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Construction of CdS/Ag_2S-based broad-spectrum photoelectrochemical immunosensor for simultaneous assessment of ochratoxins by multivariable linear regression

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ABSTRACT

A broad-spectrum photoelectrochemical (PEC) immunoassay was established to detect ochratoxins by using the CdS nanorod arrays (CdS NRs)/Ag_2S on FTO as the photoelectrode and the CuO@secondary antibody (Ab_2) label as the signal amplification. Due to the advantages of broad-spectrum antibody, such immunosensor exhibits high sensitivity to detect a low concentration of 0.67 ng/L ochratoxin A (OTA), 0.85 ng/L ochratoxin B (OTB) and 0.46 ng/L ochratoxin C (OTC), respectively. Especially, the orthogonal test was used to simulate an actual mixture containing OTA, OTB and OTC, then a multivariable linear regression model was applied to analyze the relationship of the total response with each substance. Finally, the detection of unknown mixture containing different ochratoxins was achieved by the standard addition. Such immunosensor also provided a credible selectivity and stability, suggesting its potential to detect ochratoxins or other similar compounds in food and environment.

1. Introduction

Ochratoxin A (OTA), a toxin produced by *Aspergillus* and *Penicillium* fungi, can be found in contaminated food such as coffee, wine and beverage [1]. The organ toxicity, neurotoxicity, teratogenicity and immunotoxicity of OTA have been found [2]. As the nonchlorinated and ethyl ester-substituted OTA, the analogs of OTA including ochratoxin B (OTB) and ochratoxin C (OTC) can frequently coexist with OTA in contaminated products. It is worth noting that OTB and OTC exhibit similar toxicity as same as OTA, while OTC can be hydrolyzed to OTA after oral or intravenous injection, and OTB can be conversed to OTA even at a low concentration [3–5]. Although many countries have set a restriction of OTA in cereals and relative products, the limits of other two ochratoxins haven't been established [6]. If excess OTB or OTC coexists with OTA, the accumulation of OTB and OTC may cause the possible public problems, thus it is important to detect OTA, OTB and OTC simultaneously.

Up to know, instrument analysis, chemical analysis and immunoassays have been used to detect OTA in food. In general, some traditional instrumental analysis including high performance liquid chromatography with fluorescence detection or mass spectroscopy are always time consuming and expensive [7–9], while thin layer chromatography (TLC) was no longer adopted due to its low specificity and sensitivity [10]. Recently, the immunoassay has been used to detect toxins due to the specific reaction of antigen and antibody [11]. Especially, we have found that the OTB-ovalbumin (antigen) with the antibody produced from OTB-bovine serum albumin (antibody) showed high sensitivity and broad-spectrum for the detection of ochratoxins, where the OTA calibration curve was used to express the equivalent amount of OTA, i.e., OTA, OTB_{OTA}, OTC_{OTA} [12]. However, it should be noted that the signal response was different even at the same concentration of such ochratoxins, thus an accurate and simultaneous detection of each substance is needed.

Compared with the traditional enzyme-linked immunosorbent assay (ELISA), the photoelectrical (PEC) immunosensor has been extensively explored by its high photo-electron transfer efficiency, simple instrument, low cost and high sensitivity [13,14]. With narrow band gap and broad adsorption region under visible illumination, various CdS-based PEC immunosensors have

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been established to detect RNA [15], Cu^{2+} [16], toxins [17] and tumor-associated carbohydrate antigen [18]. However, the photocorrosion of CdS and relevant unstable photocurrent are still puzzled [19], thus many CdS-based composites including CdS:Mn [20], CdS@Cu_2O [21] and CdS/ZnO [22] have been synthesized to improve the photocorrosion. Especially, with the band gap about 0.92 eV, Ag_2S can increase the absorption ability under a broad visible light (below 730 nm), thus it can be used as an efficient substance to couple CdS [23]. Also, the CdS-Ag_2S composite shows stable photocurrent due to the fact that the chemical interface energy and strain make its special crystal structure [24]. Herein, Ag_2S was decorated on CdS nanorod arrays (CdS NRs) as the photoelectrode to anchor antigens, where the effective immobilization of biomolecules including antigen and corresponding antibody can influence the PEC current to reflect the corresponding amount of small molecules based on the competitive method. In addition, the PEC current can be influenced by perfect match of energy levels between the photoelectrode material and other substances including CdS-Cu_2O [25] and CdS-ZnO [26]. As a low band gap energy of 1.7 eV, CuO was used to immobilize the secondary-antibody (Ab_2), which can further increase the PEC current change and thus amplify the signal response.

In this work, CdS NRs/Ag_2S with stable PEC current was prepared as the photoelectrode material to immobilize antigen and anchor antibody, while CuO NPs were synthesized to immobilize Ab_2 and further influence the signal. By using such immunoassay, OTA, OTB and OTC can be separately detected. After that, the orthogonal test was used to simulate a mixture containing OTA, OTB and OTC, then a multivariable linear regression model was applied to analyze the relationship of the total signal with each substance. Finally, the simultaneous detection of OTA, OTB and OTC in unknown mixture was monitored by the standard addition.

2. Experimental

2.1. Materials and apparatus

The chemicals including glutathione (reduced), ascorbic acid (AA), L-cysteine hydrochloride, thiourea and (3-aminopropyl)triethoxysilane (APTES) were obtained from Beijing InnoChem Science & Technology Co. Ltd. N-hydroxysuccinimide (NHS) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were obtained from Beijing J&K Scientific Ltd. Tween 20 and glutaraldehyde were from Acros Organics. The antigen (1 mg/mL) and corresponding antibody (1 mg/mL) were obtained from College of Food Sciences, South China Agricultural University [12]. Secondary goat anti-rabbit antibody (Ab_2, 1 mg/mL) was bought from Santa Cruz. Phosphate buffer solution (PBS) with different pH was prepared by adjusting the ratio of KH_2PO_4 and Na_2HPO_4 at 1/15 M, while PBST was prepared by the addition of 0.5% Tween-20 in PBS (0.01 M, pH 7.4) and used as the washing buffer.

All the PEC experiments were performed using an electrochemical workstation (CHI660D, Chenhua Instruments Co. Ltd., China) with a photoelectrochemical system (PEAC 200A, Ida, China) using LED as irradiation source (20 mW/cm^2). The working electrode was the modified FTO electrode, while the auxiliary electrode and the counter electrode were the Ag/AgCl electrode and Pt electrode, respectively. Laser particle size analyzer (LPSA, Zetasizer Nano ZSE, England) was used to detect particle size distribution of CuO. Scanning electron microscopy (SEM, S-4800, Hitachi, Japan) was used to characterize the morphology of nanomaterials. Energy dispersive spectrometer (EDS, SS550&SEDX-550, Shimadzu, Japan), Fluorescence spectroscopy (F-7000, Hitachi, Japan) and X-ray photoelectron spectroscopy (XPS, Escalab 250Xi, America) were employed to describe the structure, elements and surface properties of nanomaterials, respectively.

2.2. Preparation of CdS/Ag_2S on FTO

Firstly, the slices of FTO substrate were ultrasonically cleaned with ethanol and deionized water alternately, and dried under N_2. After that, 60 mL solution containing 0.462 g $Cd(NO_3)_2{\cdot}4H_2O$, 0.1843 g glutathione (reduced) and 0.335 g thiourea was added in a stainless autoclave, then the cleaned FTO was immersed. The electrode was heated at 210 °C for 6 h and then cooled down, followed by immersing in methanol solution containing 60 μM $AgNO_3$ and stirring for 5 min. Finally, CdS/Ag_2S was annealed at 400 °C for 1 h and cooled down naturally.

2.3. Preparation of the CuO@Ab_2 label

Firstly, the CuO nanopowder was synthesized by a wet method. In the typical process, 0.02 M $Cu(Ac)_2$ and 0.5 mL acetic acid were dissolved in 150 mL distilled water, and then heated to 80 °C under stirring-reflux. After boiling, the pH was adjusted to 6 by adding 0.04 g/mL NaOH. Finally, the black precipitate was formed, and washed with distilled water and ethanol. After heating at 400 °C for 1 h, the CuO nanopowder was obtained.

Secondly, CuO was modified with amino groups to further immobilize biomolecules. Briefly, the CuO nanopowder (0.01 g) was scattered in water/ethanol solution (10 mL, 1/19, V/V), and then acetic acid was added to adjust the pH as 5, followed by the addition of 600 μL APTES and sonication for 20 min. Then, the mixture was dried at 75 °C for 1 h, while the residuals were removed by centrifugation and washed with triplicate ethanol.

Finally, the amino-modified CuO was covalently associated with Ab_2 as follows. In 1 mL PBS, 1.5 mg of amino-functionalized CuO was ultrasonically dispersed, followed by the addition of 1 mL glutaraldehyde solution (2.5%). After incubation for 2 h and centrifugation at 8000 rpm, the precipitate was diluted with 1 mL PBS (pH 7.4, 0.01 M). Then, 0.01 mg of Ab_2 was added and incubated at 4 °C for 1 day under shaking. After centrifugation, the precipitate was redispersed in 1 mL of PBS (pH 7.4, 0.01 M) and the CuO@Ab_2 label was finally obtained.

2.4. Construction of the PEC immunosensor

The fabrication process of the PEC immunoassay was shown in Scheme 1. The CdS/Ag_2S was directly acted as the photoelectrode, followed by immersing in L-cysteine solution (0.5 M) to decorate carboxyl groups. After thoroughly rinsing, EDC (20 mg mL^{-1}) and NHS (10 mg mL^{-1}) in distilled water was dropped on electrode and further incubated for 1 h at 25 °C. Then, 20 μL of 10 μg/mL antigen solution was incubated on the CdS/Ag_2S photoelectrode for 1 h at 37 °C. After rinsing with PBS to remove the unbound antigen, the electrodes were further treated with 20 μL of blocking reagents for 1 h. Then, the competitive format was adopted to detect the concentration of every ochratoxin by dropping a mixture including 10 μL antibody (10 μg/mL) and 10 μL ochratoxin at different concentrations and incubating for another 1 h at 37 °C. Subsequently, 20 μL of the CuO@Ab_2 conjugate was dropped on the photoelectrode for 1 h at 37 °C. Finally, the modified photoelectrode was introduced as the working electrode, and the current-time curve was recorded in PBS solution (pH 7.4, 1/15 M) containing 0.1 M AA on a PEC workstation with the light source of 20 W m^{-2}.