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Label-free photoelectrochemical immunosensor for sensitive detection of Ochratoxin A

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ABSTRACT

A general label-free photoelectrochemical (PEC) platform was manufactured by assembly of CdSe nanoparticles (NPs) sensitized anatase TiO_2-functionalized electrode *via* layer-by-layer (LBL) strategy. CdSe NPs were assembled on anatase TiO_2-functionalized electrode through dentate binding of TiO_2 NPs to –COOH groups. Ascorbic acid (AA) was used as an efficient electron donor for scavenging photogenerated holes under visible-light irradiation. The photocurrent response of the CdSe NPs modified electrode was significantly enhanced as a result of the band alignment of CdSe and TiO_2 in electrolyte. Ochratoxin A (OTA), as model analyte, was employed to investigate the performance of the PEC platform. Antibodies of OTA were immobilized on CdSe sensitized electrode by using the classic 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride coupling reactions between –COOH groups on the surfaces of CdSe NPs and $–NH_2$ groups of the antibody. Under the optimized conditions, the photocurrent was proportional to OTA concentration range from 10 pg/mL to 50 ng/mL with detection limit of 2.0 pg/mL. The employed PEC platform established a simple, fast and inexpensive strategy for fabrication of label-free biosensor, which might be widely applied in bioanalysis and biosensing in the future.

1. Introduction

Photoelectrochemical (PEC) assay based on a photoelectric chemical phenomenon has been widely applied in chemical analysis by reason of its high sensitivity, low-cost and low background current (Brown et al., 1992; Li et al., 2012; Zheng et al., 2011). In principle, PEC can be viewed as the inverse process of electroluminescence (ECL) (Cui et al., 2003; Richter, 2004), which exhibits charge-transfer upon photoexcitation and achieves the detection purpose *via* the intensity of the photocurrent. PEC sensors have the advantages of both optical and electrochemical (Ikeda et al., 2009). Many groups have devoted much efforts in developing a novel PEC sensor for detection of chemicals and biomolecules (Yue et al., 2013).

Semiconductor is the key material in fabricating PEC sensor. As a kind of semiconductor material, TiO_2 is generally utilized in the photocatalysis and photoelectrochemistry due to its availability, nontoxicity, photostability and high electronic mobility (Fujishima and Honda, 1972). There are three main kinds of crystalline structure of TiO_2: anatase, brookite, and rutile. The difference of these three crystal structures induces tremendous diversity on the physical properties, so it leads to their specially applied field (Wang et al., 2008). Anatase phase with a high photocatalytic activity is usually used in photocatalysis (Berger et al., 2005). Yet, TiO_2 is a kind of wide band-gap semiconductor material and only has a PEC activity under ultra-violet irradiation (Wold, 1993), these features have taken a toll on its application in photoelectrochemistry. Therefore, either narrowing the band gap of TiO_2 to allow the absorption in the visible region or decreasing the electron/hole recombination is being considered. CdSe nanoparticles (NPs) are an ideal sensitizing material with a narrow-band gap and broad excitation spectrum (Cordes et al., 2006; Díaz et al., 2013); combining that with TiO_2 could greatly improve the photocurrent intensity and photostability of TiO_2 due to the enhanced charge separation efficiency (Nasr et al., 1998; Sant and Kamat, 2002).

Herein, a label-free PEC platform was developed by assembly of CdSe NPs sensitized anatase TiO_2-functionalized electrode *via* layer-by-layer (LBL) strategy. Indium tin oxide (ITO) electrode was functionalized by dropping TiO_2 NPs and calcination induced formation of normalized anatase crystalline structure. Ochratoxin A (OTA) was used as a model analyte in order to investigate the

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performance of the PEC sensor. OTA is a secondary metabolite produced by the genera aspergillus and penicillium (Van der Merwe et al., 1965); it is well known as a fungaltoxin existing in various kinds of foods, fodders, and other agricultural and sideline products (Fernández-Cruz et al., 2010). OTA is the most toxic compound in the ochratoxins group, which has a high chemical stability and thermal stability. With the development of toxicology, studies have shown that OTA is a nephrotoxin, hepatotoxin, carcinogen and teratogen (Heurich et al., 2011; Petzinger and Weidenbach, 2002). Many methods have been applied to detect OTA, such as high performance liquid chromatography (HPLC) (Soleas et al., 2001), high performance liquid chromatography with fluorescence detection (HPLC-FD) (Rhouati et al., 2011), and enzyme linked immunosorbent assay (ELISA) (Heurich et al., 2011). Nevertheless, these methods are expensive, time consuming and demanded the sample to be labeled (Van der Gaag et al., 2003). To the best of our knowledge, this is the first time to determine OTA through label-free PEC method. CdSe NPs with narrow band gap was modified on TiO_2-functionalized ITO electrode *via* dentate binding of TiO_2 NPs to –COOH groups and significantly improve photo-to-electric efficiency of the electrode. Besides, ascorbic acid (AA) acted as an electron donor for scavenging photogenerated holes and could obviously enhance PEC response. The proposed label-free PEC platform opens a new era for sensitive detection of chemicals and biomolecules.

2. Experimental section

2.1. Materials

OTA and OTA antibody were purchased from Wanger Biotechnology Co., Ltd. (Beijing, China). AA, cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) was acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Thioglycolic acid (TGA) was obtained from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China), bovine serum albumin (BSA), N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were obtained from Aladdin Reagent Database Inc. (Shanghai, China). All other chemicals were of analytical reagent grade and were used without further purification. Phosphate buffered saline (PBS) were prepared using 0.1 mol/L Na_2HPO_4 and 0.1 mol/L KH_2PO_4 stock solution. Ultrapure water (18.25 MΩ cm, 24 °C) was used throughout the experiment.

2.2. Apparatus

All PEC measurements were carried out with a home-built PEC system. A common low-cost 30 W LED lamp was used as an irradiation source. Photocurrent was measured on a CHI 760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China) using a three-electrode system which consisted of a platinum wire as a counter-electrode, a saturated calomel electrode as reference electrode and a modified TiO_2-functionalized ITO electrode as the working electrode. Electrochemical impedance spectroscopy (EIS) was obtained from IM6&Zennium electrochemical workstation (Zahner, Germany). Transmission electron microscope (TEM) image was obtained from an H-800 microscope (Hitachi, Japan). Scanning electron microscope (SEM) image was obtained using a field emission SEM (ZEISS, Germany). X-ray diffraction (XRD) patterns were performed with D8 advance X-ray diffractometer (Bruker AXS, Germany). Fourier transform infrared (FTIR) spectrum was obtained with a Perkin-Elmer 580B spectrophotometer (Perkin-Elmer, United States).

2.3. Preparation of TiO_2-functionalized ITO electrode

Before biosensor fabrication, the TiO_2 NPs were prepared according to the literature (Huang et al., 2013). 0.02 mol of tetrabutyl orthotitanate was dissolved into 30 mL of ethanol, following by adding 10 mL distilled water under magnetic stirring, After stirring for another 120 min, the suspension was transferred to the reaction kettle and maintained on the blast oven at 200 °C for 10 h. The obtained white precipitates were washed completely with distilled water and ethanol for five times and then dried in vacuum at 80 °C for 12 h to get the TiO_2 NPs.

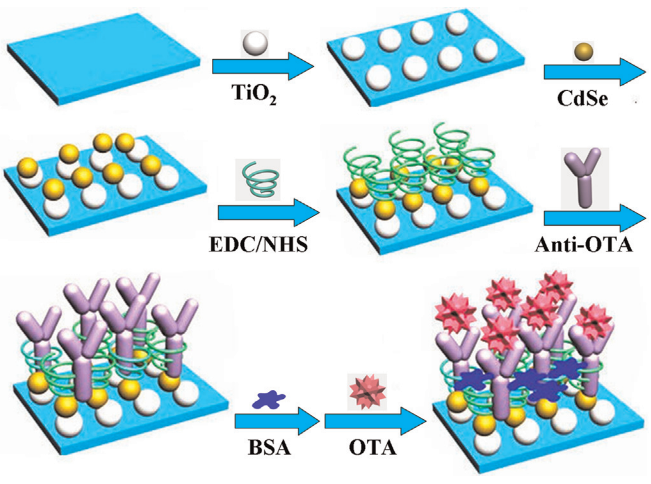
ITO substrates were cut into $3.0 \times 0.8\ cm^2$ pieces and then cleaned ultrasonically in ultrapure water, acetone, ethanol and ultrapure water for 30 min, consecutively. After being dried with a stream of N_2, 6 μL TiO_2 solution which was sonicated in water for 60 min was dropped onto a piece of ITO slice. The film was dried under an infrared lamp, followed by calcination at 450 °C for 30 min in a muffle oven, and then placed for a while to cool down to room temperature.

2.4. Preparation of TGA-stabilized CdSe NPs

The water-soluble CdSe NPs were synthesized according to the reported method (Zhang et al., 2011). Briefly, dissolving 10 mmol Se powder and 12 mmol Na_2SO_3 in 50 mL ultrapure water under magnetic stirring at 90 °C for 3 h will get the Na_2SeSO_3 solution. Then, 0.84 mL TGA diluted with 60 mL distilled water was added into 40 mL of 0.125 mol L^{-1} $CdCl_2 \cdot 2.5H_2O$ solution under magnetic stirring. Thereafter, the pH value of the solution was adjusted to 10 with 1.0 mol L^{-1} NaOH. 12 mL of the obtained Na_2SeSO_3 solution was added into the above solution. The solution was heated to boiling and refluxed for 4 h.

2.5. Fabrication of PEC biosensor via LBL strategy

Fabrication process of the proposed label-free PEC immunosensor is shown as Scheme 1. Generally, 3 μL TGA-stabilized CdSe NPs solution was dropped and immobilized on the TiO_2-functionalized ITO electrode through dentate binding of TiO_2 NPs to –COOH groups. 3 μL EDC/NHS solution was dropped on $CdSe/TiO_2$/ITO electrode to activate –COOH on the surfaces of the TGA-capped CdSe NPs. After washing the unbound EDC/NHS with deionized water, 3 μL OTA antibodies (anti-OTA) were introduced to the modified electrode surface and followed by rinsing with distilled water to remove the loosely bounded anti-OTA. Subsequently, 1% BSA was employed as the blocking buffer solution to



Scheme 1. Schematic illustration of the PEC immunosensor fabrication process.