Materials Science and Engineering C 45 (2014) 103–108

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Achieving direct electrochemistry of glucose oxidase by one step electrochemical reduction of graphene oxide and its use in glucose sensing

Mojtaba Shamsipur, Mahmoud Amouzadeh Tabrizi *

Department of Chemistry, Razi University, Kermanshah, Iran

ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form 13 August 2014
Accepted 1 September 2014
Available online 6 September 2014

Keywords:
Electrochemically reduced graphene oxide
Surfactant
Direct electrochemistry
Glucose oxidase
Glucose
Biosensor

ABSTRACT

In this paper, the direct electrochemistry of glucose oxidase (GOD) was accomplished at a glassy carbon electrode modified with electrochemically reduced graphene oxide/sodium dodecyl sulfate (GCE/ERGO/SDS). A pair of reversible peaks is exhibited on GCE/ERGO/SDS/GOD by cyclic voltammetry. The peak-to-peak potential separation of immobilized GOD is 28 mV in 0.1 M phosphate buffer solution (pH 7.0) with a scan rate of 50 mV/s. The average surface coverage is 2.62×10^{-10} mol cm^{-2}. The resulting biosensor exhibited a good response to glucose with linear range from 1 to 8 mM ($R^2 = 0.9878$), good reproducibility and detection limit of 40.8 μM. The results from the biosensor were similar (±5%) to those obtained from the clinical analyzer.

1. Introduction

A new report has found that there are now nearly 350 million people on Earth who suffer from diabetes [1]. Diabetes (diabetes mellitus) is classed as a metabolism disorder. A person with diabetes has a condition in which the quantity of glucose in the blood is too elevated. This is because the body does not produce enough insulin. This results in too much glucose building up in the blood. This excess blood glucose eventually passes out of the body in urine. Therefore, for the treatment and control of diabetes, the amount of blood glucose has to be monitored. Conventional techniques such as UV fluorescence [2], chemiluminescence [3,4] and titrimetry [5] have been reported in literatures. However, the conventional methods are generally time-consuming and difficult for an automated detection. Besides, most of these methods show limitations such as lack of sensitivity and susceptibility to interference by other substances in analyte samples. To overcome all these shortcomings, the electrochemical biosensor based on the direct electrochemistry between an electrode and the immobilized enzyme/protein is especially promising because of its simplicity, high sensitivity and selectivity [6–8]. Therefore, many methods are attempted to promote the direct electrochemistry of proteins. Among them, modifying electrode surfaces with proper surfactants and graphene has been demonstrated to be quite effective [9,10]. Surfactants are a type of amphiphilic molecules with a polar head at one end and a long hydrophobic tail at the other. They can spontaneously adsorb on the interfaces of two phases with different polarities or associate into micelles in solutions. Because of the enhancement effect and the ability to change the properties of electrode/solution interface, surfactants have been widely used in electroanalytical chemistry [11,12]. SDS can increase the immobilization glucose oxidase on ERGO due to its excellent film forming ability. The single-chain surfactant, SDS, can form a stable monolayer containing negative charges on the electrode surface and the positively charged GOD molecules can be adsorbed inside the porous surface of SDS/ERGO/GCE and provide an effective electron-tunneling pathway between GOD and the surface electrode. The biocompatible characteristics of SDS would also provide a favorable microenvironment to keep the activities of the immobilized proteins [13]. Furthermore, it is simple and inexpensive to prepare modified electrode based on surfactants and to immobilize proteins. Also, graphene is currently making an impact in the field of electrochemistry, particularly its use as a sensor substrate [14–17]. The discovery of graphene by Andre Geim and Konstantin Novoselov in 2004 has opened a new chapter in science. Graphene has shown great application potential in various fields, such as ultracapacitors [18], batteries [19], fuel cells [20,21] and bioscience/biotechnologies [22–25]. In fact, most of the graphenes that were synthesized contain other elements, such as oxygen, so it is more reasonable to call the graphene "reduced graphene oxide" (rGO). This composite can be synthesized by various methods [26–40]. Among the current methods of generating reduced graphene oxide, hydrazine

* Corresponding author. Tel./fax: +98 21 44042955.
E-mail address: mahmoud.tabrizi@gmail.com (M. Amouzadeh Tabrizi).

http://dx.doi.org/10.1016/j.msec.2014.09.002
0928-4931/

and sodium borohydride are used for the chemical reduction of exfoliated graphene oxide (GO) [41,42]. But, these reducing agents are unstable and dangerously toxic. Several groups investigated the synthesis of rGO with the eco-friendly methods [43–50]. Electrochemical methods are one promising green strategy for graphene synthesis, and several research works have been report [51,52]. To the best of our knowledge, the use of ERGO/SDS nanocomposite film for immobilization of GOD and its application as a glucose biosensor based on the direct electron transfer have not yet been reported. The proposed glucose biosensor exhibited excellent electrochemical characteristics and high analytical performance in terms of sensitivity, stability, selectivity, linear range and limit of detection. Herein, SDS increases the immobilization glucose oxidase on ERGO due to its excellent film forming ability.

We expect that the ERGO/SDS will be a suitable nanocomposite for the fabrication of biosensors.

2. Experimental

2.1. Reagents and chemicals

All chemicals were of analytical reagent grade and used without further purification. Double distilled water was used throughout. Glucose oxidase (GOD, EC 1.1.3.4, type VII from *Aspergillus niger*, 221 U mg^{-1}), D-(+)-glucose (97%) and sodium dodecyl sulfate (SDS) were obtained from Sigma (St. Louis, MO, USA). 2 mg of GOD was dissolved in 100 μL of phosphate buffer solution (0.1 M, pH = 8.0) to prepare GOD working solution. A stock solution of glucose (0.5 M) was prepared with doubly distilled water and stored at 4 °C when not in use. The glucose stock solution was allowed to mutarotate at room temperature for at least 24 h before use.

2.2. Apparatus

Cyclic voltammetry studies were performed using an Autolab potentiostat-galvanostat model PGSTAT30 (Eco Chemie, Utrecht, The Netherlands) with a conventional three electrode set-up, in which a GCE/ERGO/SDS/GOD, an Ag|AgCl|KCl_{sat} and a platinum rod served as the working, reference and auxiliary electrodes, respectively. The working potential was applied in the standard way using the potentiostat and the output signal was acquired by Autolab Nova software. Atomic force microscope (AFM) was taken on Dualscope DS 95-200. All measurements were performed at room temperature. Transmission electron microscopy (TEM) measurements were made on a HITACHI H-8100 EM with an accelerating voltage of 200 kV. X-ray diffraction (XRD) spectra were obtained using a D8ADVANCE (Bruker, Germany) with Cu Kα (1.5406 Å) radiation. Fourier Transform Infra-Red (FTIR) analysis was carried out using a Bruker (Germany) vector 22 spectrometer. Raman scattering was performed on an Almega Thermo Nicolet Dispersive Raman spectrometer using the second harmonic (532 nm) of a Nd:YLF laser source. Brunauer–Emmett–Teller (BET) analysis was carried out using a Belsorp, BELMAX (Japan) at 77 K. The ultrasonication and filtration processes were carried out using an ultrasonic cleaner (Elma — E30H, Powerful cleaning with 37 kHz cavitation) and a vacuum pump (Precision 3/4 HP Vacuum Pump Model D 150), respectively.

2.3. Synthesis of graphene oxide

A graphene oxide was obtained through natural graphite oxidation based on Hummer's method [53]. Briefly, 5.0 g of graphite powder was dispersed in a 120 mL concentrated H_2SO_4 kept at 0 °C under stirring. Then, 15.0 g of $KMnO_4$ was added gradually to the mixture kept in an ice bath to ensure that the temperature remained around 35 °C. After that, the temperature was raised to 0 °C and the mixture was stirred for 30 min and then diluted gradually with 225 mL deionized water. The mixture was re-diluted with 700 mL deionized water and treated with 3% hydrogen peroxide. The color of mixture changed to yellow-brown during the dropwise addition of H_2O_2. The mixture was filtered and washed with HCl solution (5%) and then repeatedly washed with water until neutral pH was obtained for filtrate by a vacuum pump. This solution was centrifuged at 3000 rpm for 10 min and then the filtrate was re-dispersed in water and centrifuged for several times. Finally, the dark brown GO powder was obtained through drying at 50 °C in a vacuum oven for a day. The graphite oxide was then exfoliated by ultrasonication into the ultrasonic cleaner. For this purpose, GO powder dispersed in a known volume of water was subjected to ultrasonication for 60 min to give a stable suspension of GO and then centrifuged at 3000 rpm for 30 min to remove any aggregates that remained in the transparent light brown exfoliated GO suspension. The prepared graphene oxide (GO) and electrochemically reduced graphene oxide (ERGO) were characterized by FTIR, XRD, Raman and BET (see Supporting information).

2.4. Preparation of GCE/ERGO/SDS/GOD

The surface of a GCE (i.d. = 3.05 mm, Metrohm, Herisau, Switzerland) was polished successively with 0.3, 0.1 and 0.05 μm alumina slurry (Struers, Copenhagen, Denmark) and then cleaned in ethanol and water, respectively under ultra-sonication. Then, 3 μL of the GO solution was cast on the surface of GCE and allowed to dry at ambient temperature. Then, 5 μL of the SDS solution was cast on the surface of GCE/GO and allowed to dry at ambient temperature again. Finally, the electrode was immersed into the GOD solution (3 mg/100 μL in pH 5.5 PBS) for 1 h. The electrode was also thoroughly rinsed with water to remove the unadsorbed GOD molecules and was dried in air. Then, the electrochemical reduction of GO was carried out by the Potential Step method: The potential of working electrode was kept constant at −0.85 V with respect to Ag|AgCl|KCl_{sat} for 30 min in a N_2-saturated phosphate buffer solution (0.1 M, pH = 7). The GCE/ERGO/SDS/GOD was stored at 4 °C in a phosphate buffer (0.1 M, pH 7.0) when not in use. AFM was used to characterize and compare the surface morphologies of GO and GO/SDS/GOD films on a GCE, as shown in Fig. S5. The difference of the morphologies reflects that strong interactions take place between GOD and GO/SDS, which have influence on the structure of the composite film.

3. Results and discussion

3.1. Characterization

TEM images of the prepared RGONs are shown in Fig. 1. It can be seen a transparent and flake-like sheet of GO.

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) have been used to characterize the interface properties of surface-modified electrodes. EIS has been used to characterize the interface properties of surface-modified electrodes [54–56]. The typical impedance spectrum (presented in the form of the Nyquist plot) includes a semicircle portion at higher frequencies corresponding to the electron-transfer-limited process and a linear part at lower frequency range representing the diffusion limited process. The semicircle diameter in the impedance spectrum equals the electron-transfer resistance, R_{et}. This resistance controls the electron-transfer kinetics of the redox probe at the electrode interface.

Fig. 2.A shows the EIS of the bare GCE (curve a), GCE/GO/SDS (curve b), GCE/ERGO/SDS (curve c) and GCE/ERGO/SDS/GOD (curve d), respectively. The resistance controls the electron transfer kinetics of the redox probe at the electrode. The R_{et} of bare GCE (curve a) was 377 Ω. After the GO/SDS was added to the bare GCE (curve b), the value of R_{et} increased to 8839 Ω; this is because GO composite film can decrease the rate of electron transfer. The diameter of the semicircle decreased after the formation of ERGO/SDS to 163.9 Ω (curve c), indicating that the ERGO/SDS composite film may provide higher electron conduction pathways than GO/SDS. However, when GOD was immobilized on GCE/ERGO/SDS, the