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Immobilization and direct electrochemistry of glucose oxidase on a tetragonal pyramid-shaped porous ZnO nanostructure for a glucose biosensor

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ABSTRACT

A tetragonal pyramid-shaped porous ZnO (TPSP-ZnO) nanostructure is used for the immobilization, direct electrochemistry and biosensing of proteins. The prepared ZnO has a large surface area and good biocompatibility. Using glucose oxidase (GOD) as a model, this shaped ZnO is tested for immobilization of proteins and the construction of electrochemical biosensors with good electrochemical performances. The interaction between GOD and TPSP-ZnO is examined by using AFM, N_2 adsorption isotherms and electrochemical methods. The immobilized GOD at a TPSP-ZnO-modified glassy carbon electrode shows a good direct electrochemical behavior, which depends on the properties of the TPSP-ZnO. Based on a decrease of the electrocatalytic response of the reduced form of GOD to dissolved oxygen, the proposed biosensor exhibits a linear response to glucose concentrations ranging from 0.05 to 8.2 mM with a detection limit of 0.01 mM at an applied potential of −0.50 V which has better biosensing properties than those from other morphological ZnO nanoparticles. The biosensor shows good stability, reproducibility, low interferences and can diagnose diabetes very fast and sensitively. Such the TPSP-ZnO nanostructure provides a good matrix for protein immobilization and biosensor preparation.

1. Introduction

Over the last two decades considerable attention has been paid to the development of new biocompatible materials with high porosity and large surface area for protein immobilization (Volodkin et al., 2004; Pierre et al., 2006). A series of porous materials such as clay (Carrado et al., 2004), montmorillonite (Lin et al., 2007), porous alumina (Dai et al., 2006) and sol–gel matrix (Brennan et al., 2003) have been used and proven to be promising as the immobilization matrices because of their high mechanical, thermal, and chemical stability as well as good adsorption and penetrability. The incorporation of proteins into pores could provide an active biomaterial (Xiao et al., 2003).

Zinc oxide (ZnO) is a typical semiconductor material with a wide band gap (E_g = 3.37 eV) and a large exciton binding ability (60 meV) (Wong and Searson, 1999). In the area of bioscience, the special properties of nano-ZnO have also attracted much attention gradually (Krishnamoorthy et al., 2006; Dorfman et al., 2006; Wang et al., 2006; Wei et al., 2006; Corso et al., 2007; Zhu et al., 2007). Its nice biocompatibility and fast electron transfer between the enzyme's active sites and the electrode have made the material be favor for functioning as the biomimic membrane to immobilize and modify proteins. Nano-ZnO also deserves further investigation as an important promising candidate for the supporting material in the fabrication of biosensors (Chen et al., 2007, 2008). To date, although various ZnO nanostructures, such as nanorods (Cheng and Samulski, 2004), nanowires (Rout et al., 2007), nanobelts (Height et al., 2006), nanoring (Wang et al., 2004), nanosheet (Park et al., 2004), tetrapod (Yan et al., 2003), hexagonal pyramid and cylinder (Joo et al., 2005), and radial nanowire array (Yang et al., 2005) have been prepared and ZnO nanorod-modified electrodes have been reported (Zhang et al., 2004, 2007; Wei et al., 2006), to the best of our knowledge, there are fewer reports on ZnO porous nanostructures (Polarz et al., 2007) and their application in biosensing.

Diabetes mellitus is a worldwide public health problem. The metabolic disorder results from insulin deficiency and hyperglycemia and is reflected by blood glucose concentrations higher or lower than the normal range of 4.4–6.6 mM (Wang, 2008). The determination of glucose concentration is very important in clinic for diagnosing diabetics (Yu et al., 2003). In this work, a tetragonal pyramid-shaped porous ZnO (TPSP-ZnO) nanostructure was synthesized and for the first time used for the immobilization of GOD by physical adsorption. The high isoelectric point (IP) of 9.5 of ZnO provides a friendly microenvironment for the negatively charged GOD (IP: 4.2) to retain its activity and ZnO promotes the direct electron transfer between the GOD and the electrode to a large extent.

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The immobilized GOD shows fast direct electrochemistry corresponding to its FAD/$FADH_2$ (FAD: flavin adenine dinucleotide) redox couple. The reduced form of GOD can electrocatalyze the reduction of dissolved oxygen. In the presence of glucose the electrocatalytic reaction is restrained due to the enzyme-catalyzed reaction between the oxidized form of GOD and glucose, which results in a decrease of electrocatalytic response. Based on the decrease, a new method for glucose determination is proposed. This competitive assay-like method is different from the conventional detection method based on the measurement of oxygen consumed, which is usually influenced by the concentration of the dissolved oxygen. The limitation of the solubility of the dissolved oxygen will influence the detection limit. Furthermore, the reduction of oxygen is irreversible. The peak caused by the reduction of oxygen is broad. The constructed sensor has the linear response range of 0.05–8.2 mM with a low detection limit to glucose (0.01 mM), and can operate under air without the exclusion of the dissolved O_2. It has better biosensing properties than those from other morphological ZnO nanoparticles, which might result from the larger specific surface area and the specific shape. Thus TPSP-ZnO provides an efficient strategy and a new promising platform for the further study of direct electron transfer of protein and the development of biosensors.

2. Experimental

2.1. Reagents

GOD (EC 1.1.3.4, 35.3 units mg^{-1}. Type II from *Aspergillus niger*) and β-D-(+)-glucose were purchased from Sigma and used as received. Nafion (10% in methanol with equivalent weight of about 1100) was obtained from Aldrich and was diluted to 5% with H_2O before use. All other chemicals were of analytical grade and were used without further purification. All solutions were made up with doubly distilled water. Phosphate buffer (PB, 0.1 M) solutions with various pH values were prepared by mixing stock standard solutions of K_2HPO_4 and KH_2PO_4 and adjusting the pH with H_3PO_4 or NaOH.

2.2. Electrode modification

TPSP-ZnO was prepared following a recipe similar to that reported by our precious work (Dai et al., 2008). The spherical solid ZnO particles with diameters of 30–50 nm were prepared according to the literature (Yuan et al., 2003).

10 mg of TPSP-ZnO was dispersed into 10 mL doubly distilled water to obtain a suspension of 1 mg mL^{-1} TPSP-ZnO. 2 μL of TPSP-ZnO suspensions was mixed with 2 μL of GOD (2 mg mL^{-1} in PB) thoroughly. Then 2 μL of the mixture was dropped onto the surface of a glassy carbon electrode (GCE) and allowed to dry at ambient temperature to obtain the GOD/ZnO-modified electrode. Finally, 2 μL of Nafion (5%) was cast on the GOD/ZnO-modified electrode surface and the GOD/TPSP-ZnO/Nafion-modified electrode was obtained. The solvent was allowed to evaporate before use. The modified electrodes were rinsed with doubly distilled water for twice or thrice to get rid of the non-firmly adsorbed GOD. They were then immersed into the blank 0.1 M pH 7.0 PB until a stable electrochemical response of GOD was observed. The obtained modified electrodes were stored in 0.1 M pH 7.0 PB at 4 °C in a refrigerator when not in use. The same procedure was employed to fabricate other modified electrodes.

2.3. Apparatus and measurements

The phase characterization was performed by means of X-ray diffraction (XRD) using a D/Max-RA diffractometer with Cu Kα radiation. The morphologies and particle sizes of the samples were characterized by JEM-200CX transmission electron microscopy (TEM) working at 200 kV. The X-ray photoelectron spectra (XPS) were recorded on an ESCALAB MK II X-ray photoelectron spectrometer, using Mg K-X-ray as the excitation source. Atomic force microscopic (AFM) experiments were performed with Agilient series 5100. Nitrogen adsorption isotherms were obtained using an ASAP 2000 instrument. Spectrophotometric measurements were carried out using a Hitachi (model U-2001) spectrophotometer. Cyclic voltammetric and amperometric measurements were performed on CHI 660 electrochemical workstation. A three-electrode system comprising a platinum wire as auxiliary, a saturated calomel electrode as reference and the modified electrode as working electrodes were used for all electrochemical experiments. The electrochemical behavior of GOD was performed by deoxygenating with highly pure nitrogen for 15 min, and then a nitrogen atmosphere was kept over the solutions during measurements. The detection of glucose was carried out in air-saturated solution. All experiments were carried out at laboratory temperature.

3. Results and discussion

3.1. Characterizations of the prepared ZnO

The TEM image of the prepared ZnO particles is shown in Fig. 1A. It can be seen that ZnO particles display the flat base and have the tetragonal pyramid-shaped structure (marked with arrows). The lengths of the side edge and basal edge mainly range from 70 to 90 nm and from 40 to 60 nm, respectively. The shape of some particles to be nonpyramid is attributed to their different orientations on

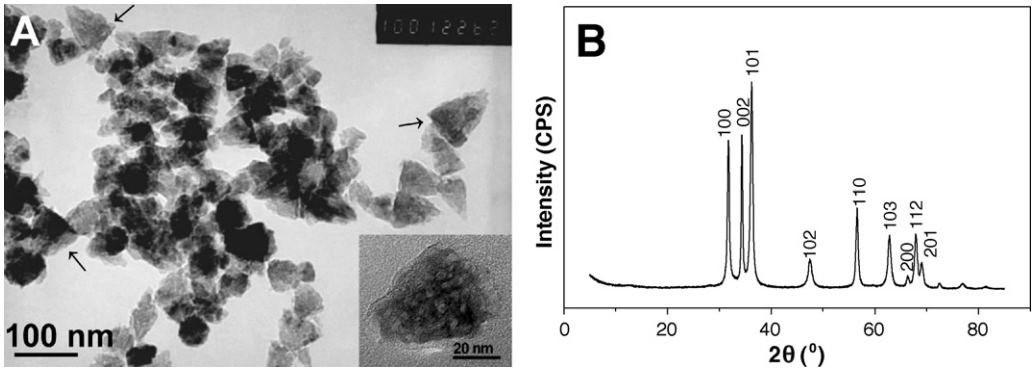

Fig. 1. TEM images at lower (A) and higher (inset in A) magnification and XRD pattern (B) of ZnO particles prepared by the reaction of $ZnSO_4$ with KOH in an aqueous solution of polyglycol.