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Broad-specificity photoelectrochemical immunoassay for the simultaneous detection of ochratoxin A, ochratoxin B and ochratoxin C

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ABSTRACT

A broad-specific photoelectrochemical (PEC) immunosensor was developed for the simultaneous detection of ochratoxin A, ochratoxin B and ochratoxin C (OTA, OTB, OTC) by using the direct growth of CdS nanorods on FTO as the photoelectrode and Au nanoflowers-modified glass carbon electrode (GCE) as the bioelectrode. The bioelectrode was used to capture antigens and then associate corresponding antibodies, followed by using SiO_2@Cu^{2+} nanocomposites to conjugate the secondary antibody (Ab_2) and a DNA strand as the initiator. After the hybridization chain reaction (HCR) and the addition of hemin, numerous DNAzymes (G-quadruplex/hemin) were produced. Due to the similar enzymatic property with horseradish peroxidase (HRP), G-quadruplex/hemin can accelerate the oxidation of 4-chloro-1-naphthol (4-CN) with H_2O_2 to yield the biocatalytic precipitation (BCP) on the bioelectrode. Then, the bioelectrode was further treated with moderate acid and thus Cu^{2+} was released, which can decrease the photocurrent of the photoelectrode by the formation of CuS. Due to the advantages of surface effect of Au nanoflowers, DNA amplification and high photoelectrocatalytic activity, the proposed broad-specificity PEC immunosensor can detect OTA, OTB and OTC with a detection limit of 0.02, 0.04 and 0.03 pg/mL, respectively. In addition, the acceptable stability and selectivity suggest its possible application in the detection of OTA, OTB and OTC in water samples.

1. Introduction

As usual, the detection of ochratoxin A (OTA) is acted as the dominant test of ochratoxins including OTA, OTB and OTC, since OTA shows higher toxicity than that of OTB and OTC. However, both OTB and OTC exhibit renal toxicity, hepatotoxicity (Heussner et al., 2006), immunotoxicity (Gan et al., 2017b), teratogenicproperties (O'Brien et al., 2005) and genotoxic effects (El-Nekeety et al., 2017) as same as OTA, while the inhibition effect of OTC on the growth of bacterium is just lower than that of OTA (Xiao et al., 1996). A maximum limit of OTA in unprocessed cereals and processed cereal products has been built up by many countries and organizations. However, a maximum limit of OTB and OTC hasn't been established yet. Therefore, the detection of OTB and OTC should be paid attention as well as OTA.

Although many analytical techniques have been developed to detect ochratoxins including thin layer chromatography (TLC) (Sultan et al., 2014), high performance liquid chromatography (HPLC) (Di Stefano et al., 2015) and liquid chromatography tandem mass spectrometry (LC−MS/MS)(Reinsch et al., 2007), they are limited by the complicated instruments, sample pretreatment or skillful operation. In addition, the simultaneous detection of OTA, OTB and OTC are restricted due to their similar structure (Köppen et al., 2010). Recently, the immunoassay has been broadly explored due to the specificity of the immuoreaction of antibody and antigen. Especially, in our previous work, we used two kinds of immunizing ochrotoxin haptens to raise four antibodies, showing that the antibody from OTB-bovine serum albumin (OTB-BSA-Ab) with the antigen, OTB-ovalbumin (OTB-OVA), exhibits high sensitivity and broad-specificity to OTA, OTB, and OTC. After theoretical calculation, it has been found that the electronegativity of the chlorine atom is the main responsible to increase the antibody binding ability. Therefore, OTB-BSA-Ab and OTB-OVA can not only response with OTB, but also response with OTA and OTC, since both OTA and OTC contain chlorine atoms at the benzene ring (Zhang et al., 2017).

The photoelectrochemical (PEC) immunosensor has been widely used to detect pollutants (Liu et al., 2016), cancer markers (Liu et al., 2017) and DNA methyltransferase (Shen et al., 2015). Among the materials for PEC detection, CdS acts as an eminent material for PEC

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analysis due to its broad absorption range in visible light region and ideal band gap (2.4 eV). Especially, CdS nanorods are excellent field emitter with high emission current density and low turn-on field (Tang et al., 2004; Wei et al., 2017). Even so, the toxicity of CdS should be still considered, since the activity of biomolecules can be severely influenced by the direct immobilization of biomolecules on the surface of CdS. Therefore, the PEC immunosensor with PEC material as the photoelectrode and the biomolecules on the counter electrode as the bioelectrode can effectively separate biomolecules from CdS, which has been used to detect HIV-1 p24 antigen (Zhao et al., 2015), since the activity of biomolecules can be preserved in the construction of the immunosensor (Qileng et al., 2018; Gao et al., 2016).

As for the separate PEC immunosensor, the deposition of non-conductive materials on the bioelectrode can greatly influence the electron transfer, taking an effect on the PEC current. Many substances such as 4-chloro-1-naphthol (4-CN) (Wang et al., 2011) and hydroquinone (Wang et al., 2015) can produce precipitation in the presence of H_2O_2 and horseradish peroxidase (HRP). Nowadays, various HRP mimic enzymes have been explored to overcome the stability of natural enzymes. For instance, with the aid of hemin, the G-rich DNA sequence can form G-quadruplex/hemin, which can exhibit a mimic HRP property with higher catalytic activity than that of hemin (Gan et al., 2017). Since it is a G-rich DNA sequence, many amplification techniques including polymerase chain reaction, rolling circle amplification and hybridization chain reaction (Wang et al., 2014, He et al., 2017) have been used to increase DNA fragments, thus the amount of G-quadruplex/hemin is increased and its corresponding enzymatic ability is promoted. Especially, by the hybridization chain reaction, after the addition of an initiator DNA, a cascade of hybridization can be triggered between two stable species of DNA hairpins and many G-rich DNA sequences can be yielded (Chen et al., 2012).

In this work, a broad-specific PEC immunosensor was constructed to detect OTA, OTB and OTC. CdS NRs with high photocurrent were directly grown on FTO as the photoelectrode, while Au nanoflowers-modified GCE was used as the bioelectrode, and then antigen, antibody and Ab_2-SiO_2@Cu^{2+}-S_0 were successively immobilized. Two auxiliary DNA strands were selected for the in-situ propagation to form a double-helix DNA through hybridization chain reaction, forming numerous DNAzymes (G-quadruplex/hemin) after the addition of hemin, which accelerated the catalytic oxidation of 4-CN by H_2O_2 to yield the precipitation, benzo-4-chlorohexadienone (BCP), on the electrode, thus the electron transfer between the bioelectrode and photoelectrode was greatly hampered. Meanwhile, the bioelectrode was treated with moderate acid, causing the release of Cu^{2+} and then forming CuS on CdS, which can block the escape of photoelectron and further quenched the photocurrent of CdS (Wen et al., 2015). By using the competitive immunoassay, the PEC immunosensor was used to detect OTA, OTB, and OTC in water sample.

2. Experimental

2.1. Materials and apparatus

$Cd(NO_3)_2$, Tris, hemin, glutathione (reduced), thiourea, 4-CN, $Cu(Ac)_2$, ascorbic acid (AA), tetraethyl orthosilicate (TEOS) and (3-aminopropyl)triethoxysilane (APTES) were purchased from Beijing InnoChem Science & Technology Co. Ltd. Tween 20 was purchased from Acros Organics. $HAuCl_4{\cdot}3H_2O$, and dopamine hydrochloride was purchased from Shanghai Macklin Biochemical Co. Ltd. The antigens of ochratoxin B (1 mg/mL) and the antibody of ochratoxin B (Ab_1, 1 mg/mL) were from College of Food Sciences, South China Agricultural University. Secondary goat anti-rabbit antibody (Ab_2, 1 mg/mL) was bought from Santa Cruz. The pH of phosphate buffer solutions (PBS) was adjusted by mixing 1/15 M stock solutions of KH_2PO_4 and Na_2HPO_4 at different ratios, while the washing buffer in the immunoassay was PBST by adding 0.5% tween-20 in 0.01 M PBS 7.4. All DNA sequences were synthesized and purified by Sangon Biological Engineering Technology & Services Co. Ltd, Shanghai. The sequences of initiator stands (S_0) and the auxiliary DNA strands (S_1 and S_2) were listed as follows:

S_0: 5′-CHO-$(CH_2)_6$-GTA CTA CAG CAG CTG-3′,

S_1: 5′-TGGG TAG GGC GGG TTG GGT ATC TCC TAA TAG CAG CAG CTG CTG TAG TAC-3′,

S_2: 5′-CTG CTA TTA GGA GAT GTA CTA CAG CAG CTG-3′.

The photocurrent was measured on a CHI660D electrochemical workstation (Chenhua Instruments Co. Ltd., Shanghai, China) with a photoelectrochemical system (PEAC 200A, Ida, China) using LED as irradiation source (20 mW/cm^2), where the FTO glass was used as the working electrode, with GCE and Ag/AgCl as the counter electrode and the auxiliary electrode, respectively. Scanning electron microscopy (SEM, S-4800, Hitachi, Japan) and transmission electron microscope (TEM, Tecnai 12, FEI, Holland) were used to characterize the morphology of nanomaterials, while X-ray diffraction (XRD, D/max-IIIA, Japan) and energy dispersive spectrometer (EDS, SS550& SEDX-550, Shimadzu, Japan) were used to characterize the structure and element types of nanomaterials, respectively. Circular Dichroism (100, Applied Photophysics, England) was used to monitor the association of the DNA and Ab_2 on SiO_2@Cu^{2+} nanocomposites. Atomic Absorption Spectrometer (SpectrAA 220FS/220Z) was used to monitor the release of Cu^{2+} after the acid treatment of SiO_2@Cu^{2+} nanocomposites.

2.2. In-situ growth of CdS NRs on FTO

CdS NRs were directly grown on FTO with a hydrothermal method. Firstly, a mixture of 0.462 g $Cd(NO_3)_2$, 0.335 g thiourea and 0.1843 g glutathione (reduced) were dissolved in 60 mL water, and transferred into a Teflon-lined stainless steel autoclave. Then, the FTO was cleaned by sonicating in ethanol and water, and immersed into the autoclave with the conducting side facing down. After the autoclave was heated at 210 °C for 6 h, cool down naturally and annealed at 400 °C for 1 h, CdS NRs were directly grown on FTO and used as the photoelectrode.

2.3. Synthesis of Au-nanoflowers

In PE test tube, 225 μL ultrapure water and 125 μL $HAuCl_4$ (2 mM) were mixed, followed by adding 120 μL dopamine hydrochloride (24 mM) and heating at 70 °C for 10 min. Then, the solution was maintained at room temperature for two days, washed and concentrated to 50 μL. The solution was stored at 4 °C and used as the substrate to immobilize the antigens.

2.4. The association of Ab_2 and S_0 on SiO_2@Cu^{2+} nanocomposites

Firstly, SiO_2@Cu^{2+} was synthesized as follows. A mixture of 4 mL TEOS, 142.8 mL ethanol, 20 mL water and 3.2 mL ammonia was stirred for 4 h at 30 °C, rinsed by water and ethanol, and then dissolved in 200 mL ethanol (Lin et al., 2011). After that, 5 mL $Cu(Ac)_2$ (0.6 g/mL) were added in the solution and stirred for 3 h at 30 °C, followed by adding 1 mL NaOH (0.02 g/mL) and stirring for another 3 h. The product was purified by washing with water and ethanol and then dissolved in 40 mL ethanol.

Secondly, SiO_2@Cu^{2+} was further modified with amino groups by treating with 1.6 mL APTES for 24 h. In 1 mL amino-modified SiO_2@Cu^{2+} solution, 10 μL 1 mg/mL Ab_2 was added and stirred. Twenty minutes later, 100 μL of 100 μM S_0 was added and stirred for another 12 h at 4 °C. After centrifugation and washing, the product was dispersed in 1 mL Tris-HAc solution for further use.