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Cathode photoelectrochemical immunoassay based on analyte-induced formation of exciton trapping for carcinoembryonic antigen detection

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ABSTRACT

A simple and sensitive sandwich-type photoelectrochemical (PEC) biosensor with CuO NPs labeled antibody for detection of protein was constructed. The approach relies on antibodies modified by CuO NPs which could release copper ions. In the presence of the target protein, the released copper ions help form the trapping sites to interfere with quantum confinement of quantum dots (QDs) and thus blocked the escape of photoelectron, leading to a "signal off" PEC method for sensitive detection. This work opens a novel highly sensitive way to detect cancer biomarker carcinoembryonic antigen (CEA) and provides an efficient applicable tool for PEC bioanalysis.

1. Introduction

Carcinoembryonic antigen (CEA) is one of the most widely used cell-surface tumor markers. According to its content, CEA can indicate a variety of tumors and estimate curative effect, patient's condition, and prognosis for some cancers [1–3]. Therefore, it is important to analyze the content of CEA. Many conventional detection techniques, such as radioimmunoassay, electrochemical immunoassay and chemiluminescence assay have been developed [4–9]. Except the high accuracy, some of these techniques involve the disadvantages such as relatively sophisticated instruments, significant sample volume, limited sensitivity, and clinically unrealistic expense and long detection time. Therefore, there is a real need to develop operationally simple, highly sensitive, and inexpensive methods to detect the levels of biomarkers for low-cost and convenient clinic diagnosis.

Recently, some useful methods on photoelectrochemical and quantum are reported [10–13]. As a highly sensitive detection methodology, PEC sensing is a newly developed technology which has drawn growing interest in many fields, such as environmental monitoring and bioanalysis [14–22]. Owing to the separation of excitation signal and detection signal, PEC sensing strategy has plenty of advantages such as low background, low potential different from electrochemiluminescence analysis, which leads to a good analytical performance. Moreover, this strategy can be very easily combined with general immunosensing methods for highly sensitive immunoassay of biomarkers [23–25].

Based on the quantum photoelectric effect of quantum dots (QDs), a "signal on" visual method has been proposed for immunoassay of protein via the formation of insoluble reduction product of nitro blue tetrazolium by the photoelectron escaped from the QDs labeled to the secondary antibody under light excitation [25], and a "signal off" PEC method has also been reported for selective sensing of trace Cu^{2+} by the analyte-induced formation of exciton trapping [26]. This work further combined the "signal off" PEC method with sandwich-type immunosensing strategy to develop a simple and sensitive PEC immunoassay method by using CuO NPs to label the secondary antibody. As shown in Scheme 1, the CdS QDs was coated on the surface of F-doped tin oxide (FTO) electrode to form CdS QDs/FTO electrode, which could produce the photocurrent by the photo induced exciton process [26]. Meanwhile, the CuO NPs were dissolved with acid after immunocomplex was formed, and the obtaining Cu^{2+} solution was dropped on the CdS QDs/FTO electrode to induce the exciton trapping sites, which blocked the escape of photoelectron and thus quenched the photocurrent of QDs. The designed "signal off" immunoassay method exhibited good performance. It extended the application of PEC sensing strategy, and possessed promising potential in clinical diagnosis and detection of low-abundant protein. Compared with CdTe [27], CdS was easier to be synthesized and more stable in air-saturated solution.

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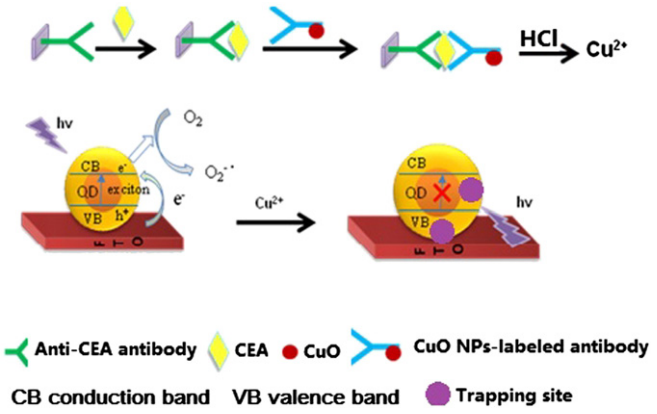

Scheme 1. Schematic illustration of photoelectrochemical immunoassay based on CuO-labeled antibody and copper induced exciton trapping.

2. Experimental section

2.1. Chemicals

Meso-2,3-dimercaptosuccinic acid (DMSA), cadmium chloride ($CdCl_2 \cdot 2.5\ H_2O$) and CuO NPs (50 nm) were purchased from Alfa Aesar China Ltd. Tellurium rod (4 mm in diameter) was purchased from Leshan Kayada Photoelectricity Co., Ltd. Carcinoembryonic antigen (CEA), anti-CEA antibody, bovine serum albumin (BSA), cupric nitrate [$Cu(NO_3)_2 \cdot 3H_2O$] and Tween-20 were purchased from Olive Twigs Biotech. Co. Ltd. (Nanjing, China). F-doped tin oxide (FTO) electrode was purchased from Beijing Midwest Group Technology Co., Ltd. (China). All other chemicals were of analytical grade without further purification. Phosphate-buffered saline (PBS, 0.01 M, pH 7.0) was used throughout the photoelectrochemical (PEC) detection. All aqueous solutions were prepared using ultra-pure water obtained from a Millipore system (≥18 MΩ, Milli-Q, Millipore). Clinical serum samples were obtained from Jiangsu Cancer Hospital.

2.2. Apparatus

A CHI 660D electrochemical workstation (CHI, USA) was used to synthesize DMSA capped CdS QDs. PEC detection was performed on a Zahner intensity modulated photo spectrometer (Zahner, German) with a LW405 LED light as the accessory light source. The X-ray photoelectron spectra (XPS) data was gained by a PHI5000 VersaProbe X-ray photoelectron spectrometer (ULVAC-PHI Co. Japan). Transmission electron microscopy (TEM) was conducted using a JEM-2100 microscope (JEOL, Japan).

2.3. Synthesis of DMSA-CdS QDs

DMSA-stabilized CdS QDs were synthesized by using a slightly modified procedure [28]. Briefly, 250 μL of DMSA was added to 50 mL of 0.01 M $CdCl_2$ aqueous solution, N_2 was bubbled throughout the solution to remove O_2 for 30 min. During this period, 1.0 M NaOH was added to adjust the pH of the above solution to 11. After that, 5.5 mL of 0.1 M Na_2S aqueous solution was injected into this solution to obtain DMSA-capped water-soluble CdS QDs and the reaction mixture was refluxed under N_2 atmosphere for 4 h. Finally, the desired DMSA-stabilized CdS QDs were obtained and then diluted with the same volume of water and stored in a refrigerator at 4 °C for further use.

2.4. Labeling the CEA antibody with CuO NPs

One milligram of CuO NPs was dispersed into 1 mL 0.01 M of PBS by ultrasonication for 10 min. 500 μL 0.2 mg/mL CEA antibody was added into the gained CuO NPs solution over a course of 3 min and vortexed for 3 h at 500 rpm [29]. Then the solution was centrifuged for 10 min at 10,000 rpm, and the solution in the upper layer which contained an unlabeled secondary antibody was disposed. After centrifugation, the antibody labeled with CuO NPs was re-dispersed in 1.5 mL PBS, vortexed for 3 min, and centrifuged for 10 min at 200 rpm to rid the excess CuO NPs as a precipitate. Two hundred microliters 10% BSA in PBS was finally added to stabilize the solution and vortexed for 30 min. The CuO NPs-labeled antibody (in the supernatant) as the probe was thus obtained and stored at 4 °C prior to use.

2.5. Immobilization of primary antibody and reaction with antigen

100 μL 50 μg/mL primary antibody was added to a 96-well plate and incubated at 4 °C overnight. The wells with PBST (PBS including 0.1% Tween 20, 3 × 200 μL) was washed and blocked with 5% BSA (200 μL) for 1 h at 37 °C. The wells were then washed with PBST (3 × 200 μL) and CEA antigen with different concentrations were added into the wells and incubated for 1 h at 37 °C. The wells were once again washed with PBST (3 × 200 μL) and the secondary antibody labeled with CuO NPs was added. After incubation for 1 h at 37 °C, the wells were washed with pure water (3 × 200 μL) for the detection experiments.

2.6. Detection protocol

Twenty microliters of HCl (1 mmol/L) was added in every well, kept the 96-well plate at 500 rpm for 10 min. Then 10 μL of the obtained solution cast onto FTO electrode together with 10 μL of QDs. The mixtures were dried at 37 °C for 20 min to obtain the modified electrode.

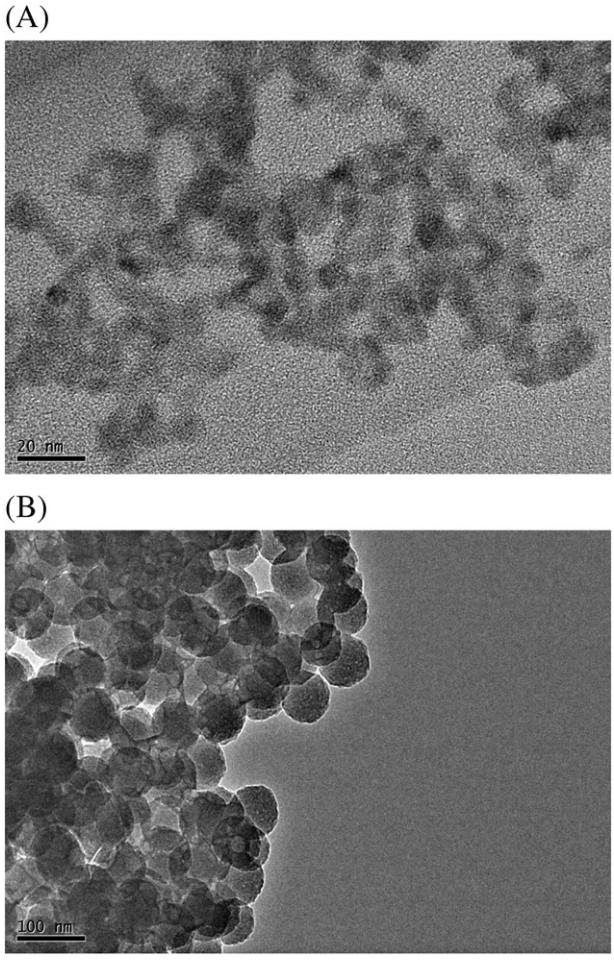

Fig. 1. (A) TEM image of the QDs. The average diameter is around 3–5 nm. (B) TEM image of the CuO NPs. The average diameter is around 50 nm.