Journal of Electroanalytical Chemistry 704 (2013) 44–49

Contents lists available at SciVerse ScienceDirect

Journal of Electroanalytical Chemistry

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

Electrocatalytic oxidation and the simultaneous determination of guanine and adenine on (2,6-pyridinedicarboxylic acid)/graphene composite film modified electrode

Chunxiang Li *, Xiyang Qiu, Yulin Ling

Chemistry and Chemical Engineering College, Hunan University of Science and Technology, Xiangtan 411201, PR China

ARTICLE INFO

Article history:
Received 29 March 2013
Received in revised form 6 June 2013
Accepted 13 June 2013
Available online 25 June 2013

Keywords:
Poly (2,6-pyridinedicarboxylic acid)
Chemically reduced graphene oxide
Guanine
Adenine

ABSTRACT

An electrochemical sensor was fabricated and used to simultaneously determine guanine and adenine in DNA. In this work, the poly (2,6-pyridinedicarboxylic acid)/chemically reduced graphene oxide modified glass electrode (PPDA/CRGO/GCE) was constructed. The PPDA/CRGO/GCE was characterized by scanning electron microscopy. The modified electrode displayed greatly improved voltammetric response to guanine (G) and adenine (A). Differential pulse voltammetry (DPV) was used for individual or simultaneous determination of guanine and adenine. The electrode showed good reproducibility with linear ranges of 0.05–4.5 μM and 0.1–6.0 μM, detection limits of 0.01 μM and 0.02 μM for guanine and adenine, respectively. The electrochemical method for the measurement of guanine and adenine in calf thymus DNA was also developed and the result was satisfactory.

1. Introduction

Since the rise of graphene in the field of carbon-based nanomaterials, it has been widely applied for chemical and biochemical sensing due to its high surface area [1,2], high electrical conductivity [3,4], and electron transfer promotion [5,6]. Recently, in order to gain faster electron transport and larger active surface areas, graphene/polymer composite films were prepared for the fabrication of electrochemical sensors and they showed great versatility as advanced electrode materials [7–10]. Graphene/polyaniline nanocomposite-based H_2 gas sensor has higher sensitivity than the sensors based on only graphene sheets and polyaniline nanofibers [11]. Graphene/p-aminobenzoic acid composite film was first employed for the sensitive determination of dopamine [12]. Poly (l-arginine)/graphene composite film was used for simultaneous determination of uric acid, xanthine and hypoxanthine [13]. In a sense, the nanocomposite film can combine the advantages of graphene and polymer, produce the synergy effect, and open more opportunities for the surface chemistry of graphene.

The main role of deoxyribonucleic acid (DNA) molecules is the long-term storage of genetic information. Their concentrations may be regarded as important index for diagnosis of different disease [14]. Guanine (G) and adenine (A) are important purine bases in DNA. Quantifying them may indicate the presence of various diseases. Many electrochemical methods have been proposed to detect purine bases in nucleic acids [15–21]. Among them, graphene nanowalls were used to determine DNA with very low detection limit [15]. Here, a simple poly (2,6-pyridinedicarboxylic acid)/chemically reduced graphene oxide modified electrode (PPDA/CRGO/GCE) was proposed. By combining the strong adsorption and electrocatalysis of PPDA and CRGO, efficient electron transfer promotion and accumulation enhancement occurred between the analytes and electrode surface. Applying differential pulse voltammetry (DPV), the PPDA/CRGO/GCE shows comparable sensitivity, linear range, and detection limit for determination of guanine, adenine and DNA. Compared with the reported [16–19], it also exhibits the more excellent analytical performances.

2. Experimental

2.1. Instruments and chemicals

All electrochemical experiments were performed with a CHI 760C electrochemical workstation (Chenhua Corp. Shanghai, China). The modified electrodes were characterized by laser-Raman spectrometer (Laboram-010, France) and scanning electron microscopy (SEM, JSM-5610L, JEOL). A conventional three-electrode system was adopted. The working electrode was a modified glass carbon electrode, and the auxiliary and reference electrodes were a platinum plate and a saturated calomel electrode (SCE), respectively. All potentials were respect with SCE. The assay cell was stirred with a magnetic stirrer and all electrochemical measurements were performed at room temperature.

* Corresponding author. Tel.: +86 0731 58290045.
E-mail address: cxli@hnust.edu.cn (C. Li).

1572-6657/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jelechem.2013.06.010

Adenine and guanine were purchased from Sigma. 2,6-pyridinedicarboxylic acid was purchased from Aldrich. Graphite powder (spectrum pure) was obtained from Shanghai Chemical Reagent Co. (China). Calf thymus DNA was obtained from Sano-American Biology Company and was used without further purification. The treatment of acid denatured DNA was according to the literature [19]. The phosphate buffer solution (PBS, 0.05 M) was prepared by KH_2PO_4 and NaOH, and pH was adjusted with 1.0 M H_3PO_4 and 1.0 M NaOH. All other reagents and solvents were of analytical grade, and doubly distilled water was used throughout.

2.2. Synthesis of chemically reduced graphene (CRGO)

Graphite oxide was synthesized from spectral graphite by using a modified Hummers method [22,23]. The synthesized graphite oxide was dispersed in water (1 mg mL^{-1}) and the further purification was done by dialysing it three times (24 h for one time) to remove the residual metal species. Exfoliated graphene oxide (GO) was prepared by ultrasonic processing for 30 min and centrifugation at 3000 rpm for 5 min. Chemical reduced graphene oxide (CRGO) was prepared by using ammonia and hydrazine hydrate as reducing agents according to the literature [24]. A hydrophobic black reduced graphene sheet could be obtained by filtration of the product and drying it in a vacuum drying oven at 60 °C.

2.3. Preparation of modified electrodes

Prior to use, the bare glassy carbon electrode (GCE, diameter 4 mm) was polished to a mirror-like surface with 0.3 and 0.05 mm Al_2O_3 powder, then rinsed with deionized water and sonicated in alcohol and deionized water for 2 min, respectively.

1 mg of purified CRGO was dispersed with the aid of ultrasonic agitation in 1 mL of *N*,*N*-dimethylformamide (DMF) to give a 1.0 mg mL^{-1} black suspension. The CRGO modified GCE (CRGO/GCE) was prepared by dropping 5 μL of CRGO solution onto the surface of GCE. The electrode was dried for approximately 2 h at room temperature.

A freshly-prepared solution for polymerization contained 0.2 M KCl and 2 M 2,6-pyridinedicarboxylic acid. Polymer film was grown on CRGO/GCE by cycling scan from +0.1 V to +1.7 V for 10 cycles at a scan rate of 20 mV s^{-1}. Thus, the poly (2,6-pyridinedicarboxylic acid) modified CRGO/GCE (PPDA/ CRGO/GCE) was constructed. The PPDA/GCE was prepared by electro-polymerizing 2,6-pyridinedicarboxylic acid on GCE. Before use, the working electrodes were treated by successive voltammetric cycles from −0.1 to +1.2 V in PBS (pH 7.0) with a scan rate of 100 mV s^{-1} until stable voltammograms were obtained.

2.4. Experimental procedure

The measurements were performed in 0.05 M PBS (pH 7.0) containing different concentrations of guanine and adenine. After an accumulation at the potential of +0.30 V for 180 s, the voltammogram was recorded from +0.3 to +1.0 V. After every measurement, PPDA/CRGO/GCE was rinsed and immersed in 0.1 M HCl for 3 min regenerate to the electrode surface. Then, the cyclic scan was repeated successively for 5 cycles within the potential range of 0.3–1.0 V to balance the surface performance of electrode for next measurement.

3. Results and discussion

3.1. Characterization

Raman spectrum is often applied to monitor the structural changes of carbon materials. Fig. 1A shows the Raman scattering of GO and CRGO. The spectrum displays the presence of D band located at 1344 cm^{-1} and G band at 1592 cm^{-1} (curve for GO), which is typical Raman features of GO. After being reduced, it exhibits that D band appears at 1343 cm^{-1} and G band at 1586 cm^{-1} (curve for CRGO). The intensity ratio of ID/IG increases. This result is consistent with the reports [24,25]. Fig 1B is the Raman spectra of GO and CRGO in the high frequency region. From the insert, we cannot see 2D peak but a D + G peak at ~2950 cm^{-1} for GO. This may be induced by the high defect. After reduction, a small 2D peak appears at ~2700 cm^{-1}. It is also an indication of reduction of the graphene oxide. But the intensity of the 2D peak with respect to the D and G peaks is small due to disorder. The result is similar to the work [26,27]. Fig. 1C displays the typical morphology of the CRGO/GCE characterized by SEM. CRGO exhibits thin wrinkled paper-like characteristics with slightly scrolled edges, which is similar to the previous works [6,28]. By comparison with Fig. 1C, the surface of PDDA/CRGO (Fig. 1D) was much different because PDDA formed a thin and homogeneous film and covered graphene. It clearly suggests that the layer of PDDA polymerized on the surfaces of graphene sheets by electrochemical method.

3.2. Electrochemical behavior of guanine and adenine

Fig. 2A shows the cyclic voltammograms (CVs) of guanine at different electrodes. An irreversible anodic peak appear at +0.70 V at GCE (curve a). In contrast, at PPDA/CRGO/GCE (curve b), a greatly enhanced anodic peak can be found with a little peak potential shift from +702 mV to +718 mV. The peak current increase greatly and the anodic peak current ($i_{p,a}$) is 5.2 folds of that at bare GCE. For the adenine, the voltammetric behavior is almost similar to the above (Fig. 2B). An anodic peak appeared at +1.01 V at bare GCE (curve a). At PPDA/CRGO/GCE (curve b), a peak can be seen at +0.98 V. The peak potential has a negative shift of 30 mV. Furthermore, the current response is higher and $i_{p,a}$ is 5.6 folds of that at bare GCE. These results suggest that fast electron transfer kinetics exist. It also shows that the PPDA and CRGO contribute to the oxidation of guanine and adenine.

In order to discern the role of different working electrodes, the voltammetric behaviors of the mixed solution of guanine and adenine were studied by linear scan voltammetry (Fig. 3). At GCE, the oxidation peaks of guanine and adenine appeared at +0.72 V and +1.02 V, respectively (curve a). In contrast, the oxidation peak currents of guanine and adenine largely increased at PPDA/GCE (curve b) and CRGO/GCE (curve c). Peak potentials had a negative shift of 20 mV for guanine and adenine at PPDA/GCE. But, at CRGO/GCE, peak potentials did not change. Furthermore, the longer the preconcentration time was, the higher the peak current would be. These results suggest that the CRGO and PPDA have strong catalytic function and adsorptive function to oxidation of guanine and adenine. We deduced the role of PPDA for the detection. The reasons may be ascribed as follows: (1) A large amount of carboxylic functional groups on PDDA can form hydrogen bonds with bases, which can facilitate the adsorption of base. (2) PPDA can also facilitate the adsorption of bases through π–π interaction. When at PPDA/CRGO/GCE, we can see the decreased capacitance current compared with that at CRGO/GCE, which was favorable for the low detection limit. In addition, the current responses for guanine and adenine were much higher (curve d) and the negative shift of peak potentials was 20 mV, which showed that PPDA and CRGO could cooperate well. This maybe was the low intermolecular distance between PPDA film and CRGO, which changed the morphology of CRGO via π–π stacking interaction, optimized the performance of CRGO in sensors, and accelerated the electron transport properties [29]. Thus, PPDA/CRGO/GCE displayed higher current response towards the oxidation of guanine and adenine because of their synergistic effects of CRGO and PPDA.