Carbon 127 (2018) 202–208

Contents lists available at ScienceDirect

Carbon

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

Self-assembly of phenoxyl-dextran on electrochemically reduced graphene oxide for nonenzymatic biosensing of glucose

Bo Li [a], Aimin Yu [a, b], Guosong Lai [a, *]

[a] Hubei Key Laboratory of Pollutant Analysis & Reuse Technology, Department of Chemistry, Hubei Normal University, Huangshi 435002, PR China
[b] Department of Chemistry and Biotechnology, Faculty of Science, Engineering and Technology, Swinburne University of Technology, Hawthorn VIC 3122, Australia

ARTICLE INFO

Article history:
Received 29 August 2017
Received in revised form
17 October 2017
Accepted 26 October 2017
Available online 1 November 2017

Keywords:
Graphene
Gold nanoparticle
Electroanalytical chemistry
Biosensor
Glucose

ABSTRACT

Electrochemically reduced graphene oxide (ERGO) has attracted considerable interest in the electrochemical biosensing field. In this work, the π–π stacking assembly of phenoxyl-dextran (DexP) and sensitive electrochemical stripping analysis of gold nanoparticles (Au NPs) on the ERGO surface are conducted to develop a novel nonenzymatic glucose biosensing method. Concanavalin A (Con A) was covalently linked with Au NP to obtain a nanoprobe, which was used for the specific biorecognition of glucose at the ERGO/DexP biosensor. Based on the glucose-Con A-dextran competition reaction, the Au NP/Con A nanoprobes were quantitatively captured onto the biosensor surface. Through the electrochemical stripping analysis of Au NPs, sensitive signal transduction was achieved. ERGO not only enables the simple preparation of the biosensor but also improves the sensitivity of the method greatly. The high specificity of the Con A biorecognition and the relatively positive potential range for the gold stripping analysis exclude well the signal interferences involving in the conventional electrochemical glucose biosensors. Thus such a nonenzymatic glucose biosensing method featuring excellent performance, low cost and convenient signal transduction provides a great potential for the diabetes diagnosis application.

1. Introduction

To fulfill the urgent demands of diabetes diagnosis and monitoring, various carbon nanomaterials have been widely employed to construct different electrochemical glucose biosensors [1–8]. Among them, graphene has attracted considerable interest due to its outstanding electrical conductivity, mechanical strength, chemical stability and unique two-dimensional sp^2-bonded carbon nanostructure [4,5,8].

Although several methods have been developed to prepare graphene, chemical conversion, namely oxidization and sonication of flake graphite to obtain graphene oxide (GO), followed by a chemically reduction step, is popularly adopted because of its obvious advantages in synthesis of bulk-quantity graphene nanosheets with low cost [9,10]. Note that, the involved chemical reduction process is unavoidable to use the highly toxic, dangerous, and unstable agents (e.g., $NaBH_4$, hydrazine) at a high temperature [11]. Additionally, the irreversible aggregation of the chemically reduced GO occurs. To prevent this, polymers are generally needed to attach as foreign stabilizers [12,13]. Unfortunately, these stabilizers are often undesirable and even disadvantageous for the graphene applications. The electrochemical reduction of the exfoliated GO was then developed for synthesizing large-scale graphene film on different substrates [11,14–17]. Due to the easy preparation and high quality of the electrochemically reduced GO (ERGO), it has been employed for the construction of various electrochemical biosensors by means of direct coating or adsorption methods [18–21]. However, these unstable and uncontrollable modification approaches affected the performance or limited the practical application of the related biosensors. In fact, the pristine π-electron-rich conjugated structure of ERGO which is responsible for its outstanding electrical conductivity, indicates that the functional molecules containing aromatic moieties are possible to be noncovalently attached onto the surface of ERGO through the π–π stacking interaction [22,23]. This provides a promising approach for the simple and controllable construction of an ERGO-based biosensor.

There are two main categories, enzyme (e.g. glucose oxidase) and nonenzyme-based models for the construction of

* Corresponding author.
E-mail address: gslai@hbnu.edu.cn (G. Lai).

https://doi.org/10.1016/j.carbon.2017.10.096
0008-6223/

electrochemical glucose biosensors [24,25]. As the former is based on the measurement of the electrochemical signal of corresponding enzyme-reaction products [1–6,18,19], the bioactivity and stability of the enzyme immobilized on the electrode surface restrict greatly its performance. The latter is relied on the direct electrochemical oxidation of glucose by the nanocomposites with excellent electrocatalytic properties [7,8,26]. Although the nonenzymatic biosensors resolve the problem of insufficient long-term stability involving in enzymatic glucose biosensors, the adsorption of glucose oxidation intermediates on the electrode surface often unavoidably blocks its electrocatalytic activity. More importantly, both biosensors often encounter the electrochemical signal interference from the coexisting substances such as ascorbic acid (AA), uric acid (UA), dopamine (DA), even dissloved oxygen in real serum samples. These interferences extensively limit the selectivity and accuracy of the constructed biosensors.

Concanavalin A (Con A) is an important lectin protein with four saccharide bind sites extracted from the jack-bean. Under neutral conditions, Con A binds specifically to certain structures found in various sugars, glycoproteins and glycolipids, mainly internal and nonreducing-terminal α-mannosyl groups [27,28]. Due to this specific affinity, Con A is believed to be an attractive receptor molecule for the development of a novel kind of biorecognition-based nonenzymatic glucose biosensor [29,30]. In this work, we employ the π−π stacking assembly method to construct an ERGO-based biosensor, and combine the monosaccharide (glucose)-Con A-polysaccharide (dextran) competition reaction with the sensitively electrochemical stripping analysis of gold nanoparticles (Au NPs) on this sensor surface to develop a new nonenzymatic glucose biosensing strategy. As illustrated in Fig. 1, the biosensor is constructed though the self-assembly of phenoxyl-dextran (DexP) on the surface of the ERGO-modifed screen-printed carbon electrode (SPCE). A gold nanoprobe prepared from the Con A-functionalization of Au NP was used for the competition recognition reaction with glucose at the biosensor. Based on the specific binding of the Au NP/Con A nanoprobe onto the biosensor surface for the electrochemical stripping analysis of Au NPs [31], convenient signal transduction is achieved for the quantitative analysis of glucose successfully.

2. Materials and methods

2.1. Reagents and materials

ConA from *Canavalia ensiformis* (Jack bean), bovine serum albumin (BSA), DexP (M_w = 40 KDa), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), AA, UA, DA and 3-mercaptopropionic acid (MPA) were purchased from Sigma–Aldrich (USA). Chloroauric acid and glucose were obtained Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). GO synthesized by the modified Hummer's method was provided by Jicang Nano Co. (Nanjing, China). A SPCE system containing a carbon working electrode (2 mm in diameter), a carbon auxiliary electrode and an Ag/AgCl reference electrode was fabricated according to the previous report [32]. The human serum samples were obtained from the Second Hospital of Huangshi, China. All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiments.

2.2. Apparatus

The FTIR spectra were recorded at a Nicolet iD5 spectrometer (Thermo scientific, USA). The scanning electron microscopy (SEM) images were obtained with a FEI Nova NanoSEM 450 scanning electron microscope (USA). The atomic force microscopy (AFM) images were obtained with a MFP-3D Origin atomic force microscope (Oxford Instruments, UK) in tapping mode. The reference levels of glucose in human serum samples were tested by an Architect C8000 Biochemical Analyzer (Abbott Diagnostics, USA). All electrochemical experiments were performed on a CHI 660E electrochemical workstation (Chenhua, China). The electrochemical impedance spectra were recorded in 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1: 1) containing 0.10 M KCl at a formal potential of 0.18 V with a frequency range from 10^{-2}–10^5 Hz and an alternating voltage of 5 mV.

2.3. Preparation of the ERGO-based biosensor

First, a 1.8-μL drop of the GO aqueous at a concentration of

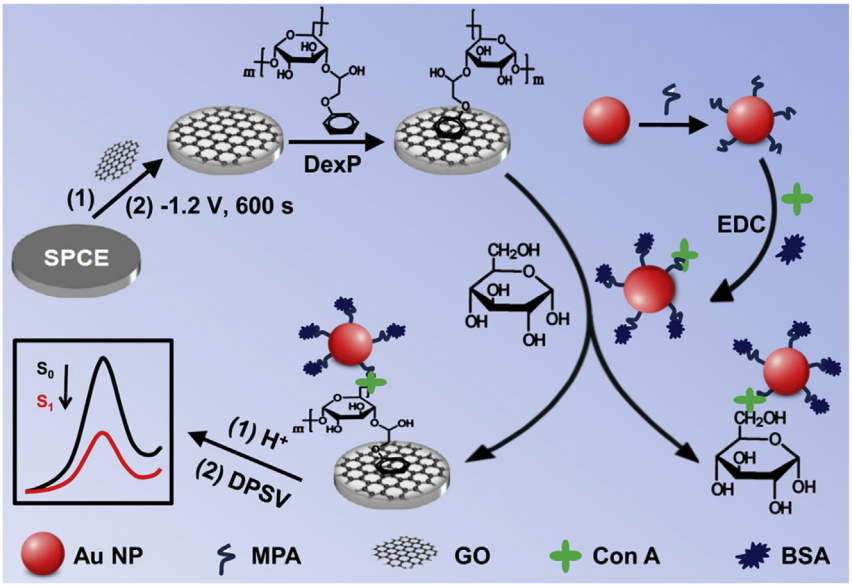

Fig. 1. Schematic illustration of the preparation processes of the biosensor and Au NP/Con A nanoprobe as well as the electrochemical detection strategy of the biosensing method. (A colour version of this figure can be viewed online.)