Biosensors and Bioelectronics 23 (2008) 1887–1890

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Amperometric glucose biosensor based on single-walled carbon nanohorns

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ARTICLE INFO

Article history:
Received 4 December 2007
Received in revised form 16 February 2008
Accepted 21 February 2008
Available online 29 February 2008

Keywords:
Single-walled carbon nanohorns
Biosensor
Glucose oxidase
Carbon nanotubes
Glucose

ABSTRACT

The biosensing application of single-walled carbon nanohorns (SWCNHs) was demonstrated through fabrication of an amperometric glucose biosensor. The biosensor was constructed by encapsulating glucose oxidase in the Nafion–SWCNHs composite film. The cyclic voltammograms for glucose oxidase immobilized on the composite film displayed a pair of well-defined and nearly symmetric redox peaks with a formal potential of −0.453 V. The biosensor had good electrocatalytic activity toward oxidation of glucose. To decrease detection potential, ferrocene monocarboxylic acid was used as a redox mediator. The mediated glucose biosensor shows a linear range from 0 to 6.0 mM. The biosensor shows high sensitivity (1.06 μA/mM) and stability, and can avoid the commonly coexisted interference. Because of impressive properties of SWCNHs, such as high purity and high surface area, SWCNHs and their composites are expected to be promising material for biomolecular immobilization and biosensing applications.

1. Introduction

Carbon nanotubes (CNTs) has been extensively used in electrochemical and biosensing studies (Sotiropoulou et al., 2003; Katz and Willner, 2004; Poh et al., 2004; Lin et al., 2005; Merkoçi et al., 2005; Wang, 2005; Balasubramanian and Burghard, 2006). For example, CNTs have been used for electrocatalysis (Chen et al., 2005; Liu et al., 2005a; Musameh et al., 2002), immobilization of proteins (Zhao et al., 2002; Davis et al., 2003; Wang et al., 2003; Hrapovic et al., 2004; Zhang et al., 2004; Tsai et al., 2005; Yan et al., 2005), and promotion of direct electrochemistry of redox proteins (Gooding et al., 2003; Patolsky et al., 2004), and so on. However, most CNT synthesis methods inevitably introduce some impurities. Tedious purification is necessary, and metal catalyst residues still existed in CNTs even after purification. The impurities resulted in conflicting reports (Shvedova et al., 2003; Banks et al., 2006; Sljukic et al., 2006; Jones et al., 2007).

Single-walled carbon nanohorns (SWCNHs) are cost-effectively produced by laser ablation of pure graphite with unparalleled purity without using any metal catalyst (Iijima et al., 1999). It is essentially metal-free and can be used directly for electrochemical and biosensing study. Typically, horn-shaped SWCNHs assemble and form dahlia-like or bud-like aggregates (Yang et al., 2005), ensuring extremely large surface area and fast mass transport. Its unique properties rapidly promote various applications (Ohba et al., 2001; Yoshitake et al., 2002; Bekyarova et al., 2003; Murakami et al., 2004).

In this study, the biosensing application of SWCNHs was exploited using glucose biosensor as a model (Heller, 1999; Wang, 2001; Newman and Turner, 2005; Wilson and Gifford, 2005). The biosensor was constructed by immobilizing glucose oxidase in Nafion–SWCNHs film. It showed high sensitivity, good selectivity, and comparable results with spectrophotometry method.

2. Experimental

2.1. Reagents

The glucose oxidase (GOx, from *Aspergillus niger*, E.C. 1.1.3.4), ferrocene monocarboxylic acid (FMCA, 97%), Nafion (20 wt.% of lower aliphatic alcohols/water mixture), lithium L-lactate, L-cysteine, glutathione, L-ascorbate acid and *p*-aminophenol were purchased from Sigma and used without further purification. β-D-Glucose (>99.8%) was purchased from Amresco and the glucose stock solutions were stored overnight at room temperature before use. Professor S. Iijima generously offers SWCNHs. Other reagents were of analytical grade and used as received. All solutions were prepared with doubly deionized water (Millipore, Bedford, MA). 0.05 M phosphate buffer solutions (PBS) were employed as supporting

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doi:10.1016/j.bios.2008.02.016

electrolyte and the pH value was adjusted to 7.5 with 0.05 M Na_2HPO_4 and 0.05 M NaH_2PO_4.

2.2. Instrumentation

Cyclic voltammetric and amperometric measurements were carried out in a conventional three-electrode cell with the 800B electrochemical workstation (CHI Inc., USA). The working electrode was a glassy carbon (GC, diameter 3 mm) electrode. An Ag/AgCl electrode and a Pt wire were used as reference and counter electrode, respectively. The amperometric measurements of glucose were performed at constant potential under stirring at approximately 25 °C. The PBS was purged with high-purity nitrogen for at least 30 min to keep a nitrogen atmosphere. Scanning electron microscope (SEM) measurements were carried out on a PHILIPS XL-30 ESEM at an accelerating voltage of 15 kV.

2.3. Preparation of Nafion–GOx–SWCNHs modified electrode

Before each electrode modification, the bare glassy carbon electrode was polished with 0.05-μm alumina slurry, sonicated in deionized water, and dried with high purify nitrogen stream to obtain a mirror surface. Then 1 mg of SWCNHs was dispersed in 0.2 ml 0.5 wt.% Nafion solution with agitation to give a stable Nafion–SWCNHs suspension. For preparation of the Nafion–GOx–SWCNHs modified electrode, 1 mg of GOx was dissolved in 20 μL of 0.05 M pH 7.5 PBS, and appropriate amount of GOx was thoroughly mixed with the Nafion–SWCNHs suspension, then 3 μL of the new mixture was dropped on the surface of a glassy carbon electrode and allowed to dry at 4 °C for 24 h to form a uniform structure. The prepared enzyme electrode was washed with copious PBS before use.

3. Results and discussion

3.1. Morphology and voltammetric characterization of Nafion–SWCNHs modified glassy carbon electrode

A typical SEM image of Nafion–SWCNHs modified glassy carbon electrode is shown in Fig. S1. Spherical SWCNHs aggregates are packed densely and homogeneously on the electrode surface. The conductivity and the inherent purity of SWCNHs make them excellent candidates for electrochemical application. Cyclic voltammetry is a useful tool for electrochemical evaluation of the transducers. $K_3Fe(CN)_6$ was used as a probe to investigate performance of Nafion–SWCNHs film (Fig. S2). A pair of well-defined oxidation and reduction peaks was observed at 0.26 and 0.15 V at the Nafion–SWCNHs nanocomposite film modified electrode. In contrast, at the Nafion modified electrode, only slight peaks were observed because Nafion film was an obstacle to diffusion on the surface of electrode. The oxidation current obtained at the nanocomposite modified electrode was over fivefold greater than that obtained at the Nafion modified electrode, and peak separation between the anodic and cathodic peaks shifted negatively by 143 mV compared with that of Nafion modified electrode. The large distinguishable response indicates that the charge transport within the composite films is relatively fast, which can be attributed to remarkable electronic properties of SWCNHs.

3.2. Electrochemistry of GOx immobilized on Nafion–SWCNHs modified glassy carbon electrode

The application of Nafion–SWCNHs for electrochemical biosensor was exploited by immobilizing GOx in Nafion–SWCNHs film. Fig. S3 shows cyclic voltammograms at the Nafion–GOx–SWCNHs modified glassy carbon electrode. The immobilized GOx displayed a pair of well-defined and nearly symmetric redox peaks in pH 7.5 PBS. The formal potential ($E^{0'} = -0.453 \pm 0.0005$ V) was independent of the scan rate ranging from 10 to 200 mV/s, agreeing with the previously reported results (Liu et al., 2005b; Yin et al., 2005). The anodic and cathodic peak currents varied linearly with the scan rate (correlation coefficient 0.998), suggesting that the reaction was a surface-controlled process on the investigated range. According to Laviron equation (Laviron, 1979a), the number of electrons involved in electron transfer reaction was calculated to be 2.0, indicating that the electron transfer was a two-electron transfer coupled with two-proton transfer process. When $n\Delta E_p < 200$ mV, the electron transfer rate constants can be obtained based on the Laviron method (Laviron, 1979b). The value was calculated to be 3.0 s^{-1} ($\alpha = 0.5$), which was larger than that obtained by immobilizing GOx on the surface of the CNTs ($1.74 \pm 0.42\ s^{-1}$) (Yin et al., 2005), and comparable to that of FAD adsorbed on the CNTs modified electrode (3.1 s^{-1}) (Guiseppi-Elie et al., 2002).

3.3. Determination of glucose by FMCA-mediated biosensor

Typical amperometric response for mediator-free glucose biosensor was shown in Fig. S4. To decrease the overvoltage, FMCA was introduced as a mediator because of its low oxidation–reduction potential. Fig. S5 shows the cyclic voltammograms at Nafion–GOx–SWCNHs modified glassy carbon electrode in 0.05 M pH 7.5 PBS containing 0.5 mM FMCA. A pair of well-defined oxidation and reduction peaks for FMCA was observed. Upon the addition of 5 mM glucose, the oxidation current increased sharply while the reduction current completely disappeared, and the magnitude of the catalytic response was more than threefold greater. The phenomena indicate that GOx retained its activity.

Effect of solution pH on the performance of biosensor was studied and the optimal pH value was 7.5 selected, consistent with literature report (Lin et al., 2004). Dependence of biosensor response on the applied potential was investigated in 0.05 M pH 7.5 PBS. With increasing the applied potential from 0.2 to 0.5 V, the signals increased and reached a maximum at about 0.3 V, then slowly decreased. The optimum potential of 0.3 V was chosen in the following experiments.

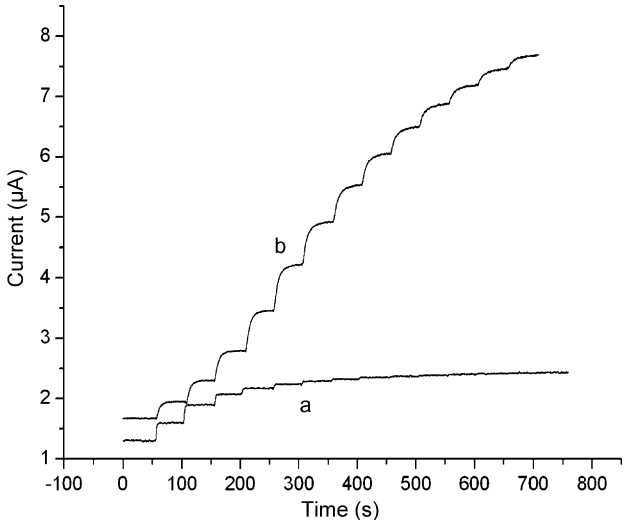

Fig. 1. Typical amperometric response of (a) Nafion–GOx modified glassy carbon electrode and (b) Nafion–GOx–SWCNHs modified glassy carbon electrode with successive addition of 0.5 mM glucose in PBS containing 0.5 mM FMCA at an applied potential of 0.3 V.

Table 1
Performance of some glucose biosensors

Glucose biosensor	Sensitivity (μA/mM)	Detection limit (μM)	K_m^{app} (mM)	Reference
Nafion–GOx–SWCNHs	1.06	6	8.5	Present work
Nafion–GOx–CNTs	0.33	4		Tsai et al. (2005)
Chitosan–GOx–CNTs	0.52		8.2	Liu et al. (2005b)
Silica sol–gel–GOx–CNTs	0.196	50		Salimi et al. (2004)
Nafion–Pd–GOx–CNTs		150		Lim et al. (2005)
Sol–gel–GOx–copolymer	0.6		22	Wang et al. (1998)
Silica gel–GOx		10	20.3	Jia et al. (2007)
TiO_2–GOx	0.144		6.08	Li et al. (2001)

Fig. 1 shows amperometric response at Nafion–GOx modified glassy carbon electrode and at Nafion–GOx–SWCNHs modified glassy carbon electrode. By comparison, the Nafion–GOx–SWCNHs modified electrode exhibited higher sensitivity, stability, and wider dynamic range than the Nafion–GOx modified electrode. This indicated that the matrix of Nafion–SWCNHs nanocomposite film had good conductivity and biocatalytic activity. The enhanced electrocatalytic response towards glucose was obviously attributed to the attractive properties of SWCNHs entrapped within the Nafion matrix. The biosensor of the Nafion–GOx–SWCNHs modified electrode exhibited good linear calibration ranged from 0 to 6.0 mM (Fig. S6, correlation coefficient, 0.993). The relative standard deviation was 2.6% for seven successive measurements of 1.0 mM glucose. For comparison, the analytical performance of the proposed sensor and some other glucose biosensors were listed in Table 1. The sensitivity of the proposed biosensor was 1.06 μA/mM, which was larger than previously reported models. The high sensitivity of the biosensor implies that SWCNHs can provide superior conductivity for electron transfer, and the Nafion–SWCNHs nanocomposite film provides biocompatible microenvironment to maintain enzymatic activity. The biosensor can detect a low concentration of 6 μM. The detection limit is comparable with that of common amperometric glucose biosensors, although not as good as some excellent performance such as the result obtained at a designed ring-disk electrode (Mano and Heller, 2005). The apparent Michaelis-Menten constant (K_m^{app}) was calculated to be 8.5 mM according to the Lineweaver-Burk plot (Fig. S6). The K_m^{app} value was quite small in comparison with that of other glucose sensors. The small K_m^{app} value indicates that diffusion of substrate and product through the membrane is much easier in the Nafion–GOx–SWCNHs film and the biosensor possesses higher affinity to glucose.

Possible interference for the glucose sensor was studied in PBS containing 2 mM glucose at applied potential of +0.3 V. 0.2 mM L-lactate, glutathione, L-cysteine, and *p*-aminophenol as well as 0.05 mM L-ascorbate acid did not interfere with the detection. The Nafion–GOx–SWCNHs biosensor showed a satisfied analysis of glucose in the presence of potential interference. Because Nafion polymer is an effectively perselective barrier, it can eliminate anionic biological interference and enhance selectivity of the glucose sensor. Additionally, the amperometric response to 1.0 mM glucose remained almost unchanged for 2 weeks, indicating that the matrix was efficient for retaining the activity of GOx and the biosensor had good stability. In order to detect glucose in real samples, human serum samples were assayed using the mediated enzyme electrode. The sample (100 μL) was added into 4 mL of PBS and a potential of 0.3 V was applied. The content of glucose in blood was calculated from working curve. The glucose level was determined to be 10.4 mM, close to the value of 10.7 mM measured by the spectrophotometric method in the hospital, and the recovery value for the assay of 0.2, 0.3 and 0.4 mM glucose was ranged from 94 to 108%. For better performance of biosensor, further effort is necessary to make SWCNHs available for clinically accurate glucose monitoring (Wilkins and Atanasov, 1996; Schmidtke et al., 1998).

4. Conclusions

In summary, we utilized SWCNHs for the first time to fabricate glucose biosensor. The glucose biosensor based on the Nafion–SWCNHs nanocomposite possessed high sensitivity, low detection limit, and good selectivity. The attractive electrochemical and structural properties of SWCNHs suggest potential application of SWCNHs for electrocatalysis and biosensor.

Acknowledgements

We are very grateful to Professor S. Iijima (Solution Oriented Research for Science and Technology in Japan Science and Technology Agency) for generous offer of SWCNHs. This work was kindly supported by the National Natural Science Foundation of China (No. 20505016), Department of Sciences & Technology of Jilin Province (20070108), and Hundred Talents Program of Chinese Academy of Sciences.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.02.016.

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