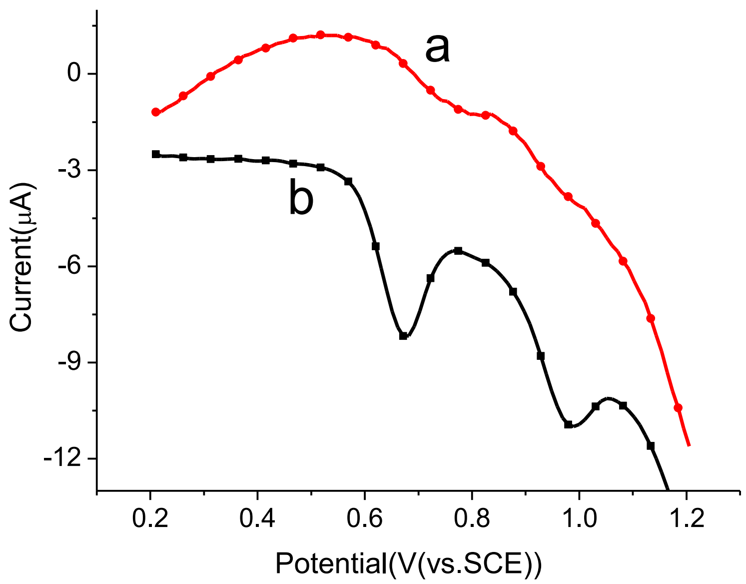
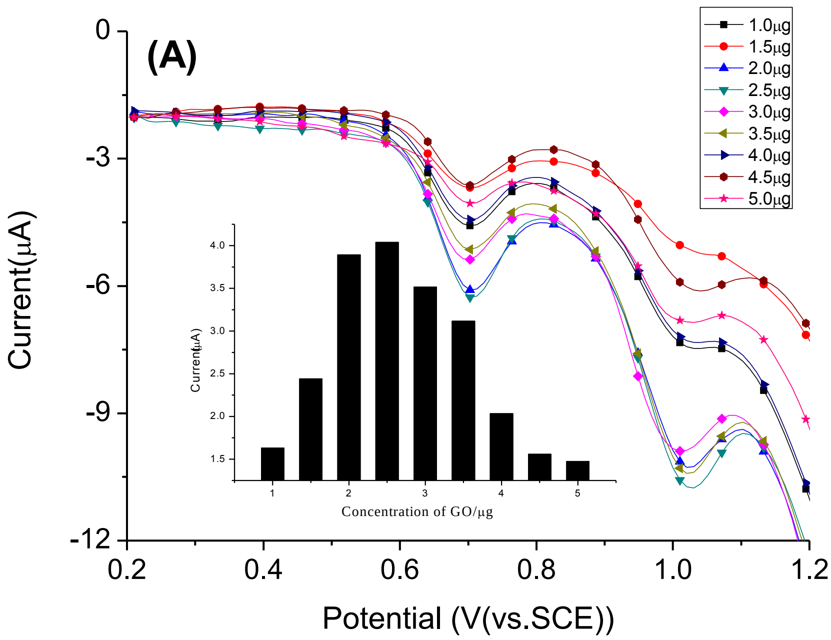


Figure 2. Differential pulse voltammograms of (a) DNA/GCE and (b) DNA/GO/GCE electrodes in 0.1 M PBS at pH 7.00.

The electrochemical oxidation behavior of DNA/GCE (a) and DNA/GO/GCE was studied in 0.1M PBS buffer solution (pH = 7.00) by DPV (Fig. 2). As can been, two anodic peaks appear at about 0.70V and 1.00V on DNA/GO/GCE, which are respectively attributed to the oxidation of guanine and adenine residues of DNA [20,21]. In comparison, the anodic peaks are very weak on DNA/GCE. Although guanine and adenine in DNA are electroactive groups, they are buried in the macromolecular structures. Thus the electron transfer between DNA residues and GCE is very sluggish. While the electrode is modified with GO, the unique nanostructure and excellent electron transfer ability of GO offer this modifier as a bridge between DNA and electrode, which can significantly promote the direct electron transfer of DNA. Because the peak current of guanine was higher than that of adenine, it was selected as the monitoring signal in the following investigation.

3.3 Effects of GO amount and DNA concentration on the response of modified electrode



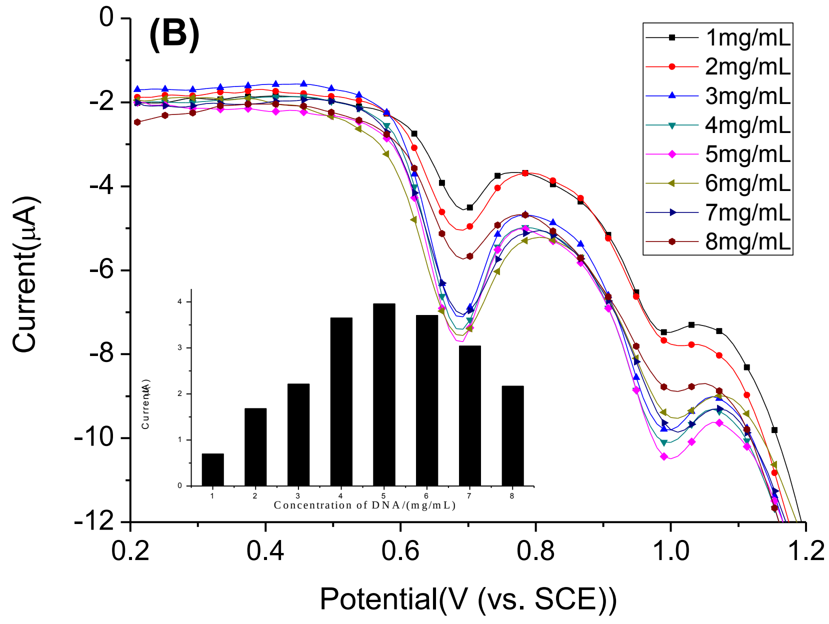


Figure 3. Differential pulse voltammograms of DNA/GO/GCE with (A) different amounts of GO and (B) different concentrations of DNA in 0.1 M PBS at pH 7.00.

The influences of GO amount and DNA concentration for preparation of modified electrodes were investigated in 0.1M PBS at pH 7.00 (Fig. 3). It can be seen from Fig. 3A that the electrochemical response increases with increasing the amount of GO from 1.0 to 2.5 μg. While the amount of GO is increased to more than 2.5 μg, the electrochemical response is decreased with further increasing the amount of GO. For the effect of DNA concentration, the electrochemical response increases with increasing the amount of GO from 1.0 to 5.0 mg/mL, and then gradually declines (Fig. 3B). It is obvious that suitable amount of GO and DNA immobilized on the electrode surface is advantageous to provide the efficient electron transfer between DNA and electrode. However, the effective surface area of the electrode decides the limited amount of DNA for electro-oxidation. Excessive amount of GO might reduce the stability of modification layer on the electrode surface [22] while excessive DNA biomolecules might increase the resistance for interfacial electron transfer [23], and thus the efficiency and sensitivity of modified electrode are decreased. Accordingly, the modified electrode prepared with 2.5 μg GO and 5mg/mL DNA exhibiting the strongest electrochemical response was used for the following experiments.

3.4 Influences of adsorption potential and adsorption time on the immobilized DNA

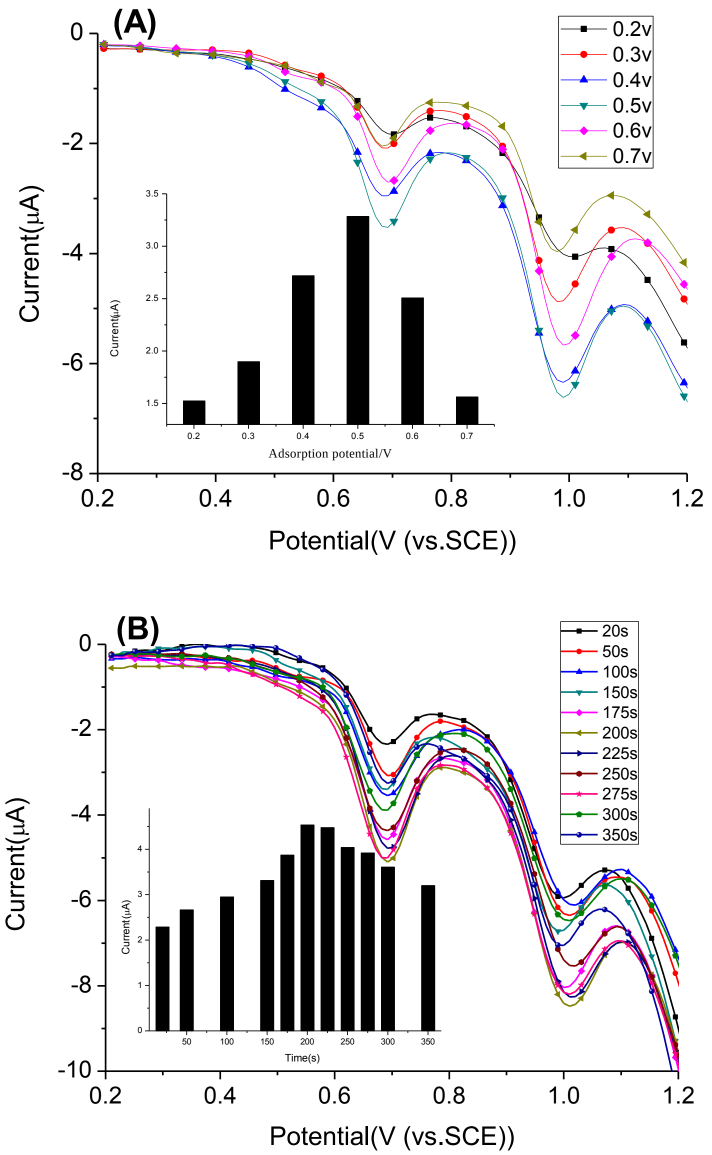
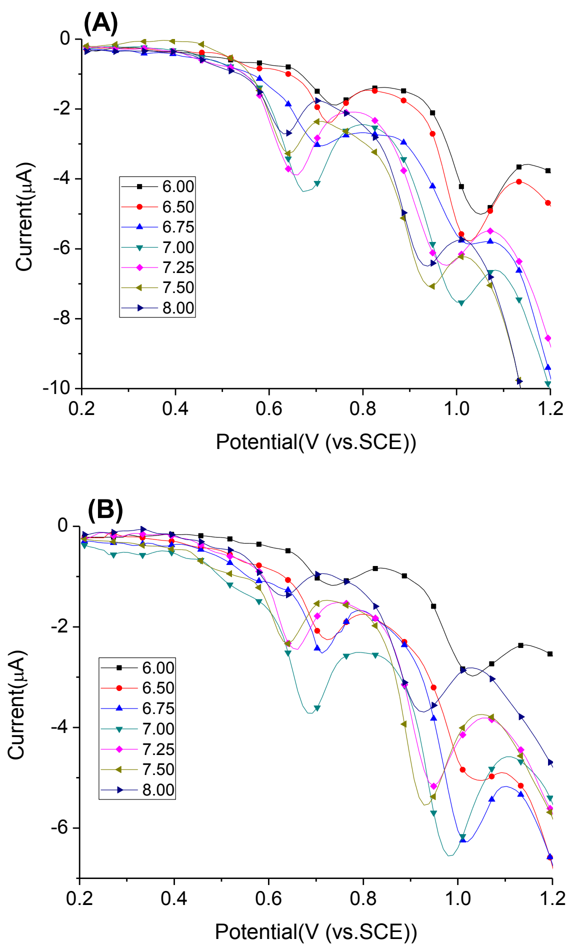


Figure 4. Differential pulse voltammograms of DNA/GO/GCE prepared with (A) different adsorption potentials and (B) different adsorption times in 0.1 M PBS at pH 7.00.

In this work, DNA was immobilized on the electrode surface by electroadsorption. Therefore, adsorption potential and adsorption time exhibited obvious impacts on the fabricated electrode. Fig. 4 shows the influences adsorption potential and adsorption time on the oxidation peak current responses of DNA/GO/GCE incubated in 0.1M PBS at pH 7.00. In view of the double helix structure of DNA containing a negatively charged phosphate group, DNA could be effectively adsorbed on the electrode surface when a positive potential was subjected to electrode. Generally, increasing the positive potential applied could increase the DNA adsorption on electrode surface via electrostatic interaction. Thus, DNA/GO/GCE showed enhanced voltammetric response with increasing the adsorption potential from 0.10 to 0.50 V (Fig. 4A). However, the voltammetric response of DNA/GO/GCE was obviously decreased as the adsorption potential was further improved to be higher than 0.50 V. This result can be attributed to the fact that guanine and adenine in the double helix structure of DNA could be electro-oxidized at about 0.70V and 1.00V, and too high positive voltage applied would cause DNA oxidation directly [24]. Meanwhile, the adsorption time also affected the immobilization of DNA on the electrode surface. The voltammetric response of DNA/GO/GCE was increased with adsorption time up to 200 s (Fig. 4B). Therefore, adsorption potential at 0.50 V and adsorption time of 200 s were suitable adsorption parameters for DNA immobilization on GO-modified electrode.

3.5 Selection of pH



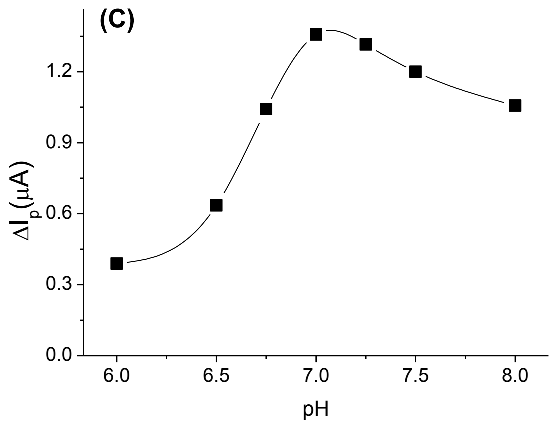
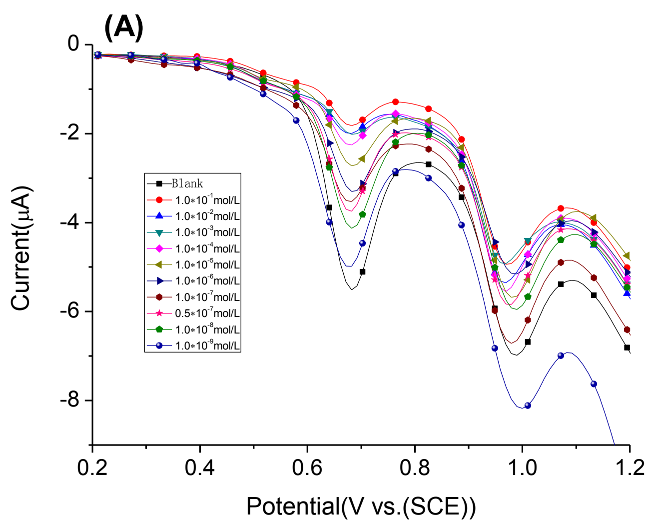


Figure 5. Differential pulse voltammograms of DNA/GO/GCE in 0.1 M PBS at different pH values (A) without and (B) with 1.0×10^{-4} mol/L AA. (C) Relationship between $\triangle I_p$ and pH. $\triangle I_p$ represents the difference for guanine oxidation peak currents before and after adding AA.

In order to examine effect of pH on the interaction between AA and DNA, the voltammetric responses of DNA/GO/GCE were studied in 0.1 M PBS at different pH values in the absence or presence of 1.0×10^{-4} mol/L AA (Fig. 5). As can be seen, in both solutions, the oxidation peaks of DNA shift to positive potential when pH decreases from 8.00 to 6.00, accompanied with variation in peak currents, implying that proton participates in the oxidation process of DNA [12,13,25]. Furthermore, to quantitatively evaluate the interaction of AA with DNA, the difference ($\triangle I_p$) for guanine oxidation peak currents before and after adding AA was calculated and used as the sensing signal of AA. Fig. 5C displays the variation of $\triangle I_p$ with pH. It is observed that $\triangle I_p$ is enhanced with increasing the pH value from 6.00 up to 7.00. Accordingly, pH 7.00 providing the highest $\triangle I_p$ value was selected as the optimum pH condition for sensing of AA on DNA/GO/GCE.

3.6 Determination of AA using DNA/GO/GCE



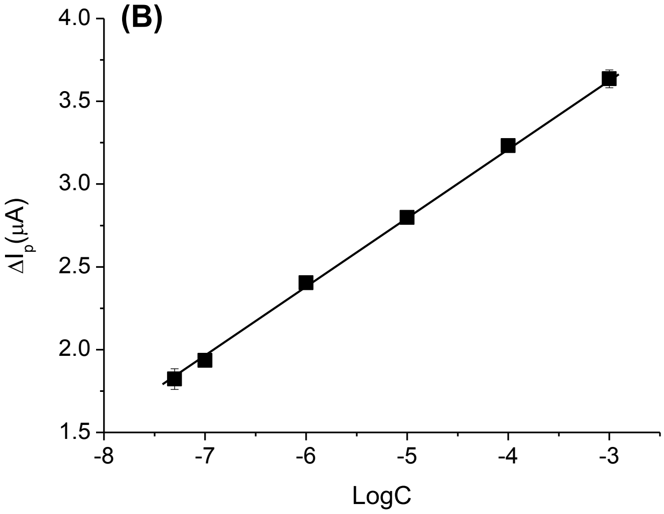


Figure 6. (A) Differential pulse voltammograms of DNA/GO/GCE in 0.1 MPBS buffer solution (pH 7.00) containing different amounts of AA. (B) Linear relationship between $\triangle I_p$ and AA concentration. $\triangle I_p$ represents the difference for guanine oxidation peak currents before and after adding AA.

Under optimum conditions, the proposed DNA/GO/GCE was utilized to determine AA. Fig. 6A shows the voltammetric responses of DNA/GO/GCE in PBS (pH 7.00) containing different amounts of AA. It is found that the signal of the biosensor ($\triangle I_p$) is linearly proportional to the concentration of AA in the range of 5.0×10^{-8} to 1.0×10^{-3} mol/L (Fig. 6B). The linear equation can be expressed as $\triangle I_p$ (μA) =4.92863+0.42447lgC (mol/L), and the correlation coefficient is 0.9989.

To check the repeatability of the sensor, the DNA/GO/GCE obtained was repeated for seven measurements in 0.1 M PBS (pH 7.00) containing1.0×10^{-4} mol/L AA and the relative standard deviation (RSD) was 0.525% (Table 1), showing a good repeatability. Moreover, the stability of the sensor was checked. The voltammetric response of DNA/GO/GCE stored at room temperature in a dry box did not show obvious decrease after 30 days (Table 2), demonstrating the high stability of the sensor.

Table 1. Responses of sensor for repeated measurements in 0.1 M PBS (pH 7.00) containing1.0×10^{-4} mol/L AA.

Repeated measurement / time	$\triangle I_p$ / μA
1st	3.2362
2nd	3.2215
3rd	3.2676
4th	3.2454
5th	3.2689
6th	3.2552
7th	3.2432
RSD (%)	0.525

Table 2. Responses of sensor in 0.1 M PBS (pH 7.00) containing1.0×10^{-4} mol/L AA during 30-day storage.

Storage time / days	$\triangle I_p$ / μA
0.5	3.2382
1	3.2212
3	3.2876
7	3.2454
15	3.2759
30	3.2115
RSD (%)	0.922

Table 3. Determination of AA in tap water sample.

Sample	Added (μmol L^{-1})	Found by proposed sensor (μmol L^{-1})	Found by spectrometry (μmol L^{-1})	Relative difference (%)
Tap water	50.00	52.94	54.84	3.46

The DNA/GO/GCE-based biosensor was applied to determine the concentration of AA in tap water. Because no AA was found in original tap water, standard AA solution was spiked to prepare a simulated water sample. As shown in Table 3, the concentration of AA in the sample found by the proposed senor was 52.94 μmol L^{-1}. On the other hand, the concentration of AA in this water sample was determined to be 54.84 μmol L^{-1} using UV spectrometry at its maximum absorption peak at 195 nm. The small relative difference between our electrochemical method and traditional spectrometry demonstrates the feasibility of the proposed sensor for the determination of AA in real water samples.

4. CONCLUSIONS

In the present work, we developed an electrochemical sensor for AA based on the interaction between AA and DNA. The electrochemical behavior of DNA immobilized on GO/GCE by electroadsorption was studied. The results showed GO significantly promoted the direct electron transfer of DNA, and the modified electrode prepared with 2.5 μg GO and 5mg/mL DNA exhibited the strongest electrochemical response. Moreover, adsorption potential at 0.50 V and adsorption time of 200 s were suitable adsorption parameters for DNA immobilization on GO-modified electrode. When AA was present in the electrolyte, the response of DNA/GO/GCE was decreased due to the interaction of AA and DNA. Thus, DNA/GO/GCE could be used as the sensor for AA determination. Furthermore, pH had an obvious effect on the electrochemical sensor and pH 7.00 providing the highest $\triangle I_p$ value was selected as the optimum pH condition for sensing of AA on DNA/GO/GCE. The response of the sensor exhibited a linear response to the concentration of AA from 5.0×10^{-8} to 1.0×10^{-3} mol/L. The developed DNA-based sensor is promising for monitoring of AA and other genotoxic pollutants.

ACKNOWLEDGEMENTS
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