Biosensors and Bioelectronics 24 (2009) 3131–3134

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Fabrication of a novel layer-by-layer film based glucose biosensor with compact arrangement of multi-components and glucose oxidase

Shanmugasundaram Komathi [a], Anantha Iyengar Gopalan [a,b], Kwang-Pill Lee [a,b,*]

[a] Department of Chemistry Graduate School, Kyungpook National University, Daegu 702-701, South Korea
[b] Department of Nanoscience and Nanotechnology, Kyungpook National University, Daegu 702-701, South Korea

ARTICLE INFO

Article history:
Received 22 January 2009
Accepted 9 March 2009
Available online 19 March 2009

Keywords:
Layer-by-layer
Thiol functionalized polyaniline
Gold nanoparticles
Multi-wall carbon nanotube
Glucose oxidase
Biosensor

ABSTRACT

Layer-by-layer (LbL) film based glucose biosensor was fabricated with alternative layers of a nanocomposite (comprising of multiwalled carbon nanotubes (MWNTs), Au nanoparticles (Au NPs) and thiol functionalized polyaniline (PANI(SH)) and glucose oxidase (GOx). The successful formation of multilayers was confirmed by UV–visible spectroscopy. The components in the nanocomposite provide adequate electron transfer path between GOx and the electrode. A high value for the rate constant of electron transfer process (27.84 s^{-1}) was observed at $\{GOx/Au\text{-}(SH)PANI\text{-}g\text{-}MWNT\}_n$/ITO electrode. The $\{GOx/Au\text{-}(SH)PANI\text{-}g\text{-}MWNT\}_n$ biosensor exhibited high sensitivity (3.97 μA/mM) for the detection of glucose over a concentration range of 1–9 mM with a low detection limit of 0.06 μM.

1. Introduction

One of the key aspects in the development of biosensors is the effective immobilization of the enzyme onto the surface of electrode. In recent years, various methodologies have been developed for the immobilization of enzyme into a suitable matrix as well for the construction of biosensors (Li and Lin, 2007; Manesh et al., 2008a,b). Among the various approaches to fabricate biosensors, layer-by-layer (LbL) deposition offers a few striking advantages, such as, precise control of composition, thickness of film, wide choice of materials, simplicity of procedure (Decher, 1997) and direct electron transfer (DET) between enzyme and underlying electrode.

Originally, the formation of LbL film is based on alternate deposition of oppositely charged polyelectrolytes from solution (Decher, 1997). LbL technique has been extended to fabricate biosensors with enzymes (Shi et al., 2006) and nanomaterials like multiwalled carbon nanotubes (MWNTs) (Wang et al., 2004; Cui et al., 2008). Recently, MWNTs were combined with gold nanoparticles (Au NPs) (Wu et al., 2007a) or an insulating polymer (Wu et al., 2009) to form LbL assembly with glucose oxidase (GOx). Also, the combination of GOx, Au NPs, an insulating polymer and LbL assembly has been utilized for fabricating a glucose sensor (Wu et al., 2007b). However, the presence of insulating polymer in the above-mentioned combinations may partially block the electrode surface and limits the electron transfer path to the electrode. On the contrary to insulating polymers, conducting polymers (CPs) are attractive for the development of enzyme based electrochemical biosensors with fast electron transfer at the electrode. The combined presence of MWNTs and CP synergistically influence the performance of biosensors (Manesh et al., 2008a,b). Au NPs with their good biocompatibility provide an environment equivalent to that of redox proteins in native system (Feng et al., 2005). However, literature reveals that the fabrication of a biosensor with combined presence of MWNT, a CP, metal nanoparticles and an enzyme in a LbL film has not been reported so far.

One of the problems associated with electrochemical biosensors is the leaching of the enzyme from the substrate during the electrode operation. The leaching of enzyme could be controlled by either anchoring the enzyme into the matrix through molecular interactions or sandwiching them in between two stable matrices. It is possible to load a large amount of enzyme in a multilayer configuration.

In the present work, we intend to exploit the synergistic advantages of MWNTs, Au NPs, CPs, and LbL assembly for achieving effective electron shuttling between the electrode and GOx. A new strategy was adapted to form LbL assembly consisting of alternate layers of a nanocomposite and GOx. Towards this purpose, thiol functionalized polyaniline (PANI(SH)) was grafted onto MWNTs

* Corresponding author at: Department of Chemistry Graduate School, Kyungpook National University, Daegu 702-701, South Korea. Tel.: +82 53 950 5901; fax: +82 53 952 8104.
E-mail addresses: algopal_99@yahoo.com (A.I. Gopalan), kplee@knu.ac.kr (K.-P. Lee).

0956-5663/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2009.03.013

and subsequently loaded with Au NPs to obtain the nanocomposite Au/(SH)PANI-g-MWNT. LbL film was successfully constructed by inducing electrostatic interactions between positively charged Au/(SH)PANI-g-MWNT and negatively charged GOx. The {GOx/Au-(SH)PANI-g-MWNT}$_n$ (n = number of stacks) LbL film based glucose biosensor (Scheme 1) exhibits excellent features for the electrochemical detection of glucose.

2. Experiment

2.1. Chemicals

MWNTs were purchased from CNT Co. Ltd., Incheon, Korea. Aniline, 4-amino thiophenol(4-ATP), gold(III) chloride, 1-butyl 3-methyl immidazolium tetrafluoro borate (ionic liquid, IL), GOx, glucose and phosphate buffer were purchased from Aldrich (USA). Indium doped tin oxide (ITO) coated glass plate (specific resistance of ~30 Ω, Corning Inc., USA) was used. Before performing each experiment, the ITO plate was thoroughly rinsed with acetone and washed with distilled water. For the LbL assembly, a constant area (1 cm^2) was used by masking the rest of surface with laquer.

2.2. Apparatus

Cyclic voltammetry, electrochemical impedance spectroscopy (EIS) and amperometric measurements were carried out with Iviumstat and Compactstat (Netherland) using a three electrode cell set up comprising Ag/AgCl/3M KCl (reference), Pt wire (counter) and LbL film (working) electrodes. Cyclic voltammetry measurements were made in quiescent solution at a scan rate of 100 mV/s in phosphate buffer solution (PBS, pH 6.8). Electrochemical impedance spectra (EIS) were recorded in the presence of $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) in the frequency range between 0.1 Hz and 100 kHz with a perturbation signal of 10 mV under open circuit conditions. Amperometric experiments were performed in PBS (pH 6.8) at a constant potential of +0.40 V (vs. Ag/AgCl/3M KCl). Glucose solution (20 μL) was successively added after the current reached a steady value. UV–visible spectra were recorded using a fast scan Varian CARY 50 (Australia) spectrophotometer in the wavelength range from 300 to 800 nm. The leaching of GOx from {GOx/Au-(SH)PANI-g-MWNT}$_{10}$ electrode to the electrolyte solution was tested by monitoring the enzyme (GOx) activity of electrolyte solution through dianisidine method (Sigma Technical Bulletin, 1983; Zhu et al., 2005). Field emission scanning electron microscopy (FESEM) observations were carried out with Pt coated vacuum ion sputter with a field emission gun operated at 15 kV (HITACHI-S4800).

2.3. Fabrication of {GOx/Au-(SH)PANI-g-MWNT}$_n$/ITO biosensor

The fabrication of {GOx/Au-(SH)PANI-g-MWNT}$_n$/ITO biosensor consists mainly of three steps (Scheme 1).

- *Step 1*: 10 mg of amine functionalized MWNT (MWNT-NH_2) (Santhosh et al., 2006) was dispersed in an aqueous solution (0.9 ml) (containing aniline (90 mM), 4-ATP (10 mM)) and IL (0.1 mL) and sonicated for 30 min. 20 μL of the slurry was drop coated onto ITO electrode and dried at room temperature.
- *Step 2*: (a) The coated electrode was immersed in 1 M HCl and electropolymerization was performed by cycling the potential between 0.00 and 0.80 V (vs. Ag/AgCl/3M KCl) at a scan rate of 100 mV/s. During electrochemical polymerization, chains of polyaniline (PANI) consisting of 4-ATP units (designated as PANI(SH)) were grafted onto the surface of MWNTs. Thus, film of MWNT-g-PANI(SH) was obtained (b). Au NPs were deposited onto MWNT-g-PANI(SH) by applying a potential of −0.30 V for 2 min. The Au-(SH)PANI-g-MWNT/ITO electrode was kept at +0.80 V for generating positive charges onto PANI(SH) backbone (Ragupathy et al., 2008).
- *Step 3*: GOx was immobilized onto Au-(SH)PANI-g-MWNT/ITO (+0.80 V) by placing the electrode into a solution of GOx (1 mg/ml) in phosphate buffer (PBS, pH 6.8) for 2 min to obtain GOx/Au-(SH)PANI-g-MWNT/ITO electrode.

Steps 1, 2 and 3 were repeated sequentially for (n − 1) times and the biosensor {GOx/Au-(SH)PANI-g-MWNT}$_n$ was fabricated.

3. Results and discussion

Cyclic voltammograms (CVs) were recorded (Supporting information: S1) during the deposition of MWNT-g-PANI(SH) on to the surface of ITO. The current values at anodic (0.40 V) and cathodic (0.20–0.30 V) peaks increased steadily with number of potential cycles, which signify the build-up of MWNT-g-PANI(SH) film on the surface of ITO (Gopalan et al., 2006). In the subsequent step, Au NPs were electrodeposited onto MWNT-g-PANI(SH) film. Au NPs are anchored onto MWNT-g-PANI(SH) film due to their interactions with –SH groups in PANI(SH) (Showkat et al., 2007). FESEM image of MWNT-g-PANI(SH) (Supporting information: S2) shows a layer of PANI(SH) over the surface of MWNTs. The average thickness of PANI(SH) was around 15 nm. Au NPs could be seen as white dots distributed on the surface of PANI(SH) grafted MWNTs. EDAX analysis reveals that nearly 25 at.% of Au are present in the MWNT-g-PANI(SH)-Au composite. Though the sizes of Au NPs could not be precisely known, the sizes of Au NPs are estimated to be lesser than 10 nm.

Further, the potential of Au-(SH)PANI-g-MWNT/ITO was kept at +0.80 V to convert the amine groups in PANI(SH) to imine sites and to generate positive charges in Au-(SH)PANI-g-MWNT. The negatively charged GOx was immobilized onto Au-(SH)PANI-g-MWNT film through electrostatic interactions.

The successful formation of {GOx/Au-(SH)PANI-g-MWNT}$_n$ LbL film was confirmed by UV–visible spectroscopy. The absorbance corresponding to polaronic band (420 nm) of PANI(SH) (Huang et al., 2006) showed a linear increase with number of layers (n) in LbL film (Supporting information: S3). This signifies the build-up of multiple layers. A hump that appears around 520 nm corresponds to the plasmon resonance of Au nanoparticles in the LbL film. The increase in absorbance values at 420 nm as well in 520 nm with 'n' confirms that layers in {GOx/Au-(SH)PANI-g-MWNT}$_n$ are well packed and adhered onto the surface of ITO. The electrostatic interactions between positive charges of amine or imine groups in PANI(SH) and GOx as well as the effective binding of Au particles through –SH groups in PANI(SH) are expected to provide compactness and stability to the LbL assembly. There was no leaching of GOx to the electrolyte for a long period (10 days). This was ensured by keeping the ITO/{GOx/Au-(SH)PANI-g-MWNT}$_n$ in the electrolyte solution for a definite period and following the enzyme activity of electrolyte solution by dianisidine test (Sigma Technical Bulletin, 1983; Zhu et al., 2005) using UV–visible spectroscopy (Supporting information: S4). This ensures long-term stability of LbL film in PBS.

The electroactivity of ITO/{GOx/Au-(SH)PANI-g-MWNT}$_{10}$ was tested using $Fe(CN)_6^{3-/4-}$ as the redox marker. CVs of ITO/{GOx/Au-(SH)PANI-g-MWNT}$_{10}$ were recorded at a scan rate of 100 mV/s for a solution of $Fe(CN)_6^{3-/4-}$ in PBS (pH 6.8) (Fig. 1). The $Fe(CN)_6^{3-/4-}$ redox processes were observed with a separation of ~64 mV between the anodic (160 mV) and the cathodic (224 mV) peaks. CVs of ITO/{GOx/Au-(SH)PANI-g-MWNT}$_{10}$were also recorded for different scan rates (10–1000 mV/s) (Fig. 1). The linear dependence of peak current on scan rate (Fig. 1, inset) implies that the electron transfer process at the electrode is a surface confined one. The surface coverage (Γ) of the electroactive component