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Construction of CdS/B-TiO_2 nanorods photoelectrochemical immunosensor for the detection of microcystin-LR using SiO_2@G-quadruplex as multi-amplifier

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ABSTRACT

A photoelectrochemical immunosensor was developed for the sensitive detection of microcystin-LR (MC-LR) by using the Au nanoclusters as the substrate and silica-functionalized DNAzyme concatamers as the label carrier. The branched TiO_2 nanorods (B-TiO_2 NRs) decorated with CdS nanoparticles were modified on FTO and acted as the photoelectrode, while the bioelectrode was prepared by in-situ electrodepositing Au nanoclusters on dopamine-modified glassy carbon electrode (GCE) to immobilize antigen. Then, silica nanospheres with excellent monodispersity were used to conjugate the secondary antibody and G-quadruplex/hemin, which can accelerate the oxidation of 4-chloro-1-naphthol (4-CN) with H_2O_2 to yield the biocatalytic precipitation (BCP) on the electrode. Thus, the photoelectrocatalytic activity of the CdS/B-TiO_2 NRs photoelectrode can be greatly retarded. By taking the advantages of surface effect of Au nanoclusters, DNA amplification and high photoelectrocatalytic activity, the proposed photoelectrochemical immunosensor can detect MC-LR in a wide range of 0.001–100 μg/L with a detection limit of 0.7 ng/L. In addition, the acceptable stability and selectivity suggested its possible application in the detection of MC-LR in water samples.

1. Introduction

Cyanobacterial blooms induce a serious threat to public health and the environment due to the release of cyanotoxins into headwaters [1]. Microcystins (MCs), the most widespread toxic material from blooming cyanobacteria in fresh and brackish water, represent a tremendous threat to the aquatic ecosystem and human health. Exposure to MCs can lead to serious injury of the liver since it can suppress the activity of protein phosphatases 1 and 2A, two key enzymes in cellular process [2,3]. Microcystin-LR (MC-LR) with 5 nonproteinogens and 2 substitutions of leucine (L) and arginine (R) at positions 2 and 4 is the most widely studied toxic compound among other 90 congeners [4–6]. The World Health Organization (WHO) has set a guideline value of 1 $\mu g L^{-1}$ for MC-LR in drinking water, and the acceptable daily intake value for MC-LR is lower than 0.04 g kg^{-1} [7]. Therefore, it is still a considerable challenge to detect MC-LR in environmental samples due to the low concentration level and the large scale analogues.

Many analytical techniques have been developed to detect MC-LR including high-performance liquid chromatography (HPLC) [8], liquid chromatography tandem mass spectrometry (LC–MS/MS) [9,10] and protein phosphatase inhibition assays [11]. Although these methods are well-proven and widely accepted, they are also complex, costly and time-consuming, especially the possible interference of similar structures and the necessary of various standards. Recently, the immunoassay techniques are of great interest in detection of various pollutants because of their specific molecular recognition without sample concentration or pretreatment. Especially, the photoelectrochemical immunosensors, due to high sensitivity, low cost, and portability, have been used to detect protein [12] and organic molecules [13].

Among the materials for photoelectrochemical detection, titanium dioxide nanorods have been used due to its large surface area, excellent light harvesting and a highly conductive pathway for charge carrier collection [14]. However, its inherent large band

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gap of 3.2 eV only allows the utilization of UV light portion of the solar light. To improve its adsorption in visible region, various modification strategies have been investigated including surface dye sensitization [15], nonmetal doping [16], and composite forming [17] including Au/TiO_2 [18], BiOI nanoflakes/TiO_2 nanoparticles [19] and CdSe/CdS/Pt/TiO_2 [20]. Especially, CdS with a semiconductor with narrow band gap is popular with harvesting photons in the visible region, thus various composites have been synthesized including TiO_2/CdS nanoparticles for interleukin-6 detection [21], TiO_2 nanotube/CdS for prostate specific antigen detection [22] and TiO_2/CdS quantum dots for C-reactive protein detection [23]. However, there are still some arguments about the toxicity of CdS. Namely, the activity of biomolecules can be influenced by CdS if they are directly immobilized on CdS. Recently, the photoelechemical immunosensor with the photoelectrochemical material as the photoelectrode and the biomolecules on the counter electrode as the bioelectrode has found application in the detection of prostate specific antigen (PSA) [24]. The separation of the photon electrode and the bioelectrode can effectively detach biomolecules from CdS, thus the activity of biomolecules can be preserved, which is very important in the construction of immunosensor.

As for the photoelectrochemical immunosensor, the effective immobilization of biomolecules can greatly influence the conductivity of the bioelectrode, which can prevent the electron transfer from the photoelectrode to bioelectrode. To immobilize enough antigens, the bioelectrode surface can be modified with different kinds of nanomaterials including Au nanoparticles [25], Au nanoflowers [26] and Au-composites [27], thus the surface specific area of the bioelectrode can be increased and biomolecules can be easily adsorbed by its affinity such as Au-S and Au-NH_2 bond. After the immunoreaction with antibody, the electrochemical detection label can be amplified by various nanomaterial-based multienzyme labels including horseradish peroxidase [28], glucose [29] or alkaline phosphatase [30]. However, the direct immobilization of enzymes on nanomaterials may influence the activity of enzymes. In addition, the amount of enzymes were also limited by the relative size between enzymes and nanomaterials. Recently, with aid of hemin, the G-rich DNA sequence can form G-quadruplex/hemin, which can exhibit a mimicking enzyme with higher catalytic activity than that of hemin [31] due to its relative stability and small size of DNA sequence.

In this work, a photoelectrochemical immunosensor with separate photoelectrode and bioelectrode was constructed to detect MC-LR. As shown in Scheme 1, the CdS/B-TiO_2 NRs with high photocurrent were synthesized and characterized as the photoelectrode. Then, the bioelectrode was prepared by immobilizing MC-LR antigen on An-nanocluster modified GCE, followed by the immunoreaction with MC-LR antibody and secondary antibody-SiO_2@G-quadruplex. After the addition of hemin, G-quadruplex/hemin can accelerate the catalytic oxidation of 4-chloro-1-naphthol by H_2O_2 to yield a precipitation, benzo-4-chlorohexadienone (BCP), on the electrode, which can greatly hamper the electron transfer between the bioelectrode and photoelectrode. By using the competitive immunoassay, the photoelectrochemical immunosensor was successfully used to detect MC-LR in water sample.

2. Experiment

2.1. Materials and apparatus

Ti[$O(CH_2)_3CH_3$]$_4$, Tris, hemin, $TiCl_3$, 4-chloro-1-naphthol(4-CN), tetraethyl orthosilicate (TEOS) and (3-aminopropyl)triethoxysilane (APTES) were purchased from Beijing InnoChem Science & Technology Co. Ltd. Dopamine hydrochloride and Tween 20 were purchased from Acros Organics. G-quadruplex (5′-CHO-$(CH_2)_6$-TTT GGG TGG GTG GGT GGG T-3′) was purchased from Sangon Biotech Co. Ltd (Shanghai). The antigen of MC-LR (Ag, 1 mg/ mL) and the antibody of MC-LR (Ab, 1 mg/mL) were from College of Food Sciences, South China Agricultural University. Secondary goat anti-rabbit antibody (Ab_2, 400 μg/mL) was bought from Santa Cruz. MC-LR and other interferences were bought from Enzo Life Sciences Company. Phosphate-buffered solutions (PBS) of various pH values were prepared by mixing 1/15 M stock solutions of KH_2PO_4 and Na_2HPO_4 at specific ratios. The washing buffer in the immunoassay was 0.01 M PBS 7.4 containing 0.5% tween-20 (PBST).

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). The FTO was used as the working electrode, while glass carbon electrode (GCE) and Ag/AgCl were used 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 G-quadruplex and Ab_2 on SiO_2 nanoparticles.

2.2. Synthesis of CdS/B-TiO_2 NRs on FTO

Firstly, TiO_2 NRs were directly grown on FTO with a hydrothermal method [16,32]. Generally, the FTO was degreased by sonicating in acetone, ethanol and water, respectively. Then, 0.8 mL titanium butoxide was added into 20 mL of 6 M HCl solution, stirred for 30 min, and transferred into a Teflon-lined stainless steel autoclave, followed by the immersion of FTO into the solution with the conducting side facing down. The autoclave was heated at 170 °C for 6 h and then allowed to cool down naturally, thus TiO_2 NRs were grown on FTO. Successively, TiO_2 NRs were branched by immersing the modified FTO in 50 mL water containing 50 μL of 12 M HCl and 500 μL of $TiCl_3$ at 80 °C for 1 h, and annealing at 350 °C for 1 h. The product was denoted as branched TiO_2 NRs (B-TiO_2 NRs).

Secondly, CdS nanoparticles were deposited on the surface of B-TiO_2 NRs by chemical bath deposition. Briefly, 0.0013 mol $CdCl_2$, 0.0076 mol NH_4Cl, 0.18 mol thiourea and 25.84 mL ammonia solution were dissolved in 500 mL water. Then the B-TiO_2 NRs modified FTO was immersed into the above solution at 80 °C for 10 min. After washing, the electrode was annealed in air at 400 °C for 1 h, which was denoted as CdS/B-TiO_2 NRs.

2.3. Synthesis Ab_2-SiO_2@G-quadruplex

Firstly, SiO_2 nanospheres were synthesized by adding 4 mL TEOS in a mixture of ethanol (142.8 mL), water (20 mL) and ammonia (3.2 mL). After stirring for 4 h at 30 °C, the product was rinsed by water and ethanol and dissolved in 40 mL ethanol. To form amino groups on SiO_2, the above solution were treated with 0.2 mL APTES and stirred for 24 h at 30 °C. Secondly, in 1 mL amino-modified SiO_2 nanospheres solution, 10 μL 1 mg/mL Ab_2 was added and stirred. Twenty minutes later, 100 μL 200 μM G-quadruplex was added and stirred for another 12 h at 4 °C. After filtration and purification, the product was dispersed in 1 mL Tris-HAc solution before use.

2.4. Fabrication of photoelectrochemical immunosensor

As shown in Scheme 1, 3 μL dopamine hydrochloride solution (1 mg/mL) was decorated on the GCE for 30 min at 37 °C. Due to self-