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An ultrasensitive photoelectrochemical immunosensor for insulin detection based on BiOBr/Ag_2S composite by in-situ growth method with high visible-light activity

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ABSTRACT

A novel ultrasensitive label-free immunosensor based on BiOBr/Ag_2S composite with high visible-light photoelectrochemical activity was prepared for the detection of insulin. After BiOBr was modified by thioglycolic acid, Ag_2S nanoparticles were grown in-situ on the surface of BiOBr hierarchical microspheres to first form novel BiOBr/Ag_2S composite. When ascorbic acid (AA) was used as an efficient electron donor for scavenging photo-generated holes, BiOBr/Ag_2S composite material showed excellent photoelectrochemical activity. In order to immobilize insulin antibody, adhesive polydopamine (PDA) film formed by self-polymerization of dopamine was fabricated onto BiOBr/Ag_2S modified electrode. Moreover, PDA film could further enhance the visible light absorption of BiOBr/Ag_2S. When the solutions of 0.08 mol $L^{-1}$$AgNO_3$ and 0.1 mol L^{-1} AA were selected respectively during fabrication and detection process of this sensor, the best photocurrent singles were obtained. Under the optimum experimental condition, the specific binding between insulin and antibody resulted in a decrease in photocurrent intensity and the intensity decreased linearly with the logarithm of insulin concentration in the range of 0.001–20 ng mL^{-1} with a detection limit of 0.2 pg mL^{-1}. The photoelectrochemical sensor ITO/BiOBr/Ag_2S/PDA/anti-Insulin/BSA/Insulin revealed facile preparation, high sensitivity, and acceptable reproducibility, which may have practical applications in the biosensor, clinical diagnosis of cancers, photocatalysis, and other related fields.

1. Introduction

Photoelectrochemical (PEC) immunoassay is a promising analytical technique and playing increasingly important roles in the detection of diverse biological targets due to its distinct advantages, such as rapid test, desirable sensitivity, low cost, simple devices and so forth (Brown et al., 1992; Wang et al., 2009a, 2009b; Freeman et al., 2013; Li et al., 2016). Efficient differentiation between excitation light source and detection signals contributes to low background interference and high sensitivity of the PEC analysis (Tu et al., 2011; Fan et al., 2015). In order to obtain rich photoelectrochemical materials with efficient PEC activity to fabricate immunosensors, many methods were employed *via* doping semiconductor with metallic or nonmetallic elements (Fan et al., 2015, 2016), sensitization with quantum dots (Fan et al., 2014a; Yang et al., 2015; Yue et al., 2013), and forming semiconductor heterojunctions (Chen et al., 2016). In the meanwhile, a series of ingenious sensing strategies have been developed for ultrasensitive detection of biomarkers, such as enhanced (Wen and Ju, 2016), quenched (Zhang et al., 2016) or blocked (Fan et al., 2014b, 2017) PEC phenomena through the interaction between substrate materials modified on the electrode and marked conjugates on the secondary antibodies (or modified strands of DNA).

TiO_2, and ZnO, as traditional semiconductor materials, have been applied widely in photoelectrochemical sensors, photocatalysis, photodegradation, and so on. However, the wide band-gaps of TiO_2 (3.2 eV) and ZnO (3.4 eV) make them show the weak visible-light response (Li et al., 2011; Das et al., 2013), and limit their applications. Bismuth oxybromide (BiOBr) has gained more attention of many researchers because of its hierarchical structures, physicochemical stability and new visible-light photocatalyst (Cheng et al., 2011; Xia et al., 2011; Wang et al., 2008). Although the band-gap of BiOBr is narrower (around 2.85 eV) than TiO_2 or ZnO. More efforts have been made in preparation of composites based on BiOBr in order to broaden its application, such as CdS/BiOBr (Cui et al., 2014), Ag/Ti-dope BiOBr (Jiang et al., 2012), and BiOBr-BiOI (Huang et al., 2015) composites.

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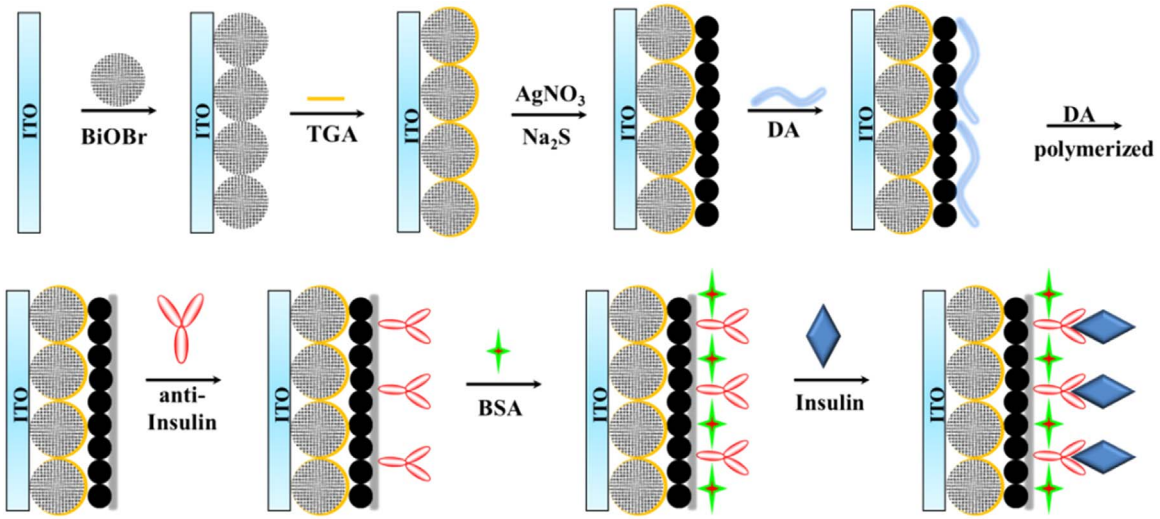

Fig. 1. Schematic representation of the preparation process of the PEC immunosensor.

Ag_2S nanomaterial, as a kind of low-toxic and narrow band-gap (about 1.0 eV) semiconductor (Neves et al., 2009), has been utilized increasingly in photocatalysis (Yadav and Jeevanandam, 2015), sensors (Zhang et al., 2014), and cell research (Hocaoglu et al., 2012). Moreover, the lower solubility product constant of Ag_2S makes it easy prepared and very stable, which contributes to fabrication of facile and stable sensors. Herein, we selected hierarchical BiOBr microflowers with high carrying capacity as the basic materials and then first prepared novel BiOBr/Ag_2S composite by in-situ growth method. The new BiOBr/Ag_2S modified ITO electrode shows excellent photoelectrochemical signal, which could lay the foundation for the fabrication of the sensitive PEC sensor.

Over here, a label-free photoelectrochemical immunosensor based on BiOBr/Ag_2S composite was fabricated, and expected to reach the goal of efficient determination of insulin. Insulin is an important polypeptide hormone, which is secreted by pancreatic β-cells, and plays an important role in maintaining blood glucose level in human beings (Habibi et al., 2016). In human, if insulin appears dysfunction, it will lead to diabetes mellitus. At the meantime, human also would be threatened with other kinds of diseases (Gobi et al., 2007). Consequently, the timely and accurate detection of insulin levels in serum is very important in monitoring and evaluation of the human condition, early warning of related diseases and another medical field (Yu et al., 2016). At present, the mainly detection methods of insulin level in serum include electrochemical immunoassay (Gerasimov et al., 2013), chromatography (Mercolini et al., 2008), radioimmunoassay (Murayama et al., 2006). In our work, this label-free PEC immunosensor system was developed and showed high sensitivity, good selectivity and stability, which exhibited its potential applications in many biological targets detection.

2. Experimental

2.1. Materials

Insulin and insulin antibody (anti-Insulin) were purchased from Shanghai Linc-Bio Science Co., Ltd. China. The details are shown in Electronic Supplementary Information (ESI†).

2.2. Apparatus

A 100 W LED lamp (white light) was employed as an irradiation source in the PEC test and its wavelength range was showed in Fig. S1 (ESI†). Scanning electron microscope (SEM) images and energy dispersive spectrometry (EDS) were obtained using a field emission SEM (Zeiss, Germany). X-ray diffraction (XRD) patterns were performed with D8 advance X-ray diffractometer (Bruker AXS, Germany). UV–vis diffuses reflectance spectra measurements were investigated with a Shimadzu UV-3101PC spectrometer (Japan). All PEC experiments were measured on a CHI760E electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd, China). A three-electrode system was used, with a platinum wire as a counter-electrode, saturated calomel electrode as a reference electrode and modified ITO electrode (2.5 × 0.8 cm^2) as a working electrode. Electrochemical impedance spectroscopy (EIS) analysis was performed with an RST5200F electrochemical workstation (Zhengzhou Shiruisi Technology Co., Ltd, China) with a three-electrode system in a 5.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution containing 0.10 mol L^{-1} KCl.

2.3. Synthesis of BiOBr

Bismuth oxybromide (BiOBr) were prepared by the reported solvothermal method (Cui et al., 2014). In detail, 1.9*g* $Bi(NO_3)_3 \cdot 5H_2O$ was dissolved in 70 mL ethylene glycol, and 2.9*g* hexadecyl trimethyl ammonium bromide (CTAB) was added to get a uniform suspension by stirring for one hour. Then, the suspension was transferred to the Teflon-lined autoclave and maintained at 140 °C for 8 h. The prepared precipitates were collected and washed by centrifugation with absolute ethanol and ultrapure water for more than 3 times and then dried at 80 °C for 8 h. In the end, the dried powder was calcined at 400 °C for 4 h and ground into powder for following use.

2.4. Fabrication of the label-free photoelectrochemical sensor

First of all, the ITO electrodes (2.5 × 0.8 cm^2) were successively washed for 30 min at 50 °C in series of ultrasonically solvents (acetone, ultrapure water, ethanol, and ultrapure water). The preparation process of the ultrasensitive PEC sensor is illustrated in Fig. 1. As follows, 10 μL of 4 mg mL^{-1} BiOBr aqueous suspension that was sonicated for 1 h was dropped onto a piece of ITO electrode. The modified electrode by BiOBr was dried naturally, followed by calcination at 400 °C for 1 h in a muffle oven. After then, 3 μL of 0.1 mol L^{-1} thioglycolic acid (TGA) aqueous solution was dropped on the BiOBr modified ITO electrode and dried under an infrared lamp for about 10 min. After rinsed with ultrapure water, the ITO/BiOBr/TGA electrode has been prepared successfully. 0.08 mol L^{-1} $AgNO_3$ solution 3 μL was dropped on the ITO/BiOBr/TGA electrode for 30 min in dark place, followed by thoroughly rinsing with ultrapure water to remove uncombined $AgNO_3$. Then, 3 μL of 0.1 mol L^{-1} Na_2S was dropped on the modified ITO electrode for 30 min to form Ag_2S sufficiently. After