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Facile synthesis of tetragonal columnar-shaped TiO_2 nanorods for the construction of sensitive electrochemical glucose biosensor

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ABSTRACT

A tetragonal columnar-shaped TiO_2 (TCS-TiO_2) nanorods are synthesized via a facile route for the immobilization of glucose oxidase (GOx). A novel electrochemical glucose biosensor is constructed based on the direct electrochemistry of GOx at TCS-TiO_2 modified glassy carbon electrode. The fabricated biosensor is characterized by scanning electron microscopy, Fourier transform infrared spectroscopy, electrochemical impedance spectra and cyclic voltammetry. The immobilized enzyme molecules on TCS-TiO_2 nanorods retain its native structure and bioactivity and show a surface controlled, quasi-reversible and fast electron transfer process. The TCS-TiO_2 nanorods have large surface area and provide a favorable microenvironment for enhancing the electron transfer between enzyme and electrode surface. The constructed glucose biosensor shows wide linear range from 5.0×10^{-6} to 1.32×10^{-3} M with a high sensitivity of 23.2 mA M^{-1} cm^{-2}. The detection limit is calculated to be 2.0×10^{-6} M at signal-to-noise of 3. The proposed glucose biosensor also exhibits excellent selectivity, good reproducibility, and acceptable operational stability. Furthermore, the biosensor can be successfully applied in the detection of glucose in serum sample at the applied potential of −0.50 V. The TCS-TiO_2 nanorods provide an efficient and promising platform for the immobilization of proteins and development of excellent biosensors.

1. Introduction

The fast and reliable determination of blood glucose levels is of considerable importance for diagnosis and therapy of diabetics (Mizutani and Yabuki, 1997; Liu and Ju, 2003). As an ideal mode enzyme, glucose oxidase (GOx) has been extensively used in fabrication of electrochemical glucose biosensors due to its catalytic ability to oxidize glucose (Vamvakai et al., 2006; Zhao and Ju, 2006; Xu et al., 2010; Jiang et al., 2012b, Li et al., 2013). The ultimate goal of glucose detection is to develop the third generation biosensor based on the direct electron transfer (DET) between the cofactor FAD of GOx and electrode surface without the mediator (Wang, 2008). Unfortunately, the redox center in biomolecules is usually seated deeply in cavity of enzyme molecules, which make it hard to realize DET of enzyme at conventional electrodes (Meng et al., 2009). Over the past few decades, various kinds of nanomaterials, such as gold nanoparticles (Jia et al., 2008; Pingarron et al., 2008), carbon nanotubes (Azamian et al., 2002; Wang and Musameh, 2003; Ammam and Fransaer, 2012), graphene (Kang et al., 2009; Shan et al., 2010a), titanium oxide (Bao et al., 2008) and tin disulfide (Yang et al., 2011) have been explored to immobilize GOx for accelerating DET of redox enzyme on the electrode surface.

TiO_2, a cheap, nontoxic and chemically stable semiconductor with a band gap energy of 3.2 eV, has attracted considerable interest in various areas such as pigment, cosmetic, catalyst, photovoltaic materials and sensors (Koo et al., 2006; Wang et al., 2008; Liang and Li, 2009; Zhang et al., 2009). One-dimensional (1D) TiO_2 nanostructures, especially nanorods, nanowires and nanotubes, are superior to their spherical and planar counterparts due to their high surface-to-volume ratio, increased number of delocalized carriers, and improved charge transport afforded by their dimensional anisotropy (Koo et al., 2006; Wang et al., 2008). To date, 1D TiO_2 nanomaterials have been synthesized by the hydrothermal method (Tian et al., 2008), controlled hydrolysis (Zhang et al., 2009), dissolution-reprecipitation process (Wang et al., 2008), sol–gel process (Koo et al., 2006; Liang and Li, 2009; Attar et al., 2009) and surfactant-templated synthesis (Chung et al., 2006). Here, we reported a facile method for the large scale preparation of tetragonal columnar-shaped TiO_2 (TCS-TiO_2) nanorods by heating a mixture of Ti and NH_4Cl powders in air at a relatively low temperature. To the best of our knowledge, TCS-TiO_2 nanorods have not yet been used to immobilize proteins for the fabrication of biosensor.

Chitosan, a natural biopolymer with unique structure features, is commonly used to disperse nanomaterials and immobilize

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enzymes for constructing biosensors due to its excellent capability for film formation, nontoxicity, biocompatibility, mechanical strength, good water permeability (Kang et al., 2009). In this work, a facile route was proposed for the preparation of TCS-TiO_2 nanorods from Ti and NH_4Cl powders in air at 400 °C for 3 h. The resulting TCS-TiO_2 nanorods were dispersed with chitosan solution, and for the first time used to modify the electrode for the immobilization of enzyme molecules. Based on DET of GOx at TCS-TiO_2/chitosan modified electrode, a novel electrochemical biosensor was proposed for sensitive detection of glucose. The TCS-TiO_2 enhanced the electron transfer between the enzyme molecules and electrode surface. A pair of obvious and well-defined redox peaks of GOx could be observed at GOx/TCS-TiO_2/chitosan modified electrode. UV–vis and Fourier transform infrared spectrum demonstrated that the TCS-TiO_2/chitosan film provided a favorable microenvironment for GOx to retain its native structure and bioactivity. The constructed biosensor showed high sensitivity, excellent selectivity, good reproducibility and could be successfully applied in reagentless glucose detection. Moreover, the TCS-TiO_2 nanorods provide a potential electrode material for electrochemical biosensing.

2. Materials and methods

2.1. Materials and Reagents

GOx (EC 1.1.3.4, 108 U mg^{-1}, from *Aspergillus niger*) was supplied by Amresco. D-(+)-Glucose and chitosan were purchased from Sigma-Aldrich. Ti and NH_4Cl powders were purchased from Sinopharm Chemical Reagent Co., Ltd. A stock solution of D-glucose was prepared and allowed to mutarotate at room temperature for 24 h prior to use. Phosphate buffer solution (PBS) was a mixture of 0.1 M Na_2HPO_4 and NaH_2PO_4 and its pH was adjusted with H_3PO_4 or NaOH solutions. All other chemicals and reagents are of analytical grade and were prepared using distilled water.

2.2. Apparatus

Electrochemical measurements were carried out on a CHI 852C electrochemical workstation (Co., CHI, Shanghai Chenhua, China). All experiments were performed with a three-electrode system using a glassy carbon electrode (GCE, $D=3$ mm) as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The cyclic voltammetric experiments were carried out at a scan rate of 100 mV s^{-1} in an electrochemical cell filled with 5.0 ml of PBS. All pH measurements were performed with S-25 digital pH-meter with glass combination electrode. Scanning electron micrographs (SEM) were obtained with a Hitachi S-4800 scanning electron microscope (Japan) at an acceleration voltage of 15 kV. Fourier transform infrared (FTIR) spectra were measured with a Tensor 27 spectrometer (Bruker Co., Germany). Electrochemical impedance spectroscopy (EIS) measurements were performed on an Autolab/PGSTAT30 (The Netherlands) in 0.1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, and the amplitude of the applied sine wave potential was 5 mV. The impedance measurements were recorded at a bias potential of 190 mV within the frequency range from 0.05 Hz to 10 kHz.

2.3. Preparation of TCS-TiO_2 nanorods and enzyme electrodes

Ti (0.02 mol) and NH_4Cl (0.05 mol) powders were mixed and ground homogeneously in a carnelian mortar, then transferred to corundum crucible. The open crucible was heated in a muffle furnace at 400 °C for 3 h, and then allowed to cool at room temperature. The resulting solid powders were collected directly as the final product. 1.0 mg of the as-prepared TCS-TiO_2 was dispersed in 1.0 ml 0.5 wt% chitosan solution with ultrasonication for 30 min. 8.0 mg of GOx was added in the suspension of TCS-TiO_2/chitosan, and homogeneously mixed under gentle stirring for 20 min for the following use.

The GCE was firstly polished successively with 0.03 and 0.05 μm alumina slurry (Buhler) followed by rinsing thoroughly with distilled water, and then sonicated in 1:1 nitric acid–water (v/v), acetone and distilled water and finally dried in air. After 5.0 μl of the resultant suspension was dropped on the surface of the pretreated GCE and dried in silica gel desiccators, the GOx/TCS-TiO_2/chitosan modified electrode was finally obtained. The fabricated enzyme electrode was rinsed thoroughly with distilled water to wash away the loosely adsorbed enzyme molecules. When not in use, the enzyme electrodes were stored at 4 °C in a refrigerator.

3. Results and discussion

3.1. Characterizations of GOx/TCS-TiO_2/chitosan modified electrode

Fig. 1A showed the SEM image of the synthesized TCS-TiO_2 nanorods, and a tetragonal columnar-shaped morphology could be clearly observed. When TCS-TiO_2 nanorods were dispersed in chitosan solution, the SEM image of the TCS-TiO_2/chitosan was shown in Fig. 1B. It could be seen that TCS-TiO_2 displayed a uniform and ordered arrays structure, which was suitable platform for the immobilization of GOx and fabrication of functional biosensor for glucose. After GOx molecules were trapped in the TCS-TiO_2/chitosan composite, its SEM (Fig. 1C) exhibited obviously different surface morphology from the TCS-TiO_2/chitosan. The aggregates of the loaded enzymes were distributed regularly and

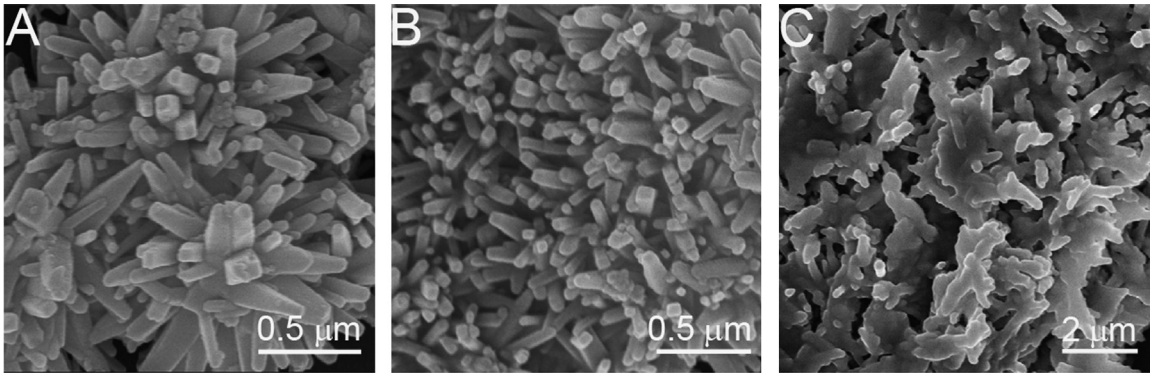

Fig. 1. SEM images of TCS-TiO_2 (A), TCS-TiO_2/chitosan (B) and GOx/TCS-TiO_2/chitosan (C).