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Glucose sensing electrodes based on a poly(3,4-ethylenedioxythiophene)/Prussian blue bilayer and multi-walled carbon nanotubes

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ABSTRACT

Here we report a new glucose sensing electrode based on a poly(3,4-ethylenedioxythiophene) (PEDOT)/Prussian blue (PB) bilayer and multi-walled carbon nanotubes (CNT). The bilayer was prepared on a flexible screen-printed carbon electrode (SPCE) by sequential electrodeposition. The inner PB layer was electrodeposited first for detecting H_2O_2 from glucose oxidation; the outer PEDOT layer was electropolymerized on a baked or an unbaked PB film to entrap glucose oxidase (GOD). It was observed that the stability of PB in phosphate buffered saline (pH 7.4) was attained by post-deposition bake at 100 °C and the outer PEDOT layer both. In addition, a baked PB film enhanced the subsequent PEDOT growth and the corresponding GOD entrapment. As a result, the bilayer enzyme electrode showed highly resolved and reproducible signals (R.S.D. = 2.54%) to glucose samples from 100 μM to 1 M during a flow-injection analysis (FIA) at −0.1 V *vs.* Ag/AgCl. The sensitivity of the linear range (1–10 mM) was 2.67 μA cm^{-2} mM^{-1}. Moreover, the electrode retained *ca.* 82% of the original response after 1-month storage in PBS, pH 6.0 at 4 °C and could determine the glucose level in human serum precisely. Besides, it was found that CNT incorporation could further improve the sensitivity and could achieve μM-range glucose detection.

1. Introduction

Glucose detection is of great importance for a variety of biological purposes ranging from blood sugar monitoring for diabetes mellitus (American Diabetes Association, 1994) to kinetic assessment of cellulose saccharification for bioethanol production (Tatsumi et al., 2006). Inspired by the innovation of Clark and Lyons in the early 1960s (Clark and Lyons, 1962), a great diversity of amperometric glucose sensors have been proposed and commercialized based on an electrode immobilized with glucose oxidase (GOD, EC 1.1.3.4) (Wang, 2008). Glucose oxidase catalyzes the oxidation of glucose ($C_6H_{12}O_6$) to gluconolactone ($C_6H_{12}O_7$) with the assistance of oxygen and its prosthetic flavin group (FAD) as summarized below

$$\mathrm{GOD(FAD)} + \mathrm{glucose} + \mathrm{H_2O} \rightarrow \mathrm{GOD(FADH_2)} + \mathrm{gluconolactone} \quad (1)$$

$$\mathrm{GOD(FADH_2)} + \mathrm{O_2} \rightarrow \mathrm{GOD(FAD)} + \mathrm{H_2O_2} \quad (2)$$

Accordingly, glucose detection can be implemented by the measurement of O_2 depletion (Clark and Lyons, 1962), H_2O_2 production (Guilbault and Lubrano, 1973) or mediator-assisted FAD regeneration without oxygen (Degani and Heller, 1987). The latter two methods are ideally suited for developing disposable sensor strips for home blood glucose testing (Newman and Turner, 2005). In particular, the measurement of H_2O_2 is relatively simple—a glucose sensing electrode is fabricated after GOD is immobilized on an electrode surface that can oxidize or reduce H_2O_2. Hence, the electroactive materials that allow ultrasensitive H_2O_2 detection all have a great potential in glucose sensor development, and Prussian blue (PB, ferric hexacyanoferrate), a classical pigment but a relatively new electrocatalyst, is one of the known examples (Karyakin and Karyakina, 1999; Ricci and Palleschi, 2005).

Rivaling naturally existed peroxidases in H_2O_2 catalysis, PB has become an important class of biosensor materials since the successful demonstration of a first PB-based glucose sensor in the middle of the 1990s (Karyakin et al., 1995). In addition to the ease of preparation, PB allows cathodic detection of H_2O_2 at a very low potential (0 to −0.1 V *vs.* Ag/AgCl) and thus greatly

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enhances the immunity against the ascorbic acid interference during blood sugar testing. On the other hand, an electrodeposited PB film is prone to decomposition and exhibits poor cycling stability at physiological pH due to the attack of hydroxide ions (Ricci and Palleschi, 2005). To solve this pH-sensitive problem, an outer polymer film, like Nafion® (Karyakin et al., 1995), polyaniline (PAni) (Garjonyte and Malinauskas, 2000a) or polypyrrole (PPy) (Garjonyte and Malinauskas, 2000b), has been coated on the PB surface to serve as a protective layer and a matrix for GOD entrapment at the same time. In addition, when PB forms a composite with a conducting polymer such as PAni and PPy, the synergistic electrochemistry is observed (Hong et al., 2008). Thus, the conducting polymer–PB composite has been an interesting topic for the biosensor field (Karyakin and Chaplin, 1994; Garjonyte and Malinauskas, 2000a,b; Zou et al., 2007; Ernst et al., 2007).

As compared to the cases of PAni/PB and PPy/PB, little literature about the application of the poly(3,4-ethylenedioxythiophene) (PEDOT)–PB composite to a biosensor has reported so far (Ernst et al., 2007). Moreover, no literature reports a glucose sensor based on the PEDOT/PB composite, although PEDOT is anticipated to have several promising advantages in addition to entrapment of GOD and protection of PB. For instance, PEDOT can be electropolymerized in a phosphate buffered saline (PBS, pH 7.4) that helps to maintain GOD's activity while entrapping GOD (Piro et al., 2001; Fabiano et al., 2002; Nien et al., 2006, 2008). Besides, PEDOT has been proven to own better aqueous stability and biocompatibility than PPy and PAni, so it is considered a promising polymer appropriate for continuous sensing and even *in vivo* implantation (Kros et al., 2005; Luo et al., 2008). For these reasons, this work targets a high-performance glucose sensing electrode based on the PEDOT/PB bilayer. Fabrication of a PEDOT/PB bilayer enzyme electrode with high operating stability in PBS, pH 7.4 is studied and discussed from both the macroscopic and microscopic viewpoints. Since multi-walled carbon nanotubes (CNT) have high surface-to-volume ratios and possess electrocatalytic activity for H_2O_2 reduction like PB (Lim et al., 2005; Liu and Lin, 2006), we also investigate how the CNT incorporation enhances the performance of a PEDOT/PB-based enzyme electrode.

2. Experimental

2.1. Preparation of the enzyme electrodes

Three kinds of PEDOT/PB-based enzyme electrodes (A, B and C) were prepared on the flexible poly(ethylene terephthalate) (PET) substrate (150 μm in thickness). Their structural configurations are given below. Electrode A: SPCE/unbaked PB/PEDOT[GOD]; Electrode B: SPCE/baked PB/PEDOT[GOD]; Electrode C: SPCE/CNT/baked PB/PEDOT[GOD], where SPCE and CNT denote screen-printing carbon electrode and multi-walled carbon nanotubes, respectively. For all of the three enzyme electrodes, SPCE was formed on the PET substrate at first. The conductive carbon ink was screen-printed onto a flexible PET sheet (3.3 cm × 1.0 cm) precleaned with diluted HCl. Then the SPCE was cured at 130 °C for 1 h, and the post-cured SPCE had a sheet resistance of *ca.* $30 \pm 3.5\,\Omega\,sq^{-1}$. The printed pattern featured a circle working electrode (dia. = 6 mm; area = 0.283 cm^2) and a bar region for conduction (2.0 cm × 0.2 cm). The bar region was insulated by a screen-printed resin dielectric. For Electrode C, the SPCE was subsequently modified with CNT (multi-walled, external dia. = 20–40 nm, length = 5–15 μm) before PB electrodeposition. The modification was done by dropping 18 μL of an aqueous suspension containing 0.1 wt% carboxylated CNT on the active area of SPCE/PET (0.283 cm^2). The CNT-modified SPCE was then dried in air at room temperature. Multi-walled carbon nanotubes were carboxylated in a 3:1 (v/v) mixture of concentrated H_2SO_4/HNO_3 with sonication at 60 °C according to the literature (Zhang et al., 2008). The CNT modification was not performed for Electrode A and Electrode B.

For the formation of a PEDOT[GOD]/PB bilayer on SPCE, a PB thin film with a surface charge density of 1 mC cm^{-2} was electrodeposited on SPCE by applying a constant potential of 600 mV (*vs.* Ag/AgCl). The deposition bath was composed of 2 mM $K_3Fe(CN)_6$, 2 mM $FeCl_3$, 0.1 M KCl and 0.1 M HCl. The as-grown PB film was rinsed with 0.1 M HCl briefly and dried under nitrogen stream. For preparation of Electrode B and Electrode C, the PB film on SPCE/PET was baked at 100 °C for 1 h (referred to as "baked PB") since it was reported in the literature (Ricci et al., 2003) that 1-h baking at 100 °C could obtain a more stable and active layer of PB. Unbaked PB films also proceeded with the preparation of Electrode A for comparison. The PEDOT film was subsequently grown on the surface of PB in the presence of abundant GOD to result in a PEDOT[GOD] composite. The monomer solution was prepared by adding 10 mM 3,4-ethylenedioxythiophene (EDOT), 1 mM polyethylene glycol (PEG) 20,000 and 1000 unit mL^{-1} GOD into PBS, pH 7.4 (Nien et al., 2006). The electropolymerization was done by sweeping the potential between 0.2 and 1.1 V (*vs.* Ag/AgCl) at a scan rate of 100 mV s^{-1} for 15 cycles. After a brief rinse with PBS, the sensing electrodes were prepared and were then stored in PBS, pH 6.0 at 4 °C prior to use. (Note: 20 mM phosphate buffer, made of Na_2HPO_4 and NaH_2PO_4, was added with 150 mM KCl to make up the phosphate buffered saline, PBS).

2.2. Characterization of the enzyme electrodes and their sensing performance

The surface microstructures of the three enzyme electrodes were characterized with a scanning electron microscope (SEM). Before SEM characterization, the three enzyme electrodes were aged by 200-s continuous FIA detection of 10 mM glucose in order to enlarge the structural differences. Cyclic voltammetry (CV) was used to assess the cycling stability of the three electrodes at physiological pH. The electrodes were subject to a successive 100-cycle CV test in PBS, pH 7.4 at a scan rate of 50 mV s^{-1}, and the CV curves ranged between +0.35 and −0.05 V (*vs.* Ag/AgCl) were measured. For evaluation of glucose sensing performance, a homemade FIA device was employed (see Fig. S1 in Supplementary Material). The carrier PBS solution (pH 7.4) was pumped into the FIA device at 1 mL min^{-1}. The FIA device was used to analyze successive injections of 150 μL H_2O_2, glucose and human serum samples in conjunction with a potentiostat/galvanostat that exerting a sensing potential at −0.1 V (*vs.* Ag/AgCl) (Ricci and Palleschi, 2005). Triplet injections were performed for each concentration to determine the average reading with a relative standard deviation (R.S.D.). The details of materials and apparatus are provided in Supplementary Material.

3. Results and discussion

3.1. Microstructural comparison between the enzyme electrodes

There is no difference in appearance between the three enzyme electrodes (A, B and C). They all show flexibility as the picture presented in Fig. 1. This makes the enzyme electrodes ideally suited for the development of flexible biosensors that have been investigated for *in vivo* implantation (Yan et al., 2007), wearable sensing devices (Mitsubayashi et al., 2003; Kudo et al., 2006; Iguchi et al.,