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An amperometric glucose biosensor based on layer-by-layer GOx-SWCNT conjugate/redox polymer multilayer on a screen-printed carbon electrode

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ABSTRACT

An amperometric glucose biosensor based on a multilayer made by layer-by-layer assembly of single-walled carbon nanotubes modified with glucose oxidase (GOx-SWCNT conjugates) and redox polymer (PVI-Os) on a screen-printed carbon electrode (SPCE) surface was developed. The SPCE surface was functionalized with a cationic polymer by electrodeposition of the PVI-Os, followed by alternating immersions in anionic GOx-SWCNT conjugate solutions and cationic PVI-O solutions. The purpose is to build a multilayer structure which is further stabilized through the electrodeposition of PVI-Os on the multilayer film. The electrochemistry of the layer-by-layer assembly of the GOx-SWCNT conjugate/PVI-Os bilayer was followed by cyclic voltammetry. The resultant glucose biosensor provided stable and reproducible electrocatalytic responses to glucose, and the electrocatalytic current for glucose oxidation was enhanced with an increase in the number of bilayers. The glucose biosensor displayed a wide linear range from 0.5 to 8.0 mM, a high sensitivity of 32 $\mu A\,mM^{-1}\,cm^{-2}$, and a response time of less than 5 s. The glucose biosensor proved to be promising amperometric detectors for the flow injection analysis of glucose.

1. Introduction

The use of effective enzyme-based biosensors is vital in many relevant applications [1] such as quality control in the food industry and amperometric glucose biosensing, a widely used testing tool for diabetes, in the medical industry. Glucose biosensors account for about 85% of the entire biosensor market [2]. However, tailoring the electrode surface to construct a sensitive and cheap biosensor remains a challenge [3–5]. In amperometric biosensors, the immobilization of the enzyme and the electrical communication among the redox centers of enzymes and electrodes is important [6,7].

A number of methods have been proposed to immobilize a redox enzyme on the electrode surface, concomitantly preserving enzymatic activity, designing efficient electron-transfer pathways between the immobilized enzyme and the electrode surface, and preventing unspecific side reactions [8,9]. One of the requisite features for the formation of well-ordered and defined biosensors is the capability to tailor the organization of sensor components as close to the molecular level as possible [10,11]. To meet this requirement, organized enzyme assemblies deposited on electrode surfaces have been described using different methods such as Langmuir–Blodgett [12], self-assembled monolayers [13], antigen–antibody recognition [14], avidin–biotin interaction [15], and electrostatic adsorption of hyperbranched multilayers [16]. Among these immobilization methods, adsorption is the simplest. Adsorption can retain well the bioactivities of the immobilized enzyme because the reaction needs no chemical reagents. Biosensors based on the adsorption of an enzyme, however, are limited by the amount of the immobilized enzyme on the electrode surface and are unstable. The sensitivity of the biosensor is directly proportional to the enzyme surface density on the electrode [17], so an increase in enzyme loading on the biosensor surface may result in a significant improvement in its sensitivity. An enzyme is a natural polyelectrolyte, so the alternate layer-by-layer (LBL) deposition technique, which involves multilayer buildup onto a solid substrate that exploits the electrostatic attraction between oppositely charged molecules, has been widely used to increase enzyme content on the electrode surface [18].

More enzymes may be immobilized on the electrode through LBL, but a thicker composite film also increases electron transfer resistance and slows down substrate diffusion [19]. To develop more sensitive biosensors, the incorporation of nano-sized materials into a sensing device has been extensively investigated [20,21]. Since their discovery in 1991, carbon nanotubes (CNTs) have led to many new technical developments and applications due to their high chemical stability, wide surface area, and unique electronic

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properties [22,23]. In particular, electrochemical biosensors made from single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) have fast electron-transfer kinetics [24,25]. Recent studies reported that incorporation of SWCNTs [26] or MWCNTs [27] into LBL films containing glucose oxidase (GOx) increases sensor response by enhancing electron diffusion through the films and/or the electrochemical surface area. Further, physical adsorption of the enzyme onto nanotubes for biosensor development has also attracted substantial research attention [28–30]; GOx adsorbed onto CNTs has been reported to reduce the distance between the FAD center and the electrode surface. CNTs may also penetrate closer to the FAD center due to their small diameter which allows improved access to the FAD center via redox mediator [31,32]. A homogeneous and porous composite film that facilitates substrate transfer may usually be obtained on the electrode surface using CNTs as a nano-sized backbone [33].

The redox polymer based on osimium $(bpy)_2$-complexed poly(4-vinylpyridine) (PVP) or poly (1-vinylimidazole) (PVI) (bpy = bipyridine or its derivative) was pioneered mainly by Heller and Feldman and has been extensively used for the "wiring" of redox enzymes [34]. Electrostatic complexes of the polycation redox polymer and enzyme have been utilized to generate amperometric biosensors with high current densities [35]. Complex samples of this type have also been utilized in LBL deposition approaches for the development of glucose biosensors [36]. Screen-printed carbon electrodes (SPCEs) are of particular importance because of their simplicity, low cost, and ease and speed of preparation. Therefore, these electrodes are very suitable for the fabrication of biosensors [37].

The present work prepared GOx-SWCNT conjugates by adsorbing the redox enzyme GOx onto SWCNT. The conjugates were incorporated into redox polymer hydrogels using the LBL method on SPCE to obtain a glucose biosensor. The analytical performance of the resulting glucose biosensor was evaluated in terms of sensitivity, reproducibility, and stability. The determination of glucose using the biosensor was also performed in a flow injection analysis (FIA) system.

2. Materials and methods

2.1. Chemicals and reagents

Glucose oxidase (GOx, EC 1.1.3.4 from *Aspergillus niger*) was purchased from Sigma–Aldrich and used without further purification. 1-Vinylimidazole, 2,2′-dipyridyl (bpy) and sodium dithionite were purchased from Sigma–Aldrich. K_2OsC1_6 and ethylene glycol were purchased from Acros. 2,2′-Azobis(isobutyronitrile) (AIBN, Polysciences) was purified by double recrystallization from methanol and stored at −20 °C. Os-$(bpy)_2C1_2$ was prepared using reported method [38]. Poly(1-vinylimidazole) (PVI) and the redox polymer, designated as PVI-Os, were synthesized according to a previously published protocol [39]. Carbon ink (Electrodag 423SS) was obtained from Acheson Colloids (Japan).

Single-walled CNTs (SWCNTs) were obtained from Shenzhen Nanotech Port Co. Ltd. (China) and the as-received MWCNTs were treated in an established way with slight modification [40]. The SWCNTs (50 mg) were purified by refluxing in 2 M HNO_3 (100 mL) for 48 h. This was followed by several ultracentrifugations and drying in a vacuum. The SWCNTs were then shortened by sonicating in a mixture of HNO_3 and H_2SO_4 (v/v, 1:3) for 6 h, followed by extensive washing in deionized water until the filtrate was neutral.

Unless otherwise stated all chemicals and reagents used are of analytical grade. Buffers were prepared using water from a Milli-Q ultra-pure water system. 0.02 M phosphate buffer (PB) was prepared by mixing the stock solution of potassium dihydrophosphate and sodium hydroxide and then adjusted to required pH with hydrochloric acid. Glucose solution was made from 1 M stock solution that has been left to maturate overnight and diluted with a phosphate buffer to expected concentration.

2.2. Apparatus

Amperometric and cyclic voltammetric (CV) experiments were performed with a CHI 832 (Shanghai, China). All experiments were carried out with a conventional three-electrode system with the screen-printed carbon electrode as working electrode, a platinum foil as counter electrode, and an Ag/AgCl (saturated potassium chloride) as reference electrode. A thermostated bath was used to control the reaction temperature at 25 ± 1 °C.

X-ray photoelectron spectroscopy (XPS) measurements were conducted with an ESCALAB MK II spectrometer (VG Co., U.K.) with a Mg Kα radiation ($h\gamma$ = 1253.6 eV) as the X-ray source and a pass energy of 20 eV. The pressure inside the analyzer was maintained at 6.5×10^{-7} Pa.

The flow injection system comprised an ISIF-C brainpower flow injection analyzer (Ruimai Company, Xi'an, China) equipped with two 3-channel peristaltic pump and a eight-port rotary injection valve with 50 μl of loops, and a home-made electrochemical flow cell [41]. Peak height of the oxidation current was used as a signal and three injections were made for each concentration of glucose standard.

2.3. Preparation of GOx-SWCNT conjugates

GOx-SWCNT conjugates were prepared according to a previously published protocol [42]. A suspension of SWCNTs was made by adding 3 mg of as-prepared nanotubes to 7 mL of 0.5 M NaCl solution. Next the mixture was sonicated for 40 min to disperse the SWCNTs. The SWCNTs solution was then centrifuged at 30,100 × *g* for 1 h at temperature 4 °C, and the supernatant containing a high fraction of individual SWCNTs was retained. Sodium phosphate was added to 5 mL of supernatant SWCNTs solution to give a concentration of 20 mM. Next 15 mg of GOx was added to the SWCNTs solution and mixed well. The sodium chlorate that was displaced as the GOx adsorbed onto the SWCNTs was removed by dialysis with a 10 kDa dialysis membrane and 1.5 L of a 20 mM phosphate buffer (pH 7.4) for 12 h at 4 °C. Next the resulting GOx-SWCNT conjugates solution was transferred to a 300 kDa dialysis membrane and dialyzed at 4 °C to remove any unabsorbed GOx. The dialysis buffer (20 mM phosphate, pH 7.4) was changed frequently (3, 6, 9, and 24 h). The resulting suspension was centrifuged at 30,100 × *g* for 1 h at 4 °C and the supernatant was retained.

2.4. Fabrication of the biosensor

The screen-printed carbon electrode (SPCE) used as a base electrode was prepared by the method described in the paper [43]. Solutions of PVI-Os (0.7 mg mL^{-1} in PB) and GOx-SWCNT conjugates were used for sensor construction. To facilitate the adsorption of the negatively charged GOx-SWCNT conjugates, the SPCE was functionalized by electrodeposition of a layer of PVI-Os on the electrode surface. The electrode was then removed and washed with deionized water. The resulted electrode was subsequently immersed in a solution of GOx-SWCNT conjugates for 40 min, followed by washing with deionized water to remove any excess material, and then immersed in PVI-Os solution for 20 min. Multilayer film of GOx-SWCNT conjugates/PVI-Os was then created by repeated alternating exposure to the GOx-SWCNT conjugates solution and PVI-Os solution. The last layer of the multilayer is PVI-Os layer.