Biosensors and Bioelectronics 66 (2015) 350–355

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Biosensor based on glucose oxidase-nanoporous gold co-catalysis for glucose detection

Chao Wu, Huihui Sun, Yufei Li, Xueying Liu, Xiaoyu Du, Xia Wang *, Ping Xu [1]

State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form
18 November 2014
Accepted 20 November 2014
Available online 24 November 2014

Keywords:
Glucose oxidase
Immobilization
Nanoporous gold
Biosensor
Glucose

ABSTRACT

Promoting the electrocatalytic oxidation of glucose is crucial in glucose biosensor design. In this study, nanoporous gold (NPG) was selected for glucose oxidase (GOx) immobilization and glucose biosensor fabrication because of its open, highly conductive, biocompatible, and interconnected porous structure, which also facilitates the electrocatalytic oxidation of glucose. The electrochemical reaction on the surface of the resulting GOx/NPG/GCE bioelectrode was attributed to the co-catalysis effect of GOx and NPG. A surface-confined reaction in a phosphate buffer solution was observed at the bioelectrode during cyclic voltammetry experiments. Linear responses were observed for large glucose concentrations ranging from 50 μM to 10 mM, with a high sensitivity of 12.1 μA mM^{-1} cm^{-2} and a low detection limit of 1.02 μM. Furthermore, the GOx/NPG/GCE bioelectrode presented strong anti-interference capability against cholesterol, urea, tributyrin, ascorbic acid, and uric acid, along with a long shelf-life. For the detection of glucose in human serum, the data generated by the GOx/NPG/GCE bioelectrode were in good agreement with those produced by an automatic biochemical analyzer. These unique properties make the GOx/NPG/GCE bioelectrode an excellent choice for the construction of a glucose biosensor.

1. Introduction

Glucose plays a crucial role in life processes (Heller and Feldman, 2008) as a direct energy source facilitating various biological activities. More specifically, the serum glucose is a clinically significant indicator of many chronic illnesses such as diabetes, obesity, hyperglycemia, and cardiovascular disease (Jiang et al., 2008; Wei et al., 2014). Therefore, it is highly desirable to develop a new method for the rapid and efficient determination of blood glucose.

As compared to the traditional methods available for glucose detection, methods based on electrochemical sensors show unique advantages such as high selectivity and sensitivity, ease of operation, fast response, and continuous real-time detection (Gu et al., 2012). Nonenzymatic electrochemical sensors have been widely used for glucose determination because of their high stability and operational simplicity (Chang et al., 2014; Fu et al., 2014). However, the selectivity and sensitivity of nonenzymatic sensors are usually inferior to those of the biosensors incorporating enzymes as the recognition elements. In 1962, Clark and Lyons (1962) developed the first biosensor system comprising glucose oxidase (GOx) coupled with an oxygen electrode. Since then, GOx has been widely used in biosensors to determine the glucose levels in serum sample in vitro (Qiu et al., 2012; Gao et al., 2014; Hsu and Wang, 2014). However, the inherent characteristics of enzymes are considered a mixed blessing, because efficient catalysis is often accompanied by a higher possibility of enzyme inactivation. Thus, the materials that link the enzymes to the electrode need to conserve the enzymatic activity by providing an efficient electron transfer and by achieving sufficient enzyme loading. However, research aimed at using inorganic materials for enzyme immobilization has been rarely carried out. Such immobilization could potentially facilitate the simultaneous electrocatalytic activities of the enzyme and the inorganic material towards the substrate.

In recent years, various nanomaterials were employed as enzyme immobilization substrates and as recognition elements in electrochemical sensors. High biosensor performance, intimate enzyme attachment, and effective electron transfer are achievable because of the high surface area, and the unique physical, electronic, and chemical properties of nanomaterials (Vijayalakshmi et al., 2008; Solanki et al., 2009). Among the various nanomaterials, nanoporous gold (NPG) has attracted much attention in the fabrication of nonenzymatic and enzyme-based electrochemical sensors because of its excellent structural continuity, higher conductivity, and general biocompatibility (Ding et al., 2004; Ding and

* Corresponding author. Fax: +86 531 88366231.
E-mail address: ghwx@sdu.edu.cn (X. Wang).
[1] Present address: Key Laboratory of Microbial Metabolism and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, PR China.

http://dx.doi.org/10.1016/j.bios.2014.11.037
0956-5663/

Chen, 2009; Scanlon et al., 2012; Yan et al., 2012; Zhang and Ding, 2013). The active surface and catalytic activity of NPG also provides us with the possibility of substrate recognition even in the absence of the enzyme. For example, hydrogen peroxide and glucose elicited a direct electrochemical response form a nonenzymatic NPG sensor (El-Daeb and Ohsaka, 2002; Liu et al., 2009). While NPG also provides a natural platform for stable enzyme immobilization because of the strong gold–sulfur covalent-type interactions, enzyme/NPG biocomposites are expected to combine the advantages of both enzymatic and nonenzymatic electrochemical sensors, and exhibit a synergistic effect that results into highly efficient glucose detection.

In our previous work, enzyme/NPG biocomposites were successfully constructed by assembling various enzymes (such as lipase, catalase, and horseradish peroxidase) onto NPG (Wang et al., 2011). A lipase/NPG biosensor was also reported to perform exceptionally well during the detection of serum triglycerides (TGs) (Wu et al., 2014). This study was devoted to confirming whether GOx and NPG could offer a synergistic electrocatalytic effect towards glucose. We also focused on the construction of a GOx/NPG co-catalytic biosensor for glucose detection. The resulting GOx/NPG/GCE bioelectrode seems to exhibit excellent sensitivity, selectivity, and stability, along with a good anti-interference ability and repeatability

2. Experimental

2.1. Chemicals

GOx (Sigma G7141 from *Aspergillus niger*; protein: 65–85%; molecular weight: 160 kDa) was purchased from Sigma-Aldrich (St. Louis, MO, USA). D-Glucose (analytical grade) was purchased from Shanghai Sangon Biotech Co. LTD (China). All other chemicals used were of analytical grade.

D-Glucose was dissolved in a PBS (50 mM, pH 7.0) solution to form a 2 M glucose stock solution. The glucose stock solution was stored in a refrigerator at 4 °C in the dark.

2.2. Preparation of the NPG/GCE electrode

NPG was made by dealloying 12-carat white gold leaves (Au50Ag50 wt%, Sepp Leaf Products, USA) in concentrated HNO_3 at 30 °C for 30 min. After that, NPG was washed with ultrapure water until the pH to 7.0. It was then kept in ultrapure water.

Glassy carbon electrodes (GCEs) were polished with a 0.05 μm alumina slurry on a piece of chamois leather. Before use, the GCEs were cleaned ultrasonically in the mixture of HNO_3 and water mixed in a 1:1 ratio (v/v), and washed with ultrapure water and absolute ethanol, respectively. The GCE was then coated with an NPG leaf in ultrapure water to form NPG/GCE. The NPG/GCE was placed in a vacuum drier for later use.

2.3. GOx immobilization

GOx solution (2000 U mL^{-1}) was freshly prepared by dissolving 34 mg GOx in 5 mL PBS (50 mM, pH 6.8) prior to being used. One unit (U) of GOx activity is defined as the amount of GOx, which oxidizes 1.0 μM of β-D-glucose to D-gluconolactone and H_2O_2 per min at pH 5.1, at 35 °C. The freshly prepared NPG/GCE was immersed immediately into the GOx solution. After a 72 h immobilization, the GOx/NPG/GCE bioelectrode was immersed into the PBS solution (50 mM, pH 7.0) and then transferred to a refrigerator (4 °C) for subsequent use.

2.4. Measurement

The morphology of NPG was characterized using a Nova NanoSEM 450 field emission scanning electron microscope (SEM) equipped with an energy-dispersive X-ray spectrometer (EDS).

All the electrochemical measurements were performed in a conventional three-electrode cell at room temperature. The cyclic voltammograms (CVs) and linear sweep voltammograms (LSVs) were measured using a three-electrode conventional cell with a CHI 760D electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China). The modified GCE was used as a working electrode, and a Pt sheet (1 cm × 1 cm) and a saturated calomel electrode (SCE) were used as counter and reference electrodes, respectively. All the potentials were referred to the SCE. The electrolyte solutions were deaerated by N_2 bubbling for 10 min prior to the electrochemical measurements, and a blanket of N_2 was maintained throughout each experiment.

2.5. Detection of GOx concentration in serum

Serum samples were offered by a school hospital affiliated to the Shandong University (Jinan, China). The serum sample (500 μL) was added to 15 mL deaerated PBS (50 mM, pH 7.0) in a three-electrode system, with the GOx/NPG/GCE as the working electrode. In order to validate the values measured by the GOx/NPG/GCE electrode, the serum samples were also analyzed with an automatic biochemical analyzer (HITACHI 7100, Japan).

3. Results and discussion

3.1. Construction and characterization of the GOx/NPG/GCE bioelectrode

The dimensions of GOx, as revealed by its crystal structure were 7 × 5.5 × 8.0 cubic nm (Hecht and Schomburg, 1993). According to theoretical calculations, the maximum stabilization of a protein can be achieved following adsorption within spherical cages whose diameter are 2–6 times those of the native molecule (Sotiropoulou et al., 2005; Wang et al., 2011). Therefore, NPG with a pore size of ca. 35 nm, was selected to link GOx for constructing the GOx/NPG/GCE bioelectrode depicted in Fig. 1. The samples before (Fig. 1A) and after (Fig. 1B) GOx loading were characterized using SEM. Fig. 1A illustrates an open three-dimensional nanoporous structure. The EDS compositional analysis reveals that only Au was detected, thus indicating that the residual Ag was below the detection limit of about 0.5% (Fig. 1C). A preferential immobilization of GOx over the ligament site with high radial curvatures was achieved, as evident from the smaller pore size and coarser surface morphology of the GOx/NPG biocomposite (Fig. 1B), relative to the bare NPG (Fig. 1A). Additionally, the EDS analysis confirmed the existence of dominant elements such as C, N, and O (Fig. 1D), thus providing the primary evidence for a successful GOx immobilization onto NPG.

3.2. Electrochemical behavior of the GOx/NPG/GCE bioelectrode

The electrochemical behaviors of the NPG/GCE electrode and the GOx/NPG/GCE bioelectrode were compared in deaerated PBS (50 mM, pH 7.0) at a scan rate of 50 mV s^{-1}. The current response of the GOx/NPG/GCE electrode was obviously reduced, relative to the NPG/GCE bioelectrode, as shown in Fig. 2A. Because of the insulating character of the enzyme, the lower current response after GOx loading indicated that GOx was successfully immobilized onto NPG (Zhang et al., 2005; Qiu et al., 2012).

To confirm the functioning of the GOx/NPG/GCE bioelectrode