Biosensors and Bioelectronics 26 (2010) 213–219

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A sensitive and stable biosensor based on the direct electrochemistry of glucose oxidase assembled layer-by-layer at the multiwall carbon nanotube-modified electrode

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ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form 20 May 2010
Accepted 11 June 2010
Available online 19 June 2010

Keywords:
Glucose oxidase (GOD)
Layer-by-layer (LBL)
Direct electrochemistry

ABSTRACT

A novel strategy for fabricating the sensitive and stable biosensor was present by layer-by-layer (LBL) self-assembling glucose oxidase (GOD) on multiwall carbon nanotube (CNT)-modified glassy carbon (GC) electrode. GOD was immobilized on the negatively charged CNT surface by alternatively assembling a cationic poly(ethylenimine) (PEI) layer and a GOD layer. And the direct electrochemistry of GOD in the self-assembled $\{GOD/PEI\}_n$ film was investigated. CNT as an excellent nanomaterial greatly improved the direct electron transfer between GOD in $\{GOD/PEI\}_n$ film and the electrode. And the ultrathin $\{GOD/PEI\}_n$ film on the CNT surface provided a favorable microenvironment to keep the bioactivity of GOD. Moreover, PEI used as an out-layer was adsorbed on the top of the $\{GOD/PEI\}_n$ film to form the sandwich-like structure ($PEI/\{GOD/PEI\}_n$), improving the stability of the enzyme electrode. On basis of these, the developed $PEI/\{GOD/PEI\}_n$/CNT/GC biosensor has a high sensitivity of 106.57 $\mu A\, mM^{-1}\, cm^{-2}$, and can measure as low as 0.05 mM glucose. In addition, the biosensor has excellent operational stability with no decrease in the activity of enzyme over a 1-week period. Therefore, the developed strategy making use of the advantages of CNT and LBL assembly is ideal for the direct electrochemistry of the redox enzymes and the construction of the sensitive and stable enzyme biosensor.

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1. Introduction

Since the first concept of an enzyme-based biosensor was proposed by Clark and Lyons (1962), many researches about biosensors have been developed. The long-term goal of these researches is to explore the biosensor for practical applications. However, it is well-known that the most critical issues in the application of in vivo biosensor systems are the stability of the biological sensing element (e.g. protein or enzyme) and the efficient and facile signal mediation and transduction for improved sensitivity and elimination interference (Vamvakaki et al., 2008). Therefore, to resolve these difficulties, the enzyme biosensor has been developed and experienced three stages in past years: the first, second and third generations. Recently, the third-generation biosensor based on the direct electrochemistry of protein/enzyme has been developed widely (Kang et al., 2009; Liu and Ju, 2003; Wu et al., 2008). This can be ascribed to these as follows: (1) comparing the first/second-generation biosensor, the third-generation biosensor has better selectivity and stability because it overcomes some drawbacks such as the possible interference from electrochemically oxidable compounds in real samples, and the un-stability of mediators (Battaglini et al., 2000; Garjonyte and Malinauskas, 1999; Yan et al., 2008); (2) the direct electron transfer between a protein/enzyme and the electrode can be considered as a mimetic process of the electron transfer through redox molecules chains in biological systems (Shen and Hu, 2005). Therefore, the study of direct electrochemistry of redox proteins/enzymes at electrodes may provide a working model for the mechanistic study of electron transfer in living systems (Gooding et al., 2003; Zhang and Hu, 2007). However, it is generally difficult to establish direct electron transfer between protein/enzyme and conventional electrodes because of the inaccessibility of the redox sites of protein/enzyme seated in the large three-dimensional structure (Hamachi et al., 1997; Shi et al., 2007). Therefore, the establishment of a fast electron transfer becomes a significant challenge in designing enzyme-based sensors. In 2008, we produced boron-doped carbon nanotubes (BCNTs) and investigated the direct electrochemistry of glucose oxidase (GOD) at the BCNTs/GC electrode. And it was found that the direct electron

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doi:10.1016/j.bios.2010.06.013

transfer between GOD and the electrode was improved obviously (Deng et al., 2009). However, it should be pointed that the biosensor based on the CNT-modified electrode may be more typical and popular than that based on the BCNTs-modified electrode, because multi-wall carbon nanotubes can be obtained more easily than BCNTs. In this case, un-doped carbon nanotube (CNT) as a classic new nanomaterial may be still attractive in the direct electrochemistry of proteins/enzymes because CNT can promote the direct electron transfer between the redox sites of protein/enzyme and the electrode (Gooding et al., 2003; Zhang et al., 2007), and improving the sensitivity of the related biosensor.

On the other hand, a novel technique of layer-by-layer self-assembly which is based on the alternate adsorption of oppositely charged species from their solutions (Decher, 1997), has aroused more interests among researchers and has been developed into a general approach for fabricating ultrathin films on solid surfaces. In recent years, the layer-by-layer assembly has been extended to build up protein or other biomacromolecule films (Lvov, 2001) and has been successfully employed for the design and construction of biodevices. Moreover, it was reported that layer-by-layer films not only provide a favorable nanoenviroment for the enzymes or proteins (Hu, 2001), but also enhance the direct exchange between the proteins and underlying electrode (Shen and Hu, 2005). Therefore, the technique of layer-by-layer assembly has been employed to achieve the direct electrochemistry of proteins/enzymes and fabricate the related biosensor. For example, nanostructured biosensors were built by layer-by-layer electrostatic assembly of enzyme-coated single-walled carbon nanotubes and redox polymers (Wang et al., 2006). Shi et al. investigated the electrochemistry and electrocatalytic properties of hemoglobin in layer-by-layer films of SiO_2 with vapor-surface sol–gel deposition (Shi et al., 2007). The direct electrochemistry of electroactive hemoglobin was carried out by layer-by-layer assembly of hemoglobin and surfactant didodecyldimethylammonium bromide (Hu et al., 2007).

In this present paper, based on the direct electrochemistry of redox enzyme, we try to integrate the excellent properties of CNT with the advantages of layer-by-layer self-assembly to fabricate a sensitive and stable biosensor. GOD was immobilized on the negatively charged CNT surface by alternatively assembling cationic poly(ethylenimine) (PEI) layers and GOD layers. Herein, CNT as an excellent nanomaterial can improve the direct electron transfer between redox sites of glucose oxidase and the electrode. The $\{GOD/PEI\}_n$ film played an important role in retaining the bioactivity of the immobilized glucose oxidase. Moreover, PEI used as a out-layer was adsorbed on the top of the $\{GOD/PEI\}_n$ film to form the sandwich-like structure (PEI/$\{GOD/PEI\}_n$), improving the stability of the enzyme electrode. And also the fabrication of the enzyme biosensor was controllable, time saving and convenient. Based on the (PEI/$\{GOD/PEI\}_n$)/CNT-modified electrode, the biosensor has high sensitivity and good stability, which is due to the advantages of nanostructured materials like CNT and the layer-by-layer assembly.

2. Experimental

2.1. Reagents and apparatus

Glucose oxidase (211 U mg^{-1}) was purchased from Amresco, and used without further purification. The solution of GOD was prepared in ultrapure water at a concentration of 10 mg mL^{-1}. Poly(ethylenimine) (PEI, MW 60,000) was dissolved in ultrapure water at a concentration of 3 wt.%. Stock solution of 1 M glucose was allowed to mutarotate for 24 h so that the α and β forms of D-glucose could reach a final stable ratio (Wang et al., 2006). Carbon nanotube with multiwall and diameter of about 20–30 nm was obtained from Shenzhen Nanotech Port Company. 0.1 M phosphate buffer solution (PBS, pH 7.0) was used as the supporting electrolyte. All other reagents were of analytical grade.

Electrochemical measurements were performed on a CHI660A electrochemical workstation (Chenhua Instrument Company of Shanghai, China) with conventional three-electrode cell. A bare glassy carbon (GC) electrode or the enzyme-modified electrode was used as the working electrode. A saturated calomel electrode (SCE) and a platinum wire were used as the reference and counter electrodes, respectively. All the potentials in this paper were in respect to SCE. Scanning electron microscopy (SEM) images were obtained on a GSM-6400F (Japan). Except the specific statement, the electrochemical measurements were carried out in a phosphate buffer solution at room temperature.

2.2. Preparation of the CNT-modified GC electrode

The multiwall CNT was first shorten and functionalized by sonicating CNT in a mixture of concentrated sulfuric acid–nitric acid (3:1, v/v) for about 3 h followed by extensive washing in deionized water until the filtrate was neutral. The treated CNT was dried in vacuum at 60 °C. The resulting black powder were sonicated in *N*,*N*-dimethylformamide (DMF) for about 1 h with a concentration of 1 mg mL^{-1}.

The glassy carbon electrode (3 mm diameter) was polished to a mirror-like surface with 1.0 and 0.3 μm alumina slurry and washed thoroughly with ultrapure water. Afterwards, a 5 μL of 1 mg mL^{-1} CNT suspension was dropped to fully cover the surface of the pretreated GC electrode and dried under an infrared lamp. Thus a thin film of CNT was formed to generate the CNT-modified electrode, and the surface was then washed carefully with ultrapure water.

2.3. Self-assembling GOD on the CNT-modified electrode

Glucose oxidase was immobilized on the negatively charged CNT surface by alternatively assembling PEI layers and glucose oxidase layers, as shown in Fig. 1A. Prior to it, the preparation conditions were optimized. And it was found that the immersing time of 10 min and 30 min was reasonable and optimal for the adsorption of positively charged PEI and negatively charged GOD, respectively. Based on the optimal preparation conditions, the positively charged polycation was first adsorbed by dipping the negatively charged CNT/GC electrode in PEI solution for 10 min (Fig. 1A-a). Then, the electrode was rinsed with water and dried in nitrogen to form the PEI/CNT/GC electrode. Secondly, the resulted PEI/CNT/GC electrode was immersed into a glucose oxidase solution (10 mg mL^{-1} at pH 7.0) for 30 min, where the negatively charged GOD (p*I* = 4.6) was adsorbed to the positively charged PEI surface through electrostatic interactions. The loosely attached GOD molecules were removed from the surface by a thorough rinse with ultrapure water, and the GOD/PEI/CNT/GC electrode was obtained (Fig. 1A-b). The desired number of bilayers (n) for $\{GOD/PEI\}_n$ films on the CNT/GC electrode can be controlled by repeating the above steps (Fig. 1A-c). Another PEI layer was adsorbed on the top of the GOD layer using the same procedure to improve the stability of the enzyme modified electrode (Fig. 1A-d). Thus, it may take ca. 2.5 h to obtain the PEI/$\{GOD/PEI\}_3$/CNT/GC electrode, which was time saving and convenient comparing with reports in literatures (Deng et al., 2009; Liu and Ju, 2003). The modified electrodes were kept at 4 °C in a refrigerator for storage when not in use. And for comparison, the GOD/CNT/GC electrodes were fabricated by immersing the CNT/GC electrodes in 10 mg mL^{-1} glucose oxidase solution for 30 min or 90 min, respectively.

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