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Aligned ZnO nanorods: A useful film to fabricate amperometric glucose biosensor

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ABSTRACT

A novel amperometric glucose biosensor has been fabricated on the basis of aligned ZnO nanorod film grown on ITO directly. Glucose oxidase immobilized on the surface of ZnO nanorods are very stable with highly catalytic activity during the measurements, Because of the novel properties of ZnO, such as biocompatibility, non-toxicity, chemical stability, electrochemical activities and high isoelectric point, and the protection effect of Nifion membrane cast on the surface of the film. This biosensor displays excellent analytical performance over a wide linear range along with good selectivity. Interference from uric acid and ascorbic acid which usually coexist with glucose in practical samples has been found to be negligible. This method may be used to construct other amperometric biosensors using aligned nanorod/nanowire films.

1. Introduction

The area of amperometric glucose biosensor based on the use of glucose oxidase (GOD) has received great attention in recently years because of the high demand for blood glucose monitoring, biological and chemical analyses, and clinical detection [1,2]. The measurement principle of oxidase-based amperometric biosensors previously relied on the immobilization of oxidase enzymes on the surface of various electrodes. However, direct electrochemistry of most redox enzymes on bare solid electrodes is difficult to achieve because of the deeply embedded active site in the proteins and the instability of the biological matrix upon interaction with an electrode surface [3,4]. Thus, synthesis and immobilization of functional materials on electrode surface, which can facilitate the immobilization of biomolecules while remaining high activity, is necessary in novel biosensor design. To realize this goal, one possible way is to employ nanostructured materials as such functional materials due to their desirable microenvironment and the direct electron transfer between enzyme's active sites and electrode. For example, palladium, gold, rhodium, copper and platinum nanoparticles have been used to construct glucose biosensors [5–9]. Besides metal nanomaterials, metal oxide nanostructures have also been attracted more and more attention in this area for their unique properties, such as high specific surface area, good biological compatibility and stability. Titania nanoporous film, titania sol–gel membrane, and nanoporous silica materials have been used to modify electrodes and further to impregnate biomolecules for biosensors [10–12]. Among these materials, ZnO, with high biocompatibility, non-toxicity, chemical stability, electrochemical activities and fast electron communication features, has shown its novel advantages for the fabrication of oxidase-based amperometric biosensors. More importantly, with an isoelectric point about 9.5, ZnO can immobilize acidic proteins (including glucose oxidase) with high bioactivity and stability by electrostatic interactions [13,14]. Up to now, there is limited work reported on the biosensors fabricated on the basis of ZnO nanostructures [15–18]. In the literatures, ZnO nanostructure modified electrodes is usually prepared by a drop coating method [16–18]. This method is simple; however, materials used can lose from the electrode surface easily during their working and result in the poor stability and short lifetime. In this work, a novel amperometric glucose biosensor has been constructed based on aligned ZnO nanorod films, which was grown on tin-doped indium oxide (ITO) glass directly. There are several reasons for us to choose ITO glass instead of other substrates, such as standard Au electrode or glassy carbon electrode. Firstly, compared with Au or glassy carbon electrodes, ITO glass is much cheaper and with low resistivity, which can be used widely rather than in the laboratory only. Secondly, the size of ITO glass can be controlled easily by special knife, which can facilitate the process of glucose-biosensor-device fabrication. Thirdly, the widely accepted solution methods for aligned ZnO nanorod films synthesis usually involve a procedure of ZnO nanoparticle seeds formation, which is performed at a temperature above 200 °C [19–21]. At such high temperature, Teflon layer of standard electrodes is melt and result in the breakage of the electrodes. Moreover, it is difficult to characterize the nanorods grown on Au or glassy carbon electrodes directly. For example, Sun and co-workers have synthesized ZnO nanorods on a standard gold electrode directly; however, the characterized

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ZnO nanorods were grown on a gold-coated glass substrate [15]. The results of our experiments show that as-prepared biosensor has a better sensitivity for glucose determination than that of previous reported results [16,22]. It is worth mentioning that the interference from uric acid (UA) and ascorbic acid (AA) which usually coexist with glucose in practical samples can be negligible. Moreover, because of the strong interaction between ITO glass and ZnO nanorods, excellent electrostatic absorption properties of ZnO nanorods and the protection effect of Nifion membrane, as-prepared glucose sensor can retain its bioactivity for several weeks. Thus, as-prepared glucose biosensor has great potential in practical measurements.

2. Experimental

2.1. Reagents and instruments

ITO glass was obtained from CSG Holding Co., Ltd (China). The substrate used in the preparation of electrode was cut about 1 cm in length and 0.5 cm in width. GOD, Ascorbic acid (AA) and uric acid (UA) were bought from Sigma. All other reagents were of analytical grade and used without further purification.

The aligned ZnO nanorod films were characterized with a high-resolution field emission scanning electron microscope (Hitachi S-4800, Japan). A single nanorod was characterized by a transmission electron microscopy (JEOL-2010 with an energy dispersive X-ray (EDX) system, Japan). X-ray diffraction measurement was performed on an XRD-6000 (Japan). All electrochemical experiments were carried out on a CHI 660 electrochemical workstation (CH Instrument Co., USA). Traditional three-electrode system involving a platinum wire counter-electrode, an enzyme immobilized ITO glass working electrode and a saturated calomel reference electrode were employed.

2.2. Preparation of enzyme immobilized aligned ZnO nanorod film electrode

Aligned ZnO nanorod films on ITO glass were synthesized via a two-step solution approach. First, a layer of ZnO nanoparticles was formed on the surface of ITO glass by thermally decomposing of zinc acetate at 320 °C for 20 min. Then the treated ITO glass was suspended in an aqueous solution containing zinc nitrate hydrate (0.025 M) and methenamine (0.025 M) at 90 °C for 3 h. On completion of the reaction, the ITO glass was removed from the solution and rinsed with deionized water. The resulting substrate was dried in air the air and applied to fabricate the glucose sensor. Typically, it was dropped into 5 ml of GOD solution (pH 7.4, 0.01 M PBS solution) under ambient conditions and then stored at 4 °C for 12 h. After this process, ITO glass was washed extensively in order to remove the unfixed GOD molecules. Finally, a droplet of 0.5% Nafion was necessary in order to form a Nafion membrane on the surface of the films.

3. Results and discussion

Vertical ZnO nanorod films were synthesized on ITO glass directly using a method reported by Yang with tiny modifications [19]. Fig. 1a shows the typical SEM top-image of as-prepared ZnO films, indicating that the nanorod arrays are highly uniform and densely packed. Fig. 1b is a cross-sectional view of the sample, showing that the nanorods grow almost perpendicularly onto the ITO glass, and their diameters are in the range of 50–100 nm. A typical XRD pattern of the sample is shown in Fig. 1c. All of the reflection peaks of the XRD pattern can be well-indexed to wurtzite-type ZnO (JCPDS Card file No. 36-1451). The EDS spectrum of the ZnO nanorods shows that atomic ratio of Zn and O is ca. 1:1, indicating the perfect stoichiometric chemical composition of the Zn and O in the nanorods (Fig. 1d). The typical TEM image of ZnO

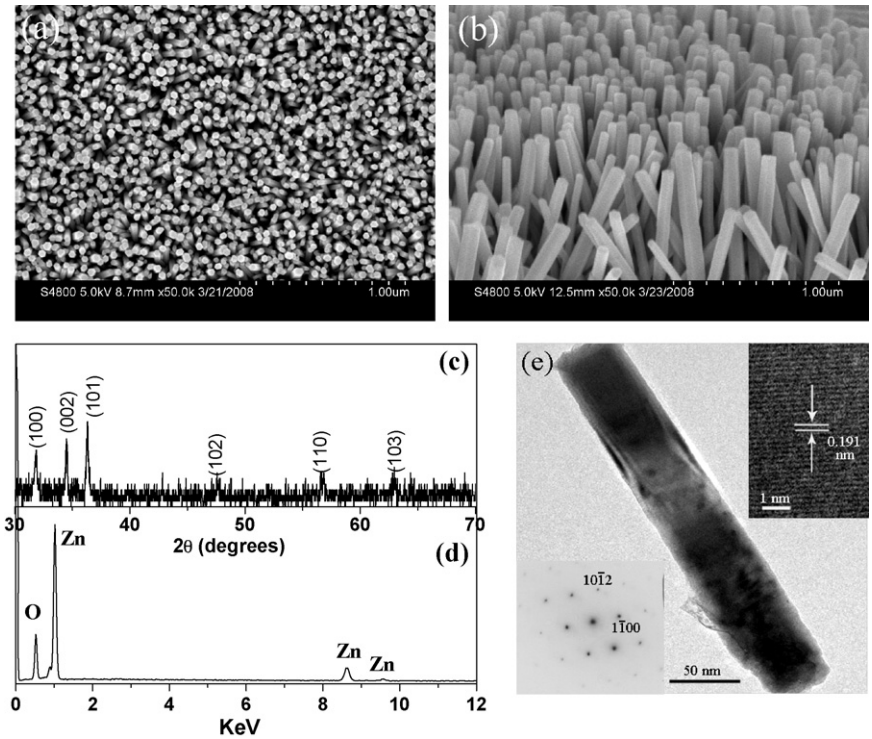

Fig. 1. (a) SEM top-image of the as-prepared aligned ZnO nanorod Films. (b) Cross-sectional view of the aligned ZnO nanorods. (c) XRD pattern of the as-synthesized nanorod films. (d) EDS spectrum of the as-prepared ZnO nanorods. (e) Low magnification TEM image of a single ZnO nanorod, insets exhibit the high-magnification TEM image and the SAED pattern image of the corresponding structures.

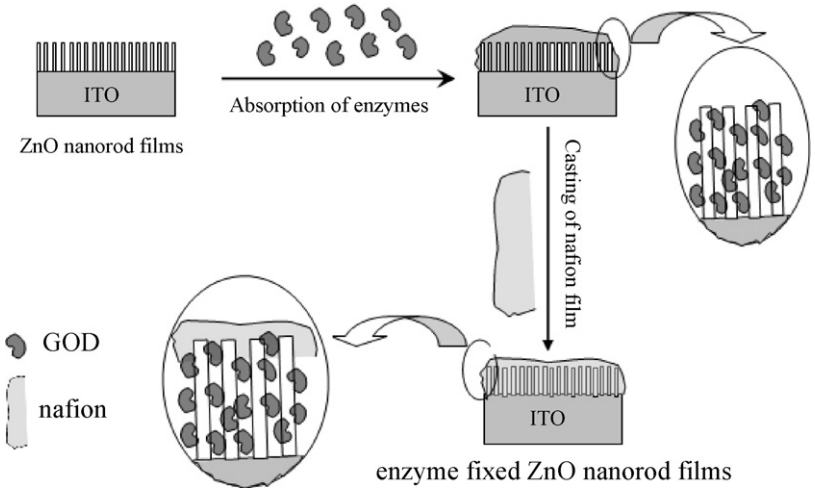

Fig. 2. Schematic illustration for the preparation procedure of the enzyme immobilized aligned ZnO nanorod film electrode.

nanorods is shown in Fig. 1e, which reveals that the diameter of the rod is about 50 nm with length about 300 nm. The corresponding HRTEM image (upper right inset of Fig. 1e) shows clearly the (1 0 2) planes of hexagonal ZnO with an inter-planar spacing of ca. 0.191 nm along the length of the nanorod. The inset of Fig. 1e presents the selected area electron diffraction (SAED) pattern taken from a single nanorod, which can be indexed as single-crystalline hexagonal structural ZnO.

The enzyme fixed ZnO nanorod film electrode was prepared based on above ZnO nanorod arrays following the procedure shown in Fig. 2. ITO glass was immersed in 5 ml of GOD solution (pH 7.4, 0.01 M phosphate buffered saline solution) under ambient conditions and then stored at 4 °C for 12 h for protein absorption. After absorption, the electrode was washed extensively to remove the unimmobilized GOD, and then 5 μl 0.5% Nafion was placed on the surface of the films to form a Nafion membrane. The electrode is ready for use after the drying of the Nafion membrane.

The strong interaction between the ZnO nanorods and GOD molecules can be confirmed by the IR spectrum differences among the as-prepared ZnO nanorods, and pure GOD and GOD-fixed ZnO nanorods. The pure ZnO nanorods (Fig. 3a) exhibit obvious absorption band centered at 1382 cm^{-1}, which attributes to the symmetrical vibration of $-COO^-$, indicating some acetic ions are adsorbed on the surface of ZnO nanorods which came from acetic zinc used in our synthesis procedure. Fig. 3b shows the IR spectrum of pure GOD, which has some characteristic absorption bands of GOD, such as, typical amide I absorption band round 1648 cm^{-1} and amide II absorption near 1544 cm^{-1} [23]. After being immersed into GOD solution, the two absorption bands of amide are appear in the IR spectrum of the ZnO nanorods (Fig. 3c), which demonstrate that GOD molecules are effectively fixed on the surface of ZnO nanorods.

The cyclic voltammograms of two different electrodes in 0.01 M phosphate buffered saline (PBS) solution at 40 mV s^{-1} were investigated (Fig. 4). The Nafion/ZnO nanorod film/ITO glass only exhibit typical capacitive, squared CV curves, indicating that both ZnO nanorods and Nifion membrane are not electrochemically active in this potential window. However, Nafion/GOD/ZnO nanorod film/ITO glass shows a pair of well-defined redox peaks at −300 mV and −20 mV, which result from direct electron transfer at the immobilized GOD for the conversion of FAD to $FADH_2$, suggesting that most of the GOD molecules retain their catalytic activity.

The effect of scan rate on the electrochemical response of the immobilized GOD is shown in Fig. 5a. The peak currents increase linearly with an increase of the scan rate from 20 to 120 mV s^{-1} (Fig. 5b), and the anodic and cathodic peak potentials of the GOD-immobilized ITO glass show shifts in positive and negative directions, respectively, demonstrate that the GOD redox reaction is a surface-controlled electrochemical process. The peak-to-peak separation of the cyclic voltammograms increases slightly with an increase of the scan rate, possibly because of the resistance of the ZnO nanorod films.

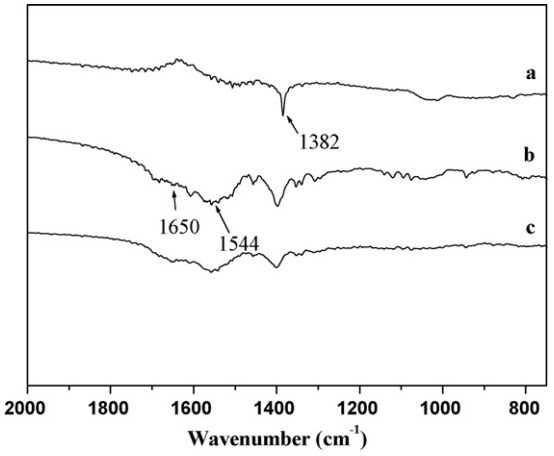

Fig. 3. IR spectra of (a) ZnO nanorods, (b) pure GOD, and (c) COD-fixed ZnO nanorods.

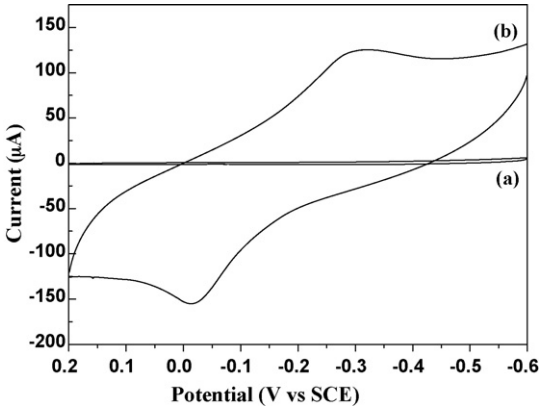

Fig. 4. Cyclic voltammograms of Nafion/ZnO nanorod film/ITO glass (a) and Nafion/GOD/ZnO nanorod film/ITO glass (b) in 0.01 M pH 7.4 PBS solution at 40 mV s^{-1}.

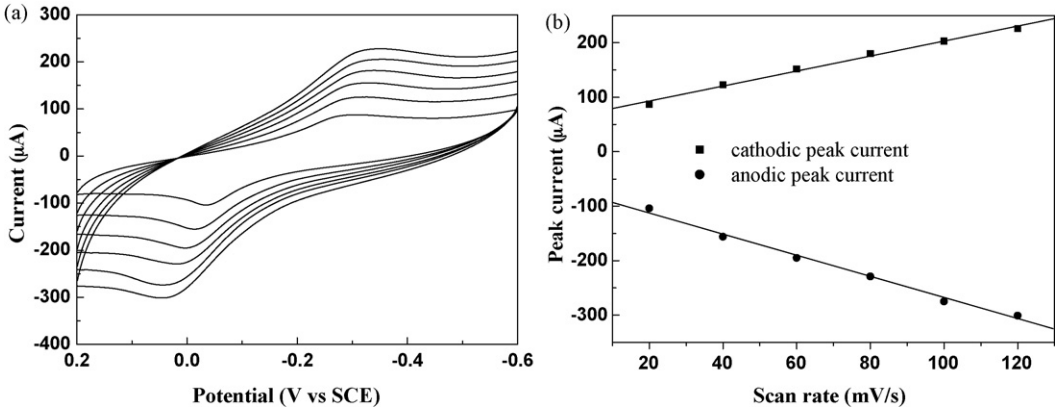

Fig. 5. (a) Cyclic voltammograms of Nafion/GOD/ZnO nanorod film/ITO glass in 0.01 M pH 7.4 PBS solution at scan rates of 20, 40, 60, 80, 100, and 120 mV s^{-1} (from bottom to top). (b) Plots of peak currents versus scan rate.

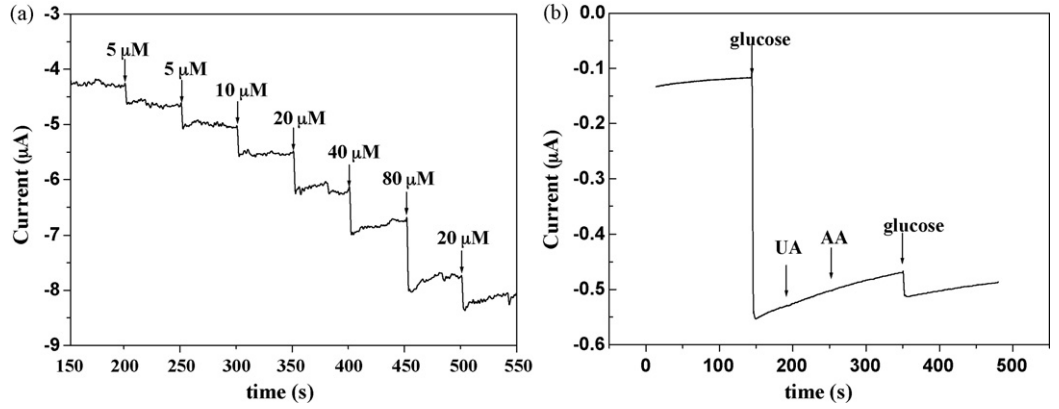

Fig. 6. (a) Typical steady state response of the biosensor on successive addition of specific concentrations of glucose to air-saturated and stirred 0.01 M pH 7.4 PBS solution at −100 mV, and (b) effect of interfering species on the biosensor response.

The glucose biosensors are generally based on the detection of the oxidation signal of hydrogen peroxide or the reduction signal of dissolved oxygen, which is produced or consumed in the oxidation process of β-D-glucose to D-glucono-δ-lactone catalyzed by GOD, respectively. The measurement principle of our electrode relies upon the immobilization of GOD on the surface of ZnO nanorod films and the detection of the current associated with decrease of O_2 concentration in the biological reaction. Fig. 6a shows a typical current–time plot for the Nafion/GOD/ZnO nanorod film/ITO glass on successive additions of a glucose solution into an air-saturated and stirred 0.01 M pH 7.4 PBS solution at −100 mV. Each successive injection of glucose results in a decrease in the steady-state current. The time required for reaching 95% of the steady-state response is less than 5 s, indicating a fast response, which is similar with Sun's result [15]. The biosensor exhibited a linear response to glucose from 5 μM to 300 μM and a limit of detection of 3 μM. Such fast response and low limited detection is due to the large surface area of the nanorod films. As we can see from Fig. 6a, this biosensor still has a fast repose for a little amount of glucose after the response of a large quantity of it. Exhilaratingly, as-prepared sensor also shows a good reproducibility in detection of glucose, with a relative standard deviation (RSD) about 3.0% in more than 15 measurements.

The stability of the electrode was investigated by measuring its cyclic voltammogram at intervals during the storage of it in 0.01 M pH PBS at 4 °C. No obvious decrease was observed in the response to glucose after one month of storage. The response current was still remained at 90% value of initial response. The good stability of as-prepared electrode was further confirmed by UV–vis spectrometry. After the preparing of the electrode, the ITO glass was immersed into 3 ml distilled water with stirring for several hours. No detectable absorption around 280 nm was observed, indicating that the leaching of GOD is negligible. There are two main reasons for these phenomena, one is the strong electrostatic interaction between GOD molecules and ZnO nanorods, and the GOD molecules remain their activity during the interaction. The other is the protection effect of the Nafion membrane formed on the surface of the film, which can prevent the loss of the immobilized enzyme.

The glucose biosensors are usually interfered by electro-active compounds such as UA and AA in the analysis of serum samples. However, this biosensor shows good selectivity for glucose. In an air-saturated and stirred 0.01 M PBS containing 5 μM glucose, there is no significant change of the amperometric response by injection of 10 μM UA and AA, respectively (Fig. 6b), as the Nifion membrane can prevent the majority of the anionic interferences such as UA and AA [24].

4. Conclusions

In summary, we have constructed a novel amperometric glucose biosensor based on aligned ZnO nanorod films formed on the surface of ITO glass directly, which exhibits favorable conductivity for electron transfer and biocompatibility for protein immobilization. Due to the strong electrostatic interactions between GOD and ZnO nanorods and the protection effect of Nifion membrane, enzymes immobilized on the surface of ZnO nanorods are very stable and with highly catalytic activity during the measurements. This biosensor displays excellent analytical performance over a wide linear range along with good selectivity. Interference from

UA and AA which usually coexist with glucose in practical samples has been found to be negligible. This method may be used to construct other amperometric biosensors based on aligned nanorod/nanowire films.

Acknowledgments

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