Construction of Amperometric Glucose Sensor using Carbon Nanotubes and IO$_4$-oxidized GOx

YingYing Sun, Jie Wang and Xin Ge

ABSTRACT

Multi-wall carbon nanotubes (MWNTs) functionalized with amino groups were prepared via silane treatment. The resulting amino terminated MWNTs (AMWNTs) exhibited an improvement of dispersion in water, which was clearly indicated by changes visible to the naked eye. Here, AMWNTs were applied to construct glucose biosensors with IO$_4$-oxidized glucose oxidase (IO$_4$-oxidized GOx) through the layer-by-layer (LBL) covalent self-assembly method without any cross-linker. The marked decrease in the overvoltage for the reduction of dissolved oxygen facilitated convenient low-potential stable detection of the glucose. The enzyme electrode exhibited good electrocatalytic response toward the glucose and that the response increased with the number of GOx/AMWNTs bilayers, suggesting that the analytical performance such as sensitivity and detection limit of the glucose biosensors could be tuned to the desired level by adjusting the number of deposited GOx/AMWNTs bilayers.

Introduction

Rapid, selective and accurate determination of glucose concentration is very important in clinical, biological and chemical samples [1]. Glucose oxidase (GOx) has been widely used in the construction of glucose biosensor by virtue of the high selectivity to glucose and high activity over broad range of pH values. Recently, the combination of MWNTs and GOx is receiving considerable interest, since studies have proven that MWNTs is a very suitable matrix to trap GOx for glucose biosensors with fast response time, high sensitivity, and great versatility in analytical tools [2].

Layer-by-layer (LBL) self-assembly based on molecular recognition has emerged as the most popular and versatile approach for the fabrication of organized multilayer films [3]. Recently, this technique has found wide applications in preparation of MWNTs/GOx multilayer biosensors. Despite successful fabrication has been realized, the driven force in those LBL assembly processes is electrostatic interaction, which certainly leads to the poor stability and the limited application. Besides, the cross-linkers employed to combine MWNTs with GOx increase the distance between the FAD center of GOx and nanotubes, which results in reduced electron transfer rate and thus the poor analytical properties. To overcome these drawbacks, directly LBL
covalent attachment of GOx on MWNTs without any cross-linker for fabricating ordered multilayer films is a practical method.

In this paper, we report the chemical modification of MWNTs by covalent bonding of an amino terminated silane (3-aminopropyltrimethoxysilane, APS) onto activated carbon nanotubes. By using silane-coupling chemistry, the amino terminated MWNTs (AMWNTs) maintained the carbon skeleton structure, while grafting new functional groups. The resultant AMWNTs were uniformly dispersed in water and exhibited no aggregation over several weeks, indicating the introduction of amino groups greatly increased the hydrophilicity of MWNTs. Here we applied such AMWNTs to the construction of glucose biosensors. LBL method was employed for direct attachment of IO$_4^-$-oxidized glucose oxidase (IO$_4^-$-oxidized GOx) on the AMWNTs, based on the covalent Schiff-base bonding between the aldehyde groups of IO$_4^-$-oxidized GOx and amino groups of AMWNTs. In this way, the cross-linkers, PDDA and glutaraldehyde were avoided, and the thickness of multilayer films was in nanometer. The resulting electrode showed a porous structure and exhibited outstanding bioelectrocatalytic response to the oxidation of glucose and very effective electrochemical communication between the successive layers.

**Experimental**

Glucose oxidase (GOx, EC 1.1.3.4, from Aspergillus niger, 100 U mg$^{-1}$) was purchased from Amersco. 3-aminopropyltrimethoxysilane (APS, 99%), Cystamine dihydrochloride (CA) and ferrocenemethanol were obtained from Aldrich. Multi-wall carbon nanotubes (MWNTs, >95%, 10-30 nm diameter) came from Shenzhen Nanotech Port Ltd. Co. (Shenzhen, China). The other chemicals were of analytical grade and were used as supplied.

The process started by oxidizing as received MWNTs in 10 M H$_2$SO$_4$ containing 0.04 g mL$^{-1}$ K$_2$Cr$_2$O$_7$ for 30 min at 80 $^\circ$C. Then, 10.0 mg OMWNTs was dispersed by ultrasonication in 20.0 mL of ethanol. Then 1.0 mL of 1.0 wt% ethanol solution of APS was added and stirred for 12 h at 40 $^\circ$C for silanization. The amount of APS used was 1:1 in weight with respect to the OMWNTs. Thereafter, the resultant amino terminated MWNTs (AMWNTs) were washed with water three times by resuspension/centrifugation to ensure removal of excessive APS.

The layer forming process started with the introducing of amine functionalities on the Au surface by dipping a clean Au electrode into an aqueous solution of cystamine (10 mg mL$^{-1}$) in darkness for 2 h. After thiol adsorption, LBL assembly of GOx/AMWNTs was conducted by alternatively dipping the modified electrode into the IO$_4^-$-oxidized GOx solution and the above AMWNTs solution each for 1 h. After each dipping step, the electrode was carefully washed with distilled water for three times to remove the excess of assembling materials and then dried with nitrogen. This sequence was repeated until the desired GOx/AMWNTs bilayer number was obtained.

**Results and discussion**

The success of the attachment was confirmed by FTIR, as shown in Fig. 1. For the OMWNTs (Fig. 1a), the bands at 1730 and 1380 cm$^{-1}$ were attributed to the C=O stretching and O-H bending vibration presented in carboxylic groups [4], while the stretching vibrations of –OH and C-OH bands appeared at 3440 and 1180 cm$^{-1}$ [5], respectively. This confirmed that many carboxylic and hydroxyl groups were present on the OMWNT. After the silane treatment, the IR spectrum changed remarkably, showing the disappearance of the bands at 3440 and 1180 cm$^{-1}$ (Fig. 1b) and a
corresponding appearance of bands at 3424, 1586 and 1226 cm$^{-1}$ due to the stretching mode of N-H bond and in-plane vibration mode of the N-H and C-N bonds [6], respectively. This phenomenon undoubtedly demonstrated that APS molecules reacted with the hydroxyl groups of OMWNTs and hydrophilic amino groups were introduced on the MWNTs. In addition, the appearance of new band at 1110 cm$^{-1}$ that was corroborated to Si-O-C stretching further supported the chemical interactions between silane and MWNTs.

![FTIR spectra of (a) OMWNTs and (b) AMWNTs.](image)

Figure 1. FTIR spectra of (a) OMWNTs and (b) AMWNTs.

The need for oxygen reduction at catalytic surfaces has been recognized in fuel cells, batteries, and many other electronic applications. Hence, oxygen reduction at nanotube surfaces is of great interest. Fig. 2 depicts cyclic voltammograms for dissolved oxygen reduction at the Au/CA/(GOx/AMWNTs)$_4$ and bare Au electrodes in 0.1 M PBS. When the Au/CA/(GOx/AMWNTs)$_4$ was in N$_2$-saturated solution, no redox peak in the potential range 0.2 to $-0.8$ V were observed (curve a). While in the presence of O$_2$, a remarkable catalytic reduction peak appeared at $-0.28$ V (curve c), which was documented to be a two-electron reduction process to produce hydrogen peroxide. As a control, the reduction of oxygen on the bare Au electrode resulted in an ill-defined peak at about $-0.55$ V with smaller current, shown in Fig. 2b. Compared with the bare electrode, the Au/CA/(GOx/AMWNTs)$_4$ not only improves the redox currents of O$_2$ but also decreases the overvoltage by at least 0.28 V, which indicates a more facile reaction occurring at the multilayer films. This result is consistent with literature for other CNTs modified electrode, demonstrating that the present strategy of using the covalent functionalization does not affect the inherent electrochemical properties of CNTs, and CNTs retain excellent electrocatalytic ability towards O$_2$. The larger lowering of the overvoltage observed in the presence of CNTs is attributed to the oxide defects on the
tube sidewalls and ends which can be involved in the redox reaction and makes the CNTs more hydrophilic so that the sample can contact the surface better.

Figure 2. Typical cyclic voltammograms obtained at Au/CA/(GOx/AMWNTs)₄ (a, c) and bare Au electrodes (b) in 0.1 M PBS saturated with N₂ (dotted lines) or air (solid lines). Scan rate: 10 mV s⁻¹.

Summary

This work is the first attempt to apply the amino terminated MWNTs to glucose biosenors via layer-by-layer covalent self-assembly method without any cross linker between the AMWNTs and GOx. Based on the silane treatment, APS molecules containing amino-ended groups were covalently bonded on OMWNTs through Si-O-C linkages. Such amino-termination of AMWNTs can be reacted with the aldehyde groups of IO₄⁻-oxidized GOx through the formation of Schiff base. The resulting multilayer film showed a porous structure and the assembled AMWNTs exhibited an electrocatalytic activity to the reduction of dissolved oxygen, which facilitated convenient low-potential stable detection of the glucose.

Acknowledgement

This research was financially supported by the National Science Foundation. The research work was supported by National Natural Science Foundation of China under Grant No. 21205081, the Foundation of Liaoning Educational Committee (No. L2015541).

REFERENCES