Colloids and Surfaces B: Biointerfaces 82 (2011) 543–549

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Novel electrochemical sensor based on functionalized graphene for simultaneous determination of adenine and guanine in DNA

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ARTICLE INFO

Article history:
Received 27 June 2010
Received in revised form
14 September 2010
Accepted 7 October 2010
Available online 15 October 2010

Keywords:
Carboxylic acid functionalized graphene
Guanine
Adenine
Simultaneous determination

ABSTRACT

A nano-material carboxylic acid functionalized graphene (graphene-COOH) was prepared and used to construct a novel biosensor for the simultaneous detection of adenine and guanine. The direct electrooxidation behaviors of adenine and guanine on the graphene-COOH modified glassy carbon electrode (graphene-COOH/GCE) were carefully investigated by cyclic voltammetry and differential pulse voltammetry. The results indicated that both adenine and guanine showed the increase of the oxidation peak currents with the negative shift of the oxidation peak potentials in contrast to that on the bare glassy carbon electrode. The electrochemical parameters of adenine and guanine on the graphene-COOH/GCE were calculated and a simple and reliable electroanalytical method was developed for the detection of adenine and guanine, respectively. The modified electrode exhibited good behaviors in the simultaneous detection of adenine and guanine with the peak separation as 0.334 V. The detection limit for individual determination of guanine and adenine was 5.0×10^{-8} M and 2.5×10^{-8} M (S/N = 3), respectively. Furthermore, the measurements of thermally denatured single-stranded DNA were carried out and the value of (G + C)/(A + T) of single-stranded DNA was calculated as 0.80. The biosensor exhibited some advantages, such as simplicity, rapidity, high sensitivity, good reproducibility and long-term stability.

1. Introduction

Deoxyribonucleic acid plays an important role in the storage of genetic information and protein biosynthesis. Guanine and adenine are important components found in deoxyribonucleic acid and play fundamental roles in life process [1]. They have widespread effects on coronary and cerebral circulation, control of blood flow, prevention of cardiac arrhythmias, inhibition of neurotransmitter release and modulation of adenylate cyclase activity [2]. The abnormal changes of the bases in organism suggest the deficiency and mutation of the immunity system and may indicate the presence of various diseases. Their concentration levels are considered as important parameter for diagnosis of cancers, AIDS, myocardial cellular energy status, disease progress and therapy responses [3]. Therefore, the determination of these bases has great significance to the bioscience and clinical diagnosis [4].

Many methods have been developed for the detection and quantification of purine bases in nucleic acids, such as high-performance liquid chromatography (HPLC) [5], capillary electrophoresis (CE) [6], spectroscopic methods [7,8], chemiluminescence (CL) [9], and mass spectrometry (MS) [10]. Although these methods are sensitive, complicated instruments and time-consuming sample pretreatment are required. Compared to these methods, the electrochemical technique is attractive owing to its high sensitivity, inherent simplicity, miniaturization and low cost, and some electrochemical methods have been developed for the determination of the guanine and adenine.

Graphene, a single layer of carbon atoms in a closely packed honeycomb two-dimensional lattice, is a novel and fascinating carbon material. Recently, it has attracted considerable attention from both the experimental and theoretical scientific communities due to its many unique and excellent properties [11–13]. It exhibits extremely high thermal conductivity, good mechanical strength, high mobility of charge carriers, high specific surface area, quantum hall effect and upstanding electric conductivity [14–16]. Much research effort has been made to explore its fascinating applications in fabricating various electrical devices, such as battery [17], field-effect transistors [18], ultrasensitive sensors [19], electromechanical resonators [20] and electrochemical biosensors [21]. Some works have demonstrated that graphene possesses excellent electrochemical catalytic activity, and should be a novel electrode modified material with excellent performance. However, many of the interesting and unique properties of graphene can only be realized after it is integrated into more complex assemblies [21–25]. A useful technique to incorporate graphene into such assemblies is through chemical functionalization of the graphene, which enables

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doi:10.1016/j.colsurfb.2010.10.014

chemical covalent bonding between the graphene and the material of interest. Functionalized graphene are also typically easier to disperse in organic solvents and water, which can improve the dispersion and homogeneity of the graphene within the polymer and yield novel types of electrically conductive nanocomposites [26,27].

In this work, functionalized graphene was used to develop a simple and sensitive method for the simultaneous determination of guanine and adenine. Graphene was firstly functionalized via chemical modification of carboxyl groups on its surface. The functionalized graphene nanosheets were easily dispersed in water and then used to modify the glassy carbon electrode by simple drop moulding procedure. The negatively charged graphen-COOH nanofilm could adsorb the positive charged guanine and adenine, and this led to effectively improving the sensitivity of proposed method. Based on the unique properties of graphene-COOH, the fabricated modified electrode facilitated the electron transfer of guanine and adenine, resulting in the increase of oxidation signals. The method proved to be simple, reliable, and inexpensive for the individual or simultaneous determination of guanine and adenine in DNA at low levels.

2. Experimental

2.1. Chemicals and materials

Graphite powder (99.95%, 325 mesh), hydrazine solution (50 wt%) and ammonia solution (28 wt%) were purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Guanine, adenine, herring sperm, $SOCl_2$ and $NH_2(CH_2)_2NH_2$ were purchased from Sigma (Saint Louis, MO, USA). All other chemicals were of analytical grade and used without further purification. Acetate buffer solutions (ABS) were prepared by mixing of 0.1 M CH_3COOH and CH_3COONa and adjusting the pH with NaOH. Ultrapure water (18.2 MΩ) was obtained from a Milli-Q water purification system and used throughout. Prior electrochemical experiments, solutions were deaerated with high purity nitrogen and maintained under nitrogen atmosphere during measurements.

2.2. Apparatus

CHI660A electrochemical workstation (CH Instruments, USA) and a standard three-electrode cell contained a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and the modified electrode as working electrode were employed for electrochemical studies. All potential values given below refer to SCE. FT-IR spectroscopy was obtained using Bruker Tensor 27 Spectrometer (Germany).

2.3. Preparation procedure

Graphene oxide was synthesized from graphite according to Hummers and Offeman method [28]. In a typical procedure for chemical conversion of graphene oxide to graphene, the resulting graphene oxide dispersion (100 mL) was mixed with 70 μL of hydrazine solution (50 wt% in water) and 0.7 mL of ammonia solution (28 wt% in water). The mixture was stirred for 1 h at the temperature of 95 °C. Finally, black hydrophobic powder of graphene was obtained by filtration and dried in vacuum.

To prepare carboxylic acid functionalized graphene (graphene-COOH), graphene oxide was firstly washed with HCl solution (5%, v/v), and then repeatedly washed with water until the pH of filtrate was neutral. Then through extremely rapid heating and successful splitting of graphene oxide, wrinkled graphene sheets functionalized with hydroxyl and carboxylic groups were obtained.

Dried graphene-COOH was chlorinated by refluxing for 12 h with $SOCl_2$ at 70 °C. After evaporating any remaining $SOCl_2$, amine functionalized graphene (graphene-NH_2) were obtained by reaction with $NH_2(CH_2)_2NH_2$ in dehydrated toluene for 24 h at 70 °C. After washing with ethanol and deionized water several times, graphene-NH_2 powder was obtained from drying at 50 °C in vacuum for 24 h.

The sheet of graphene, graphene-COOH and graphene-NH_2 have lateral dimensions from a few hundred nanometers to several micrometers, and the average thickness of single-layer graphene sheets was <1 nm.

2.4. Preparation of modified electrode

For electrode preparation, 1 mg of graphene-COOH or graphene-NH_2 or graphene was dispersed in 1 mL of water using an ultrasonic bath to give a black suspension. Before modification, the GC (3 mm in diameter) electrode was polished to a mirror-like with 0.3 and 0.05 μm of alumina slurry, and then washed successively with ultrapure water, anhydrous alcohol and ultrapure water in an ultrasonic bath and dried in N_2 blowing. The cleaned GC electrode was treated by dropping 6 μL of the resultant suspension and then dried in air. The obtained electrodes were noted as graphene-COOH/GCE or graphene-NH_2/GCE or graphene/GCE.

2.5. Preparation of DNA samples

Thermally denatured dsDNA was produced according to the previous report [29]. In short, native herring sperm dsDNA samples were dissolved in water and then the solution was heated in a boiling water bath (100 °C) for about 10 min. Finally, the solution was rapidly cooled in an ice bath. Generally, thermal denaturation involves the rupture of hydrogen bonds, the disturbance of stacking interaction but not any breakage of covalent bond. So thermally denatured dsDNA may act as single-stranded DNA (ssDNA).

2.6. Experimental procedure

The electrochemical experiments were performed in 0.1 mol L^{-1} ABS buffer solutions (pH 4.5) with different concentrations of adenine or guanine or their mixtures. The preconcentration procedures of stock solution at the modified electrodes were carried out on open-circuit for 100 s. After 2 s of the quiet period, the differential pulse voltammograms were recorded at the scan rate of 100 mV s^{-1}.

3. Results and discussion

3.1. FTIR characterization of functionalized graphene

The IR spectrum of the graphene oxide samples (Fig. 1a) shows the presence of –OH (3421 cm^{-1}), C=O (1722, 1626 cm^{-1}) and phenol or alcohol or ether (1384, 1063 cm^{-1}). Fig. 1b shows the reduction of the carboxyl groups (graphene-COOH) to hydroxymethyl (graphene-CH_2OH) as indicated by the disappearance of the C=O bands (at 1720 cm^{-1}) and the appearance of bands at 2924 and 2854 cm^{-1} corresponding to the C–H stretch vibrations of the methylene group. Fig. 1c shows FTIR results for the graphene-COOH. The peak at 1722 cm^{-1} is attributed to the C=O stretch of the carboxylic (COOH) group. The appearance of a strong and broad band at 3418 cm^{-1} confirms the present of carboxylic group. The IR spectrum of the amide-functionalized graphene samples, graphene-NH_2 (Fig. 2d), shows the disappearance of the band at 1722 cm^{-1} and a corresponding appearance of a band with lower frequency (1638 cm^{-1}) assigned to the amide carbonyl (C=O) stretch. The NH_2 stretch band appears at 3395 cm^{-1}. The small 3296 cm^{-1} peak may be due to the NH_2 symmetric stretch of the

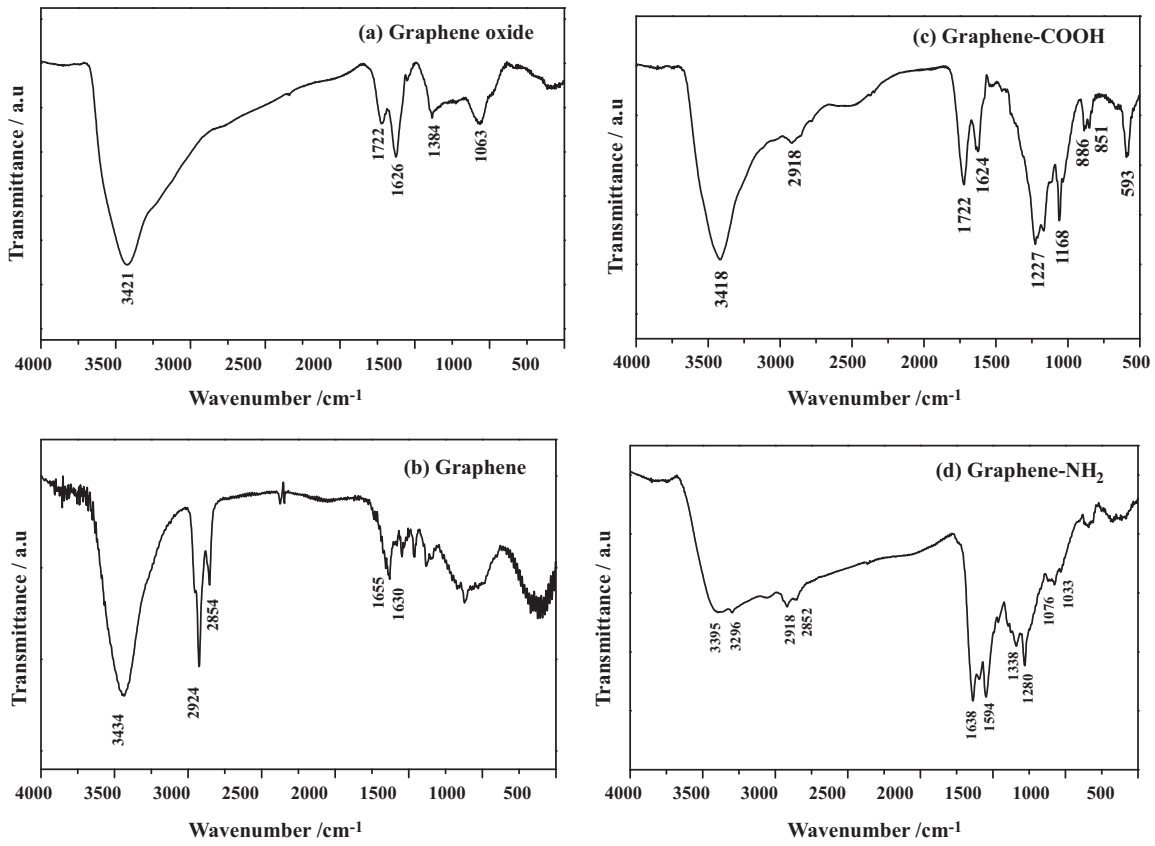

Fig. 1. FTIR spectra of graphene samples. (a) Graphene oxide, (b) graphene, (c) graphene-COOH, (d) graphene-NH_2.

amine group. Bands at 2918 and 2852 cm^{-1} represent the stretching of the CH_2 group, while C–N bond stretch vibrations appear at 1033 cm^{-1}. In addition, the presence of new bands at 1594 and 1280 cm^{-1}, corresponding to N–H in-plane and C–N bond stretching, respectively, further confirms the presence of the amide functional group.

3.2. Electrochemistry of the biosensors

Electrochemical impedance spectroscopy (EIS) provides detailed information on the change of the surface property of modified electrodes. The impedance spectra include a semicircle portion and a linear portion. The semicircle diameter at higher frequencies corresponds to the electron-transfer resistance (R_{et}), and the linear part at lower frequencies corresponds to the diffusion process. Fig. 2 shows the impedance spectra corresponding to the different electrodes. It was observed that the EIS of the bare GCE displayed a small well defined semi-circle at higher frequencies, which indicated small interface impedance. However, after immobilization of graphene-COOH on the GCE, an almost straight line in the Nyquist plot of impedance spectroscopy appeared, which characteristics of a diffusion-limited electron-transfer process. Because graphene-COOH was an excellent electric conducting material, it could accelerate the electron transfer and resulted in the reduction of R_{et}. The EIS change of the modified process also indicated that the graphene-COOH was firmly immobilized on the modified electrode surface.

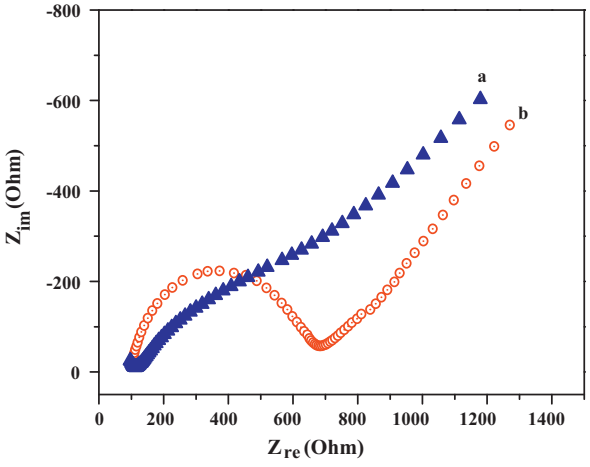

Fig. 2. Nyquist plots of the different electrodes in a PBS (pH 7.0) solution containing 0.1 M KCl and 5.0 mM $Fe(CN)_6^{4-/3-}$. The frequency range was from 10^{-1} to 10^5 Hz with perturbation amplitude of 5 mV. (a) Graphene-COOH/GCE; (b) GCE.

Fig. 3 shows the differential pulse voltammograms and cyclic voltammograms of 1×10^{-4} M guanine and 1×10^{-4} M adenine in 0.1 mol L^{-1} ABS buffer solutions (pH 4.5) at a scan rate of 100 mV s^{-1}. As shown in Fig. 3A and B, no redox peak was observed in the range from 0 to 1.6 V at graphene-COOH/GCE in the absence of guanine (Fig. 3A, curve f) and adenine (Fig. 3B, curve g), indicating that graphene-COOH was non-electroactive in the scanned potential window. When guanine and adenine were added into ABS, the modified electrode gave two obviously oxidation peaks for guanine (Fig. 3A, curve e) and adenine (Fig. 3B, curve h), respectively. Fig. 3a–d shows the differential pulse voltammograms of guanine and adenine at different electrodes, respectively. Compared to the electrochemical response of the bare GCE (Fig. 3b), the oxidation peak currents of guanine and adenine obtained at the graphene/GCE (Fig. 3c) obviously increased and the corresponding oxidation peak potential shifted negatively (the ΔE_p of guanine and adenine was 92 and 110 mV). This indicated that graphene

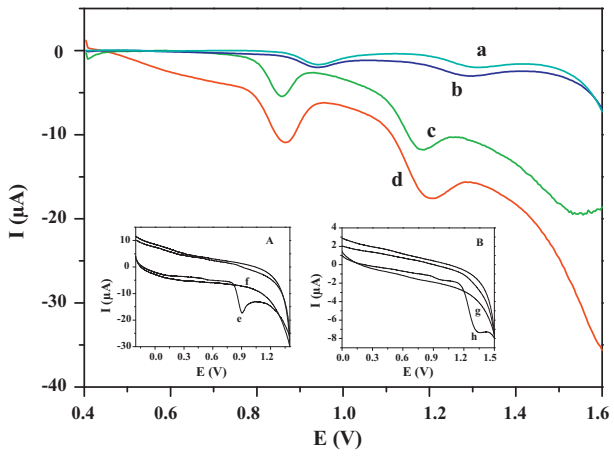

Fig. 3. Differential pulse voltammograms of 1×10^{-4} M guanine and 1×10^{-4} M adenine on graphene-NH_2/GCE (curve a), GCE (curve b), graphene/GCE (curve c) and graphene-COOH/GCE (curve d) in 0.1 mol L^{-1} ABS buffer solutions (pH 4.5) at a scan rate of 100 mV s^{-1}. The inset (A) is the cyclic voltammograms of 1×10^{-4} M guanine in ABS (curve e) and 0.1 mol L^{-1} ABS buffer solutions (pH 4.5) (curve f). The inset (B) is the cyclic voltammograms of 1×10^{-4} M adenine in ABS (curve g) and 0.1 mol L^{-1} ABS buffer solutions (pH 4.5) (curve h).

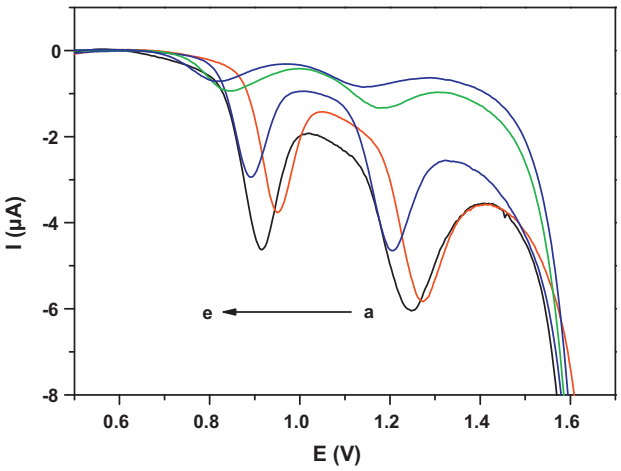

Fig. 4. Differential pulse voltammograms of 1×10^{-4} M guanine and 1×10^{-4} M adenine 0.1 mol L^{-1} ABS buffer solution with different values of pH (from a to e: 3.0, 4.5, 5.0, 5.5, 6.0).

facilitated the electron transfer of guanine and adenine. However, the oxidation peak currents of guanine and adenine obtained at graphene-NH_2/GCE (Fig. 3a) showed no change compared to GCE. Because positive charged graphene-NH_2 film prevent adsorbing of the positive charged guanine and adenine molecules, so the oxidation signals at graphene-NH_2/GCE was more weak than that at graphene/GCE and similar to GCE. However, the oxidation peak currents obtained at graphene-COOH/GCE (Fig. 3d) enhanced significantly compared to the above electrodes. The value of oxidation peak potential negative shift was 90 and 105 mV of guanine and adenine, respectively, compared to the bare GCE. Generally speaking, the graphene surface is difficult to contain negatively charged surface at pH 4.5. However, because graphene-COOH has been treated with strong hydrochloric acid when prepared, this may result in many negative electric charges retaining on graphene-COOH surface including some strong acids, which would keep ionization at pH 4.5. So the negatively charged graphene-COOH film would adsorb more the positive charged guanine and adenine molecules to enhance the oxidation signals. The results also indicated that graphene-COOH showed excellent electrocatalytic activity towards the oxidation of guanine and adenine. Moreover, the peak-to-peak separation of guanine and adenine was 321 mV. The large peak-to-peak separation could effectively decrease the interaction between guanine and adenine in simultaneous determination and increase the determination selectivity.

To obtain the kinetic parameters of guanine and adenine redox at graphene-COOH/GCE, the scan rate effect was investigated by cyclic voltammetry. Only oxidation peaks were observed, indicating that the oxidation of guanine and adenine was a totally irreversible electrode process. Along with the increase of the scan rate, the oxidation peak currents of guanine and adenine increased gradually and the relationship of the oxidation peak current with scan rate were established with linear regression equations as I_{pa} (μA) = 8.429υ(mV s^{-1}) + 1.799 (R = 0.996) and I_{pa} (μA) = 0.024υ (mV s^{-1}) + 1.028 (R = 0.9956) in the range of 20–350 mV s^{-1}, which indicated that the electrooxidation of guanine and adenine was an adsorption controlled process.

The adsorbed amount of guanine and adenine on the surface of graphene-COOH/GCE was further calculated by the following equation [30]: $i_p = nFQv/4RT = n^2F^2Av\Gamma_c/4RT$. Where n is the number of electron transferred, F is the Faraday's constant, A is the area of the electrode, Γ_c is the surface concentration of the guanine and adenine, Q is the quantity of charge consumed during the electrooxidation reaction and v is the scan rate.

Based on the relationship of i_p with υ, the values of the electron transfer number (n) of the guanine and adenine were calculated as 2.13 and 2.24, respectively. The surface concentration of the guanine and adenine (Γ_c) were obtained with the results as 2.86×10^{-10} mol cm^{-2} and 2.84×10^{-10} mol cm^{-2}, respectively.

The relationship of the oxidation peak potential E_p with ln υ was further constructed for the calculation of the electrochemical parameters. According to the Laviron's equations [30,31]: $\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(RT/nF\upsilon) - \alpha(1-\alpha)nF\Delta E_p/2.3RT$, $E_p = E_0 + RT/\alpha nF \times \ln(RTK_s/\alpha nF) - RT/\alpha nF \times \ln\upsilon$, the values of the charge transfer coefficient (α) of guanine and adenine on the graphene-COOH/GCE were calculated as 0.54 and 0.60, respectively. The results showed that the values of α of guanine and adenine on the GCE were 0.46 and 0.51, respectively. The electrode reaction standard rate constant (k_s) of guanine and adenine on the graphene-COOH/GCE and GCE were obtained as 9.82×10^{-4} s^{-1}, 1.02×10^{-3} s^{-1} and 3.86×10^{-4} s^{-1}, 4.12×10^{-3} s^{-1}, respectively. As showed in Table 1, the α and k_s of guanine and adenine on graphene-COOH/GCE were comparable to that on the other modified electrodes.

The effect of buffer pH on the electrooxidation of guanine and adenine was also investigated in the range of pH 3.0–6.0 (Fig. 4). The results indicated that the oxidation peak potential of guanine and adenine shifted negatively with the increment of the solution pH, which indicated that protons were involved in the electrode reaction. Good linear relationship was established between the oxidation peak potential of guanine and adenine and the solution pH with the linear regression equation as E_{pa} (V) = −0.0536 pH + 1.098 (R = 0.997) and E_{pa} (V) = −0.0606 pH + 1.448 (R = 0.992), respectively. The slope of 536 and 616 mV/pH implied that the electron transfer was accompanied by an equal number of protons in the electrode reaction process [31]. Integrating the results obtained in scan rate investigation, it could be concluded that the electrooxidation of guanine and adenine on graphene-COOH/GCE was a two-electron and two-proton process. In addition, the results showed that both the maximum current responses of guanine and adenine were obtained at pH 4.5. Therefore, pH 4.5 was chosen for the subsequent analytical experiments.

The peak currents of both guanine and adenine increased with accumulation time growing at the graphene-COOH/GCE, but after 100 s they kept almost unchanged for a 0.1 mM guanine plus 0.5 mM adenine solution, meaning that the accumulation of the

Table 1
The electrode reaction parameters of guanine and adenine at different electrodes.

Modified electrode	Charge transfer coefficient (α)	Standard rate constant (k_s/s^{-1})			Reference
	Guanine	Adenine	Guanine	Adenine	
Molybdenum(VI) complex–TiO_2 nanoparticle modified carbon paste electrode	0.36	–	–	–	[33]
Multi-walled carbon nanotubes modified carbon ionic liquid electrode	0.66	–	2.94×10^{-4}	–	[34]
Carbon ionic liquid electrode	0.65	0.58	2.39×10^{-3}	7.42×10^{-4}	[35]
Ordered mesoporous carbon modified carbon ionic liquid electrode	0.75	0.85	3.13×10^{-3}	3.17×10^{-3}	[36]
Graphene-COOH/GCE	0.54	0.60	9.82×10^{-4}	1.02×10^{-3}	This work

analytes at the graphene-COOH surface reached saturation. The peak currents almost did not vary with accumulation potential ranging from −0.3 to +0.6 V. The accumulation of guanine and adenine was therefore carried out on open-circuit.

The thickness of the graphene-COOH cast film on the GCE surface, which is determined by the amount of graphene-COOH suspension, has effects on the current responses of analytes. It showed that the current responses increased notably when the amount of graphene-COOH suspension increased from 0 to 4 μL. However, with further increase of the amount of graphene-COOH suspension to 6 μL, the current responses increased slightly. It conversely showed gradual decline when the amount of graphene-COOH suspension exceeds 6 μL. With the amount of graphene-COOH increased, the reactive site of the modified electrode to the analytes also enhanced. But the ability of electron exchange and transfer would decrease when the graphene-COOH film was too thick. Hence, the oxidation peak current decreased accordingly. So 6 μL of graphene-COOH suspension was selected for the fabrication of the biosensors in this work.

3.3. Individual determination of adenine and guanine

Under the optimal experiment conditions established above, the calibration curve of guanine and adenine in ABS were measured by DPV. As shown in Fig. 5, the anodic peak current of guanine was linearly related to the concentration over the range of 0.5–200 μM. The linear regression equation was I_{pa} (μA) = 0.0103C + 0.4774 (μM), with a correlation coefficient of R = 0.9941. The detection limit (S/N = 3) was 5.0×10^{-8} M. As to adenine, similar studies were also carried out in the case of guanine and the calibration curve yielded a linear range from 0.5 to 200 μM (Fig. 6). The linear regression equation was I_{pa} (μA) = 0.0069C + 0.4227 (μM), with a correlation coefficient of R = 0.9951. The detection limit (S/N = 3) was 2.5×10^{-8} M.

3.4. Simultaneous determination of adenine and guanine

Fig. 7 is the DPV obtained when the concentrations of adenine and guanine simultaneously increased at the modified electrode. It could be seen that two well-defined voltammetric peaks appeared at about +0.90 and +1.23 V, corresponding to the oxidation of guanine and adenine, respectively. The inset of Fig. 7 shows the linear range of the dependence of the DPV peak currents on the concentration of guanine (curve a) and adenine (curve b). The DPV experiments showed that the curves of current versus concentration of either guanine or adenine exhibited good linearity in the range of 0.5–200 μM. The regression equation for guanine was I_{pa} (μA) = 0.0075C (μM) − 0.3678, with a correlation coefficient of 0.9985. The detection limit was 5×10^{-8} M. The regression equation for adenine was I_{pa} (μA) = 0.0065C + 0.3370 (μM), with a correlation coefficient of 0.9979. The detection limit was 2.5×10^{-8} M. Thus, this proposed method allowed both simultaneous and sensitive determination of guanine and adenine.

3.5. Stability and reproducibility of the modified electrode

The successive stability of the proposed sensor was evaluated. Ten successive scans in the solution containing 1.0 μM guanine and 1.0 μM adenine were performed. The RSD values were found

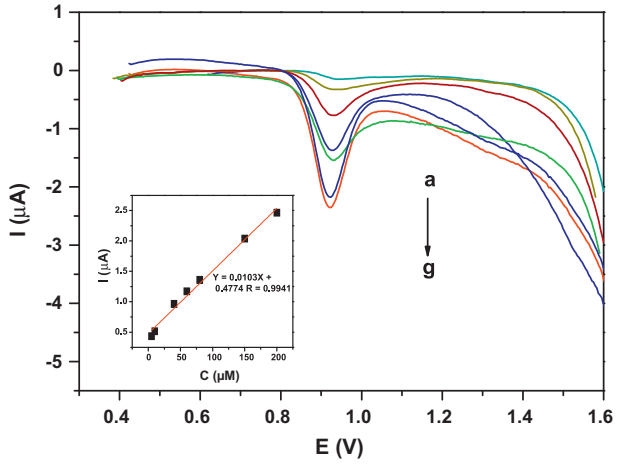

Fig. 5. Differential pulse voltammograms of guanine (from a to g: 0.5, 5, 30, 60, 80, 150 and 200 μM) in 0.1 M ABS at graphene-COOH/GCE. Inset: calibration plots of the oxidation peak current versus different concentration of guanine.

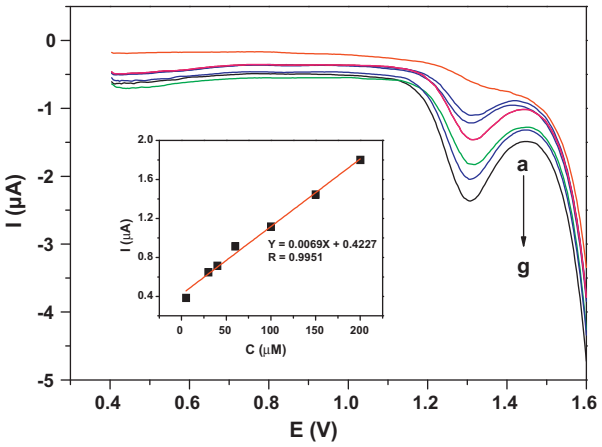

Fig. 6. Differential pulse voltammograms of adenine (from a to g: 0.5, 5, 30, 60, 80, 150 and 200 μM) in 0.1 M ABS at graphene-COOH/GCE. Inset: calibration plots of the oxidation peak current versus different concentration of adenine.

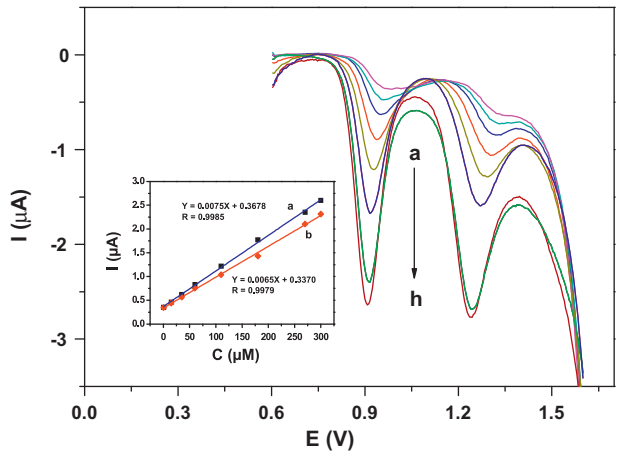

Fig. 7. Differential pulse voltammograms for the simultaneous determination of guanine and adenine in 0.1 M ABS at graphene-COOH/GCE with guanine and adenine concentration ranging from 0.5 to 200 μM (from a to h: 0.5, 5, 30, 60, 80, 150, 180 and 200 μM). Inset: calibration plots of the oxidation peak current versus different concentration of guanine and adenine.

to be 2.62% and 3.18% for guanine and adenine, respectively, indicating excellent stability of the modified electrode. The long-time stability of the modified electrode was also studied on a 15-day period. The electrode can be used for next measurement after continuous sweep for three cycles in the range of 0.4–1.6 V. At the different storage periods between 15 days, the modified electrode was used to detect the same guanine and adenine concentration, the analytical performances did not show an obvious decline (RSD ≤ 5%), demonstrating that the electrode had good stability.

The reproducibility of the modified electrode was estimated by determining 1.0 μM guanine and 1.0 μM adenine with five modified electrodes which were made at the same electrode and used the same suspension of graphene-COOH suspension. Five measurements from the batch resulted in a relative standard deviation of 3.8%. The experimental results indicated good reproducibility of the fabrication protocol.

3.6. Applications

In order to evaluate the validity of the proposed method, the graphene-COOH/GCE was applied to determine the guanine and adenine content of thermally denatured DNA. The thermally denatured DNA gave two well-defined oxidation peaks at the modified electrode, which was due to the oxidation of guanine and adenine residues, respectively. The standard addition methods were used to determine guanine and adenine concentration. In short, 50 μL of thermally denatured DNA solution was added in a 10-mL buffer solution and the peak currents of the guanine and adenine were measured. Subsequently, 30 μM guanine and 30 μM adenine were added in above mixture and the peak currents of the guanine and adenine were recorded again. From the differences between the peak currents of guanine and adenine, the concentration of guanine and adenine in DNA could be obtained by the calibration graph. The contents of adenine and guanine in thermally denatured DNA were calculated as 22.2% and 27.8% (in the molar ratio, mol%), respectively. The value of (G + C)/(A + T) was calculated as 0.80 for thermally denatured DNA sample, which coincided to the standard value of 0.77 [32].

For further validation of the application of the proposed method, an experiment has been attempted to use the graphene-COOH/GCE in the media including blood serum and plasma. However, the results showed that some compounds existed in these media seriously interfered with the determination of guanine and adenine. Furthermore, the matrix of the blood easily contaminated the modified electrode. In order to improve the selectivity of the proposed method, antibody or aptamer that can selectively recognised the analyte should be introduced in the construction of the biosensor.

4. Conclusions

In this work, a novel electrochemical sensor was fabricated based on graphene-COOH modified GCE for the sensitive determination of guanine and adenine. After optimizing the experimental parameters, guanine and adenine exhibited well separated and well-defined oxidation peaks. Remarkable enhancement effects on the oxidation peak currents were observed with the negative shift of the oxidation peak potentials. The results were attributed to the specific characteristics of graphene-COOH. The developed biosensor exhibited wide linear detection range, acceptable reproducibility, high sensitivity, long-term stability, and low detection limit. The proposed method was further used for the simultaneous detection of adenine and guanine in thermally denatured DNA with satisfactory results. This work demonstrated that the proposed graphene-COOH was highly useful for bioelectrochemical and biosensing applications.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (20805040), Program for Science & Technology Innovation Talents in Universities of Henan Province (2010HASTIT025), Excellent Youth Foundation of He'nan Scientific Committee (104100510020), and Foundation of He'nan Educational Committee (2009A150023).

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