Talanta 134 (2015) 496–500

Contents lists available at ScienceDirect

Talanta

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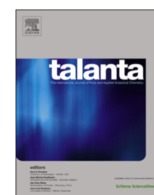

Ultrasensitive photoelectrochemical immunoassay through tag induced exciton trapping

Guangming Wen [a,b], Huangxian Ju [a,*]

[a] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China
[b] School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, PR China

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form
17 November 2014
Accepted 19 November 2014
Available online 27 November 2014

Keywords:
Photoelectrochemistry
Immunoassay
Quantum dots
Exciton trapping
α-Fetoprotein

ABSTRACT

The development of photoelectrochemical (PEC) sensors with novel principles is of significance in realizing sensitive and low-cost detection. This work uses CuO NPs labeled antibody to construct a simple and sensitive sandwich-type immunobiosensor for the detection of protein. The detection signal is produced by dissolving the CuO NPs to release copper ions, which are then added on a quantum dots (QDs) modified F-doped tin oxide to quench the photocurrent of QDs via copper ion-induced formation of exciton trapping. The formed exciton trapping blocks the escape of photoelectron and thus leads to a "signal off" PEC method for sensitive immunoassay. The proposed method shows a detectable range from 0.05 to 500 ng/mL for α-fetoprotein (AFP) with a detection limit (LOD) of 0.038 ng/mL. This work further extends the application of exciton trapping-based PEC biosensing strategy in bioanalysis. The sensitive analytical performance of the designed route implies a promising potential of the PEC sensing in clinical diagnosis.

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1. Introduction

As the levels of tumor markers in serum are associated with the stages of tumors, sensitive and accurate determination of tumor-related biomarkers is critical to clinical diagnosis [1]. In particular, the clinical measurement of tumor biomarkers shows great promise for early diagnosis, cancer monitoring, and highly reliable prediction [2]. It also offers opportunities for understanding the fundamental biological process involved in monitoring patient response to the therapy method [3]. As a tumor marker, α-fetoprotein (AFP) is an oncofetal glycoprotein with a molecular mass of approximately 70 kDa [4], and is mainly produced by the liver, yolk sac, and gastrointestinal tract of a human fetus. It may be found at high levels in the sera of adults having certain malignancies. The increased AFP concentration in adult plasma is usually considered as an early indication of hepatocellular carcinoma [5] or endodermal sinus tumor [6]. Thus, developing a rapid and sensitive detection method for AFP is of great importance in clinical research. Many conventional methods, including enzyme-linked immunosorbent assay (ELISA) [7], electrochemistry [8], electrochemiluminescence (ECL) [9], mass spectrometry [10], quartz crystal microbalance (QCM) [11], and surface plasmon resonance (SPR) immunoassays [12], have been reported for the detection of AFP. 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 clinical diagnosis.

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 [13–21]. 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 [22–24].

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 [24], 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 [25]. This work further combined the "signal off" PEC method with sandwich-type immunosensing strategy to develop a simple and sensitive PEC

* Corresponding author. Tel./fax: +86 25 83593593.
E-mail address: hxju@nju.edu.cn (H. Ju).

http://dx.doi.org/10.1016/j.talanta.2014.11.041
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immunoassay method by using CuO NPs to label the secondary antibody. As shown in Fig. 1, the CdTe QDs was coated on the surface of F-doped tin oxide (FTO) electrode to form CdTe QDs/FTO electrode, which could produce the photocurrent by the photo-induced exciton process [25]. Meanwhile, the CuO NPs were dissolved with acid after immunocomplex was formed, and the obtaining Cu^{2+} solution was dropped on the CdTe 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.

2. Experimental

2.1. Materials and reagents

Meso-2,3-Dimercaptosuccinic acid (DMSA), cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) and CuO NPs (40 nm) were purchased from Alfa Aesar China Ltd. Tellurium rod (4 mm in diameter) was purchased from Leshan Kayada Photoelectricity Co., Ltd. α-Fetoprotein (AFP), anti-AFP antibody and bovine serum albumin (BSA) were purchased from Nanjing Olive Twigs Biotech. Co., Ltd. (China). FTO electrode was purchased from Beijing Midwest Group Technology Co., Ltd. (China). All other chemicals were of analytical grade without further purification. Phosphate buffer 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 ($\geq$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 CdTe 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) were gained by a PHI5000 VersaProbe X-ray photoelectron spectrometer (ULVAC-PHI Co. Japan). The X-ray source was Al target with an applied power of 25 W 15 kV.

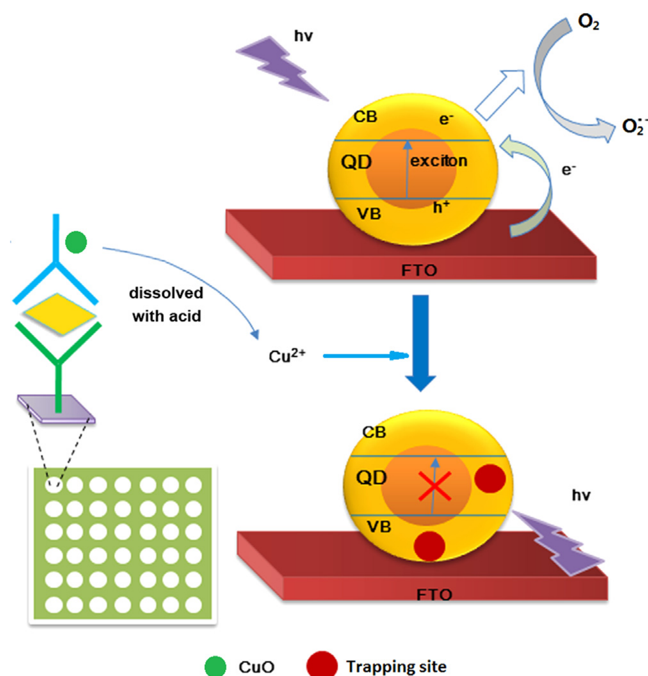

Fig. 1. Schematic illustration of photoelectrochemical immunoassay using CuO NPs-labeled antibody and copper ion-induced formation of exciton trapping on a CdTe/FTO electrode.

2.3. Synthesis of DMSA-capped CdTe QDs

The DMSA capped CdTe QDs were synthesized with an electrolysis method according the previous report [26] using a CHI 660D electrochemical workstation (CH Instruments Inc.). Firstly, 6.5 mg of DMSA, 200 μL of 1 M NaOH, and 120 μL of 0.1 M $CdCl_2$ were added into 20 mL of water in sequence. After being bubbled with highly pure N_2 for 20 min, this solution was used as electrolyte by applying a constant potential of −1.0 V (vs. saturated calomel electrode) on a Te electrode until an electric charge of 0.5 C was reached. During the electrolysis process, the solution was continuously bubbled with highly pure N_2. After the resulting solution was refluxed at 50 °C for 24 h, an equal volume of isopropyl alcohol was added, and the mixture was centrifuged at 8000 rpm for 5 min. The obtained precipitate was washed with a 1:1 mixture of isopropyl alcohol and water and then redissolved in 20 mL of water, which was kept at 4 °C prior to use. After storage for 2 months, the solution remained clear and stable.

2.4. Preparation of CuO NPs-labeled antibody and immunoreaction wells

1 mg of CuO NPs was dispersed into 1 mL 0.01 M of PBS by ultrasonication for 10 min. 500 μL 0.2 mg/mL AFP antibody was then added into the dispersion over a course of 3 min and vortexed for 3 h at 500 rpm. The mixture was centrifuged for 10 min at 10,000 rpm to obtain the CuO NPs-labeled antibody, which 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. 200 μL 10% BSA in PBS was finally added and vortexed for 30 min to stabilize the solution. The CuO NPs-labeled antibody was stored at 4 °C prior to use.

100 μL 50 μg/ml primary antibody was added to each well of 96-well plate and incubated at 4 °C overnight. The wells were washed with PBST (PBS including 0.1% Tween 20, 3 × 200 μL) and blocked with 5% BSA (200 μL) for 1 h at 37 °C, which were then washed with PBST (3 × 200 μL) to obtain the immunoreaction wells.

2.5. Detection protocol

After 10 μL AFP solutions or samples were added into the wells and incubated for 1 h at 37 °C, the wells were washed with PBST (3 × 200 μL) and 10 μL CuO NPs-labeled antibody was added in each well, which were incubated for 1 h at 37 °C and thrice washed with water. 20 μL HCl (1 mmol/L) was then added in every well to react at 500 rpm for 10 min. 10 μL of the obtained solution was finally used to perform the PEC detection in 10 mL pH 7.0 PBS at an applied voltage of −0.2 V vs. SCE and an illuminating wavelength of 405 nm and intensity of 50 W m^{-2} with an CdTe QDs/FTO electrode as working, a platinum wire electrode as the auxiliary and a saturated calomel electrode as the reference electrodes. The CdTe QDs/FTO electrode was prepared by dropping 10 μL of DMSA-capped CdTe QDs solution and drying at 37 °C for 20 min.

3. Results and discussion

3.1. PEC biosensing mechanism

The PEC response of the CdTe QDs/FTO electrode comes from the light-induced formation of excitons under light excitation, which release negatively charged electrons into the vacant conduction and then are adopted by dissolved oxygen [25] (Fig. 1). The unpassivated surface of QDs possesses low surface energy level

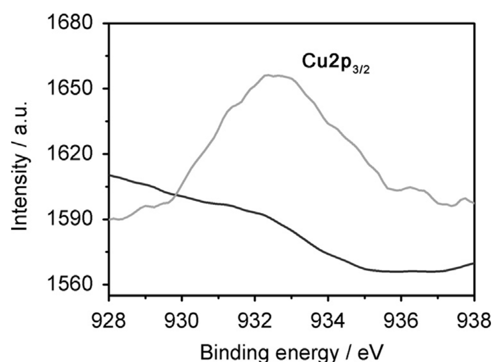

Fig. 2. XPS Cu 2p spectra of antibody before (lower) and after (upper) labeled with CuO NPs.

and narrow band gap, which makes the surface electron transfer easier [27,28] and thus produces sensitive PEC response. In the presence of copper ions, the cupric ions can be captured on the surface of CdTe QDs due to the competition between Cu^{2+} and Cd^{2+} of QDs to S atom in DMSA molecule, where the sedimentation equilibrium constant of CuS (8×10^{-36}) is much smaller than that of CdS (7×10^{-27}). Under light excitation the excitons formed from the QDs can reduce the Cu^{2+} to produce a mixture of CdS^{-}–Cu^{+}, in which S comes from DMSA on the surface of QDs, thus resulting in the formation of the trapping sites [25]. The formed trapping sites inhibit the formation of exciton and thus blocked the escape of photoelectron, leading to a “signal off” PEC method for sensitive immunoassay.

3.2. XPS characterization of the CuO NPs-labeled antibody

The labeling of CuO NPs to the secondary antibody could be demonstrated with XPS measurements. No specific peak for copper element was observed before the labeling process (Fig. 2, blue line), indicating that the antibody did not contain Cu (II). After the labeling reaction of CuO NPs to the antibody, a broad peak around 932.6 eV occurred (Fig. 2, red line), which was attributed to the electronic binding energy of Cu (II). Therefore, the CuO NPs were successfully labeled to AFP antibody.

3.3. Optimization of experimental conditions

The applied potential is an important factor that is relevant to the photocurrent response. On account of the elimination of interference from other reductive species that coexisted in the samples, low applied potentials ranging from −0.2 V to +0.1 V vs. SCE were examined (Fig. 3A). At irradiation intensity of 50 W m^{-2} the response increased with the changing potential from +0.1 V to −0.2 V, at which the response was large enough to achieve the sensitive immunoassay. Therefore, −0.2 V was selected for the photocurrent measurements, at which the electron was transferred from the FTO electrode to the orbital hole in the valence band of QDs to form the exciton on the surface of QDs, the exciton released electron into the vacant conduction and then was accepted by O_2 to produce the photocurrent [25].

The effect of irradiation intensity on photocurrent was examined from 0 to 100 W m^{-2}. As shown in Fig. 3B, the photocurrent response at an applied potential of −0.2 V sharply increased with the increasing irradiation intensity, and trended a pseudo-plateau at 50 W m^{-2}. Thus, 50 W m^{-2} was chosen as the optimized light intensity.

The photocurrent response also greatly depended on the pH of electrolyte. At too acidic solution, the QDs could easily be decomposed; thus the photocurrent was measured at in the pH range

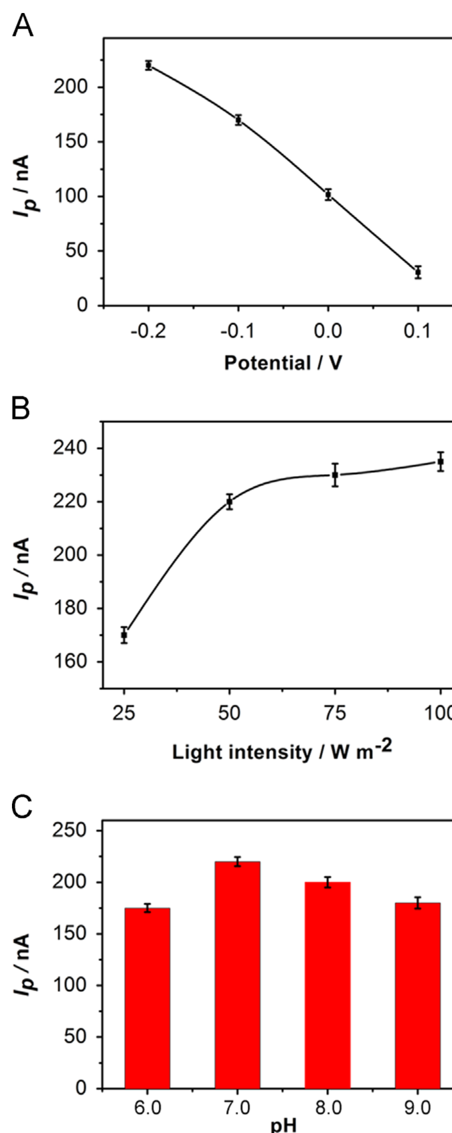

Fig. 3. Effects of (A) potential, (B) light intensity, and (C) pH of 0.01 M phosphate buffer saline on photocurrent response of CdTe/FTO electrode (error bar represents three independent measurements).

of 6.0–9.0 at an irradiation intensity of 50 W m^{-2} (Fig. 3C). With the increasing pH, the response increased and reached the maximum at pH 7.0. At high pH, Cd^{2+} exposed at the surface defect sites would adsorb the OH^{-}, which hindered the electron transfer from QDs to O_2, and thus lowered the photocurrent. This work used 0.01 M pH 7.0 phosphate buffer throughout the following experiments.

3.4. Detection of AFP

Under optimized conditions, the CdTe QDs/FTO electrode showed sensitive photocurrent response, which decreased due to the copper ion-induced formation of exciton trapping (Fig. 4A). With the increasing AFP concentration, the amount of CuO NPs in the immunocomplex increased; thus the concentration of Cu^{2+} in the acidic solution increased, which decreased the photocurrent to a greater degree. The plot of I_p vs. the logarithm of AFP concentration showed a good linear relationship (Fig. 4B). The linear range was 0.05–500 ng/mL with a detection limit (LOD) of 0.038 ng/mL calculated from 3σ. The linear response range was wider than those based on fluorescence resonance energy transfer (0.8–45.0 μg/L) [29], time-resolved fluoroimmunoassay (0.1–100 μg/L) [30], near-infrared fluorescence

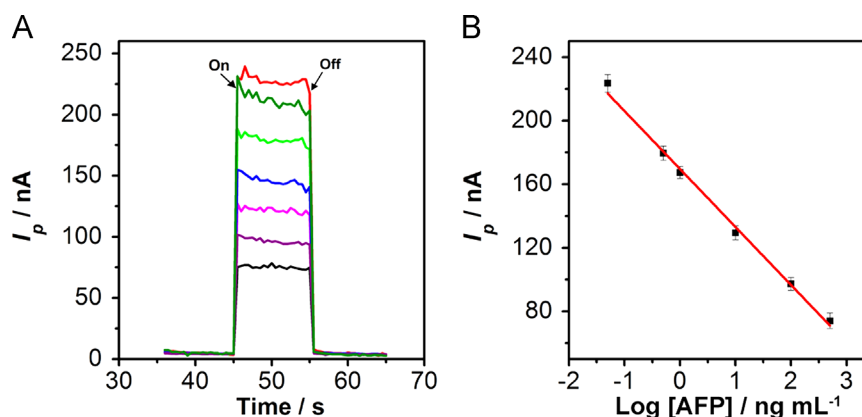

Fig. 4. (A) Photocurrent responses of CdTe/FTO electrode to 0, 0.05, 0.5, 1, 10, 100 and 500 ng/mL AFP (from up to bottom) in air-saturated pH 7.0 0.01 M PBS at −0.2 V vs. SCE. (B) Calibration curve for AFP immunoassay.

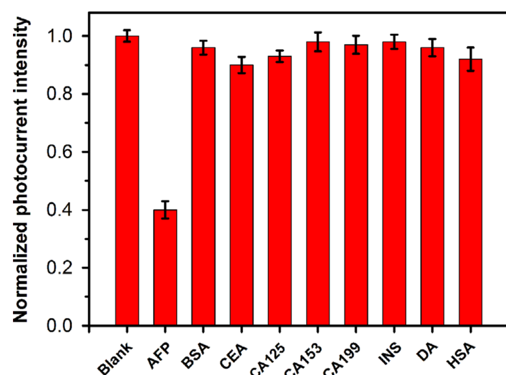

Fig. 5. Normalized photocurrent intensities of CdTe/FTO electrode in the presence of 100 ng/mL individual interference under the same conditions as in Fig. 4 (error bar represents three independent measurements).

immunoassay (1.9–51.9 ug/L) [31] and chemiluminescent immunoassay (0.1–5.0 μg/L or 1.0–120 μg/L) [32,33]. The LOD was also lower than those of fluorescence resonance energy transfer (0.41 μg/L) [29], time-resolved fluoroimmunoassay (0.1 μg/L) [30], chemiluminescent immunoassay (0.01 μg/L, 0.16 μg/L) [32,33], fluorescence quenching (11.7 μg/L) [34], laser-induced fluorescence (1 μg/L) [35] and gold nanoparticles based immunoassay (49.3 μg/L) [36], showing better analytical performance. The wide linear range and low LOD extended the practical application of the proposed method.

To test the utility of the proposed approach for real samples, this method was employed to detect AFP in a human serum sample. The analytical result of 75 ng/mL for AFP was comparable with the result of 70 ng/mL obtained by enzyme-linked immunosorbent assay (ELISA), indicating an acceptable accuracy.

3.5. Interferences

To evaluate the selectivity of the presented sensing system, the interferences of HSA, BSA, CEA, CA125, CA129, CA153, insulin (INS), and dopamine (DA) were tested at the same experimental conditions (Fig. 5). These proteins did not show obvious interference for the detection. Thus, the photoelectrochemical strategy showed good selectivity in the detection of AFP.

4. Conclusions

By integrating CuO NPs-labeled antibody for sandwich-type immunoreaction, a PEC immunoassay method is proposed with a CdTe QDs/FTO electrode. The immunocomplex can release copper ions to react with QDs at an optimal irradiation intensity of 50 W m^{-2} and applied potential of −0.2 V, which forms trapping sites on QDs surface to inhibit the formation of exciton and the escape of photoelectron, thus decreases the photocurrent. The proposed "signal off" PEC immunoassay method shows high sensitivity, low detection limit, and wide detectable concentration range. Moreover, the method can be simply extended for the detection of other targets, providing a highly efficient tool for bioanalysis.

Acknowledgments

This research was financially supported by National Basic Research Program of China (2010CB732400), National Natural Science Foundation of China (21135002 and 21121091), and China Postdoctoral Science Foundation (2014M561619).

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