Multilayer assembly of positively charged polyelectrolyte and negatively charged glucose oxidase on a 3D Nafion network for detecting glucose

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Received 29 September 2006; received in revised form 4 December 2006; accepted 10 January 2007
Available online 19 January 2007

Abstract

In this paper, a novel amperometric glucose biosensor was constructed by alternative self-assembly of positively charged poly(diallyldimethylammonium chloride) (PDDA) and negatively charged glucose oxidase (GOx) onto a 3D Nafion network via electrostatic adsorption. The amount of Nafion in the electrode and the number of the (PDDA/GOx) n multilayers were optimized to develop a sensitive and selective glucose biosensor. Under optimal conditions, the glucose biosensor with (PDDA/GOx) 5 multilayers exhibited remarkable electrocatalytic activity, capable of detecting glucose with enhanced sensitivity of 9.55 A/H262 M and a commendably low detection limit of 20 M (S/N = 3). A linear response range of 0.05–7 mM (a linear correlation coefficient of 0.9984, n = 20) was achieved. In addition, the glucose biosensor demonstrated superior selectivity towards glucose over some interferents, such as ascorbic acid (AA) and uric acid (UA), at an optimized detection potential of 0.6 V versus Ag/AgCl reference.

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Keywords: Layer-by-layer; Multilayer self-assembly; Glucose biosensor; Nafion; 3D network

1. Introduction

The development of electrochemical biosensors has progressed rapidly during the past decade with the apt combination of biological systems and the inherent advantages of advanced electrochemical transducers. To date, the most common glucose amperometric biosensors are based on the electrochemical detection of hydrogen peroxide produced by the enzymatic oxidation of glucose (Wang et al., 1998, 2003; Yao and Takashima, 1998; Lei et al., 2004; Topçu Sulak et al., 2006; Wu et al., 2006). Due to the concomitant electro-oxidation of many interference species, such as uric acid (UA) and ascorbic acid (AA), a variety of permselective polymer films have been investigated to prevent or alleviate the interference from endogenous electro-active species (Hoshi et al., 2001; Garjonyte and Malinauskas, 1999; Yuan et al., 2005). Among them, Nafion film owns high permselectivity towards anionic interferents by its negatively charged sulfonic functional group, receiving particular interests for the construction of biosensors (Mailley et al., 2000; Yuan et al., 2005; Schuvailo et al., 2006).

The glucose oxidase can be immobilized onto the electrode by physical adsorption (Losic et al., 2002), ionic and covalent bonding (Caruso and Schuler, 2000; Davis et al., 2003; Liu and Lin, 2006; Zhang et al., 2005), cross-linking (Iwuoha et al., 1997), and entrapment (Mitala and Michael, 2006; Shan et al., 2006). The sequential adsorption of oppositely charged ions by layer-by-layer (LBL) self-assembly is an effective method to prepare multilayer thin films with controlled thickness, unique mechanical properties, and ordered molecules (Jiang and Tsukruk, 2006). The simplicity and efficiency of this technique has resulted in wide applications of multilayer films in sensors/biosensors (Hodak et al., 1997; Suye et al., 2005; Zhao and Ju, 2006; Xu et al., 2006). In most cases, the multilayer films were LBL assembled on planar modified electrodes. For example, Au electrode was modified with 3-mercaptop-1-propanesulfonic acid to introduce negative charge on the Au surface (Zhao et al., 2005a). However, the results reported by Zhao et al. (2005a) showed that the resistance of LBL-assembled multilayer films increased due to the increase of the film thickness and the amount of non-conductive GOx. We believe therefore that LBL immobilization...
of GOx on a 3D network would significantly increase the GOx loading while still maintaining the advantages of LBL technique and subsequently improve the sensitivity of the enzyme electrode. However, to our knowledge, preparation of polyelectrolyte/GOx multilayer self-assembled films on a 3D network has been rarely reported.

The studies presented here are to report a new idea to develop a high sensitive and selective amperometric glucose biosensor, targeting for clinical application. Nanosized platinum supported on carbon black (hereafter named as PtC) is bonded with Nafion to serve as the porous electro-catalyst for the H2O2 oxidation owing to its high electrocatalytic activity, large surface area and unique chemical and physical properties (Antolini, 2004). Nafion plays multiple roles in this electrochemical glucose biosensors: (1) bonding and adhering the PtC catalyst onto the glassy carbon electrode; (2) eliminating the interference effects of some anionic interferents by charge exclusion; (3) acting as 3D platform for the LBL assembly of (PDDA/GOx)n multilayer films. This PDDA/GOx multilayered configuration on 3D Nafion network integrated the high electroactivity of the nanosized platinum, advantages of the LBL technique, and biocompatibility and permselectivity of Nafion.

2. Materials and experimental procedure

2.1. Reagents and instrument

Glucose oxidase (EC 1.1.3.4, from Aspergillus niger, 200 units/mg), β-D-(+)-glucose, ascorbic acid (AA), uric acid (UA) and poly(diallyldimethylammonium chloride) PDDA (MW = 200,000–350,000 g/mol) in 20% aqueous solution were purchased from Sigma–Aldrich and used without further purification. The PtC catalyst (nanosized platinum supported on carbon black with Pt loading of 50 wt.%) was purchased from Alfa. The buffer solutions were prepared from 0.08 M K2HPO4 and 0.02 M KH2PO4 (99.9%, Sigma–Aldrich) and 0.02 M KH2PO4 (99.9%, Sigma–Aldrich). All of the solu-
tions were prepared and kept overnight at room temperature before use to remove unassembled materials and then dried in N2 atmosphere. This sequence was repeated until the desired (PDDA/GOx)n multilayer films were obtained on the GCE/PtC/Nafion electrode. Multilayer films of (PDDA/GOx)n were also LBL self-assembled on the Nafion membrane in the same way described earlier. The morphology of the (PDDA/GOx)n multilayer films on the Nafion membrane was characterized by Atomic Force Microscope (AFM Nanoscope IIIa, Digital Instruments Inc.).

2.2. Preparation of the Nafion bonded PtC electrode

PtC catalyst was ultrasonically dispersed into 2-isopropanol for 30 min to form a uniform suspension with Pt concentration of 5 mg/mL. Six microliters of PtC suspension was coated on the surface of glassy carbon electrode and dried in air. Different amounts of Nafion solution (5 wt.%, Dupont) was then spread onto the PtC catalyst surface to form a GCE/PtC/Nafion electrode.

2.3. Immobilization of glucose oxidase on the 3D Nafion network supported by PtC catalyst

Layer-by-layer (LBL) self-assembly of PDDA and GOx on the negatively charged 3D Nafion network (supported by PtC catalyst) was conducted by alternatively dipping the GCE/PtC/Nafion electrode into a PDDA aqueous solution (10 mg/mL containing 0.5 M NaCl) and a glucose oxidase solution (10 mg/mL, in 0.1 M phosphate buffer solution pH 7.4) for 30 min. After each dipping step, the electrode was carefully washed with Milli Q water for three times to completely remove unassembled materials and then dried in N2 atmosphere.

3. Results and discussion

3.1. LBL self-assembly of (PDDA/GOx)n multilayer films

Before LBL self-assembling (PDDA/GOx)n multilayer films on the 3D Nafion network, the amounts of Nafion in the GCE/PtC/Nafion electrode were optimized in relation to the amperometric response of the electrode to 1 mM H2O2 and 0.5 mM AA (shown in Table S1 as Supporting Information). At applied potential of 0.6 V versus Ag/AgCl, KCl (3 M) electrode as reference and platinum foil as counter electrode. The amperometric response measurements were carried out at 0.6 V versus Ag/AgCl reference electrode under stirring. All measurements were carried out at room temperature (about 24 ± 2 °C) in phosphate buffer solution (PBS), pH 7.4. The biosensor was kept in refrigerator at 4 °C when not in use.

3.2. Characterization and amperometric measurement of the enzyme electrode

The electrochemical measurements were performed using the Autolab electrochemical analyzer (Autolab/PGSTAT30, ECO CHEMIE, The Netherlands). A conventional three electrode configuration was employed with a self-assembled enzyme electrode (Ø 2 mm) as working electrode, an Ag/AgCl, KCl (3 M) electrode as reference and platinum foil as counter electrode. The amperometric response measurements were carried out at 0.6 V versus Ag/AgCl reference electrode under stirring. All measurements were carried out at room temperature (about 24 ± 2 °C) in phosphate buffer solution (PBS), pH 7.4. The biosensor was kept in refrigerator at 4 °C when not in use.
alternatively self-assembled onto a charged substrate to form multilayer films. Because of an isoelectric point (pI) of 4.2, GOx is negatively charged in phosphate buffer solution (PBS), pH 7.4, so that it could be self-assembled onto a positively charged polyelectrolyte surface. A schematic diagram of the LBL self-assembly process of (PDDA/GOx)$_n$ multilayer films is shown in Scheme 1. The 3D Nafion network with negatively charged sulfonic functional group was acted as the platform for the multilayer assembly of positively charged PDDA and negatively charged GOx. Because the quantity of GOx can be precisely controlled by adding or reducing the layers of GOx by this LBL technique, hence the amperometric response can be controlled by different layers of GOx (Trau and Renneberg, 2003; Zhao et al., 2005b). Furthermore, large amount of GOx can be immobilized on the carbon black surface using LBL method since the nanosized carbon black has large surface area and the Nafion film can retain the bioactivity of GOx. This is obviously different from other LBL methods to prepare amperometric glucose biosensor in which a modified planar Au electrode was generally used as the substrate (Ram et al., 2000; Zhao et al., 2005a, 2005b; Yang et al., 2006).

Fig. 1a and b shows AFM images of Nafion membrane and (PDDA/GOx)$_n$ multilayer films on the Nafion membrane. The Nafion membrane surface was found to be rough and irregular (Fig. 1a). In contrast, after the adsorption of PDDA/GOx multilayers, a uniform layer of multilayer films with homogeneous distribution of “particles” was obtained, suggesting the alternative assembly of PDDA and GOx on the Nafion film surface (Fig. 1b).

3.2. Amperometric response of the (PDDA/GOx)$_n$ multilayer films modified GCE/PtC/Nafion electrode

The hydrodynamic voltammograms of the (PDDA/GOx)$_n$ multilayer films modified GCE/PtC/Nafion electrode in 2 mM glucose with the layers from 1 to 6 are shown in Fig. 2. It is found that the more the numbers of the multilayer films, the higher the response current will be in the studied potential range of 0.4–0.7 V versus Ag/AgCl reference electrode. The oxidation current reaches a plateau around 0.6–0.7 V. With the consideration of sensitivity, 0.6 V was chosen in the following study as the operating potential for the biosensor to detect glucose. As shown in the insert of Fig. 2, the response current does not increase linearly with the increase of the multilayer numbers at applied potential of 0.6 V. The current increment from one to two layers was 0.31 μA, which reduced to 0.05 μA from five to six layers. This phenomenon was also observed on the (PDDA–Prussian Blue/GOx)$_n$ multilayer films (Zhao et al., 2005b). The authors attributed it to the increased resistance of the LBL-assembled multilayers owing to the increase of the film thickness and the amount of nonconductive GOx. For the (PDDA/GOx)$_n$ multilayer films modified GCE/PtC/Nafion electrode, the enzymatically produced H$_2$O$_2$ should diffuse through the multilayer films and the Nafion film to reach electrocatalyst surface (nanosized Pt), then the oxidation of H$_2$O$_2$ could happen. The above phenomenon could be attributed to the additional diffusion resistance of H$_2$O$_2$ and glucose with the increase of the multilayer numbers.
The (PDDA/GOx)$_n$ multilayer films modified GCE/PtC/Nafion electrodes were tested in glucose to evaluate the performance of the biosensor at applied potential of 0.6 V. After the stabilization of the background current, different amounts of 1 M glucose solution was successively injected into the stirring PBS and the current was recorded continuously. Fig. 3 shows the steady state amperometric response of the (PDDA/GOx)$_n$ multilayer films modified GCE/PtC/Nafion electrode to the successive addition of glucose into the stirring PBS. A subsequent addition of glucose to the solution provoked a remarkable increase in the oxidation current. The response time was around 25 s for the biosensor to reach 95% steady state current. The long response time when compared to the planar electrode may be due to the increased diffusion resistance of H$_2$O$_2$ through the (PDDA/GOx) multilayers and Nafion film. As shown in the
insert of Fig. 3, the calibration curve for this glucose biosensor shows a linear response to the concentration of glucose in the range of 0.05–7 mM \((r=0.9984, n=20\) and the sensitivity \(= 9.55 \mu A/mM cm^2\), with a low detection limit of 20 \(\mu M\) (estimated based on \(S/N = 3\)). This sensitivity is noticeably higher than 0.2 \(\mu A/mM cm^2\) at self-assembled GOx/polypyrrole films (Ram et al., 2000), 3.9 \(\mu A/mM cm^2\) at Au nanoparticle–GOx multilayer biosensor (Zhao et al., 2005b) and 5.72 \(\mu A/mM cm^2\) at six layers of GOx/Colloidal gold nanoparticles biosensor (Yang et al., 2006).

### 3.3. Interference and repeatability of the glucose biosensor

The interference of 0.1 mM AA and UA was completely prevented and 0.5 mM AA only provoked current response of 0.04 \(\mu A\), accounting for 3.45% interference in a 2 mM glucose solution. The (PDDA/GOx)\(_n\) multilayer films modified GCE/PtC/Nafion electrode showed better selectivity than the GCE/PtC/Nafion electrode, in which 0.32 \(\mu A\) oxidation current was produced with addition of 0.5 mM AA at applied potential of 0.6 V, indicating that the LBL self-assembled (PDDA/GOx) multilayer films also can prevent the diffusion of interferents. The relative standard deviation (RSD) of the amperometric response to 2 mM glucose was 2.24% for the 10 successive measurements. The biosensor was kept in refrigerator at 4\(\circ\)C when not in use and tested once every week. The biosensor retained around 85% and 70% of its initial current response in 2 mM glucose after 1 and 4 weeks, respectively. The decreased current response may be due to the leaching of the enzyme from the electrode.

### 4. Conclusions

This paper reports a novel multilayer self-assembly technique to construct an amperometric glucose biosensor on a 3D Nafion network by using its negatively charged sulfonic acid group. With the combination of large specific surface area and perselectivity of the 3D Nafion network, a glucose biosensor with high sensitivity and selectivity was successfully demonstrated experimentally. Improvement on the highly positive performance of this kind of biosensor is under work, especially to reduce the response time and extend the upper linear detection limit, by optimizing the configuration of the (PDDA/GOx)\(_n\) multilayer films modified GCE/PtC/Nafion enzyme electrode.

### Acknowledgements

We thank Dr. Tian Ziqun for his valuable discussion on the nanosized platinum catalyst. This project is financially supported by the Singapore Millennium Foundation.

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2007.01.006.

### References