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A novel signal-on photoelectrochemical immunosensor for detection of alpha-fetoprotein by in situ releasing electron donor

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ABSTRACT

A signal-on photoelectrochemical (PEC) immunosensor was constructed for detecting tumor marker in this work. α-fetoprotein (AFP) was chosen as a model analyte to investigate the prepared procedure and the analytical performance of the exploited sensor. In order to construct the sensor, CdSe QDs were used as photoactive material, biotin conjugated AFP antibody (Bio-anti-AFP) as detecting probe, streptavidin (SA) as signal capturing unit, biotin functionalized apoferritin encapsulated ascorbic acid (Bio-APOAA) as amplification unit, which were assembled onto the electrodes. The sensing strategy was based on in situ enzymatic hydrolysis of Bio-APOAA to release ascorbic acid (AA) as sacrificial electron donor to produce photocurrent. The photocurrent from the immunosensor was monitored as a result of AFP concentrations. The constructed sensing platform displayed high selectivity and good sensitivity for detecting AFP. Under optimal conditions, a wide linear range from 0.001 to 1000 ng/mL and a low detection limit of 0.31 pg/mL were obtained. The developed immunosensor is expected to be used to determine AFP and other tumor markers in human plasma in clinical laboratories either for pre-cancer screening or cancer monitoring. Moreover, this sensing platform further has the potential to use for the detection of trypsin activity and the corresponding inhibitor-screening.

1. Introduction

Currently, cancer is one of the main threats to human beings and their health. The keys of clinical recovery are early diagnosis, early treatment and early detection of tumor markers (Du et al., 2010). AFP as a widely used tumor marker is an oncofetal glycoprotein, which produced by the yolk sac and the liver during fetal life (Zheng et al., 2016). The elevated AFP concentrations in adult plasma is generally considered to be an early indicator for prognostics and diagnosis of some cancerous diseases including hepatocellular cancer, endoderm carcinoma, testicular cancer, teratoma, yolk sac cancer, ovarian cancer and liver metastasis from gastric cancer (Giannetto et al., 2011). There are several conventional and international methods for detecting AFP, such as radioimmunoassay (Chuang et al., 2004), enzyme-linked immunosorbent assay (Ci et al., 1995), electrochemiluminescence assay (Zhou et al., 2015), electrochemical (Gao et al., 2015; Xie et al., 2016) and fluorescence detection (Wang et al., 2014). Although high accuracy has been obtained, some of these protocols involve some disadvantages, such as relatively sophisticated instruments, limited sensitivity, high cost and so on. Thus, developing novel, highly sensitive, easy operational and inexpensive methods to detect trace amount of AFP for early discovery and diagnosis of cancers remains a challenge for humans.

Photoelectrochemical (PEC) biosensing is a newly developed analytical method, which couples photoirradiation with electrochemical detection (Dai et al., 2017; Haddour et al., 2006; Zhang et al., 2017; Zeng et al., 2014). The photocurrent generating mechanism is that the photocatalytic reduction or oxidation of biomolecules induces the detectable photoelectronic transfer between the target and electrodes under light irradiation (Wang et al., 2017; Wu et al., 2017; Yang et al., 2017; Yu et al., 2015). In recent ten years, more and more PEC immunosensors have been used for detecting AFP owing to their rapid response, easy operation and low-cost (Dai et al., 2015; Liu et al., 2015; Li et al., 2012; Sun et al., 2015; Wen and Ju, 2015; Xu et al., 2015; X. Yang et al., 2015). However, most of these exploited immunosensors belong to the kind of signal-off. It has been reported that the signal-on mode demonstrated better accuracy, higher sensitivity and selectivity for practical sample when compared with signal-off mode (Song et al., 2016; Z. Yang et al., 2015; Zhao et al., 2012a). The reason is that the maximum signal from signal-off sensors can only reach the 100% of the original signal under any experimental conditions (Pan et al., 2012; Yu et al., 2012). Considering from the aspect, it is meaningful to exploit

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signal-on immunosensors in future application.

It has been reported that the output anodic photocurrent of a typical PEC sensor was directly proportional to the quantity of the solution-solubilized electron donor in a certain range (Hao et al., 2012; K. Wang et al., 2012; Zhao et al., 2012a). Therefore, the photocurrent intensity could be changed by controlling the concentration of the electron donor. On a PEC immunosensing platform, the insulated protein multilayer, which formed on the electrode surface, severely blocks the electron transfer from the electron donor in the solution toward the electrode surface and decreases the photocurrent intensity (Haddour et al., 2004; Liu et al., 2017; Tan et al., 2017; Zhang et al., 2011; Zhao et al., 2012b). Therefore, we wanted to prepare the electron donor encapsulation, which could be assembled to the surface of the electrode, and the electron donor was released by enzymolysis of the corresponding encapsulation, thereby generating photocurrent. In this strategy, increased antigen/antibody concentration results in the enhanced loading of the electron donor encapsulation, and thus boosts the electron donor generation for improved photocurrent responding. Therefore, a signal-on PEC immunosensor could be obtained.

To construct the PEC immunosensor, CdSe QDs were used as photoactive material, Bio-anti-AFP as detecting probe, SA as signal capturing unit, Bio-APOAA as amplification unit, which were assembled onto the electrodes. The sensing strategy was based on in situ enzymatic hydrolysis of Bio-APOAA to release AA as sacrificial electron donor to simulate the photocurrent generation. In this work, the preparation of the electron donor encapsulation is very important. It has been reported that apoferritin (APO) as a protein cage had been widely used to synthesize bio-inorganic or bio-organic nanocomposites (Blazkova et al., 2013; Kim et al., 2011). AA was one of the molecules, which have been trapped in the cavities of APO (Chen and Zhao, 2017; Yin et al., 2014). Interestingly, enzyme can specifically hydrolyze the obtained encapsulation. The in situ release of AA as a sacrificial electron donor was employed. The immunosensor relied on monitoring the increasing photocurrent intensity as a result of adding AFP because of the immune recognition reaction between AFP and Bio-anti-AFP. The more AFP was connected, although the insulated protein multilayer blocked the interfacial electron transfer, the more APOAA connected simultaneously. Thus the more AA released and the higher of photocurrent intensity we obtained. Therefore, we may gain a signal-on, sensitive and specific immunosensor for detecting of AFP.

2. Experimental section

2.1. Materials and reagents

AFP and Bio-anti-AFP, prostate specific antigen (PSA) and carcinoembryonic antigen (CEA) were obtained from Beijing Key-Bio. Biotech. CO., LTD. (Beijing, China). CdSe QDs were bought from Wuhan Jiayuan Quantum CO., LTD. (Wuhan, China). Human serum samples were presented from Wuhu center blood station. APO and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich (America). Chitosan (CS) and Glutaraldehyde (GLD, 25%) were obtained from Sinopharm Chem. Re. Co. Ltd. (Shanghai, China). SA and biotin-N-Hydroxysuccinimide (Bio-NHS) were obtained from Aladdin reagent Co. Ltd. (Shanghai, China). Phosphate buffer (PBS, 0.1 M, pH 7.80) was used as detection buffer for the photocurrent measurements. PBS (10 mM, pH 7.40) was used as washing buffer. All other reagents were of analytical grade and used without further purification as received. All aqueous solutions were prepared using deionized water (≥18 MΩ cm^{-1}), which was obtained from a Milli-Q water purification system.

2.2. Apparatus

The ITO slices (Wuhu, China, type STN, ITO coating 30 ± 5 nm, sheet resistance ≤10 Ω/square) were presented from Wuhu Token Sciences Co., Ltd. The photocurrent was measured on a home-made PEC system. A 500 W Xe lamp was served as the light source on the front of the electrode and equipped with a monochromator. Photocurrent measurements and Electrochemical impedance spectroscopy (EIS) were performed by coupling the CHI electrochemical workstation (660b, Shanghai Chenhua Instrument Co., Ltd., China) with a conventional three-electrode system: a CdSe QDs modified ITO electrode, a platinum wire and an Ag/AgCl (saturated KCl solution) electrode were as the working, counter and reference electrodes, respectively. The photocurrent detections were conducted at a constant potential of −0.5 V with a 0.1 M PBS (pH 7.80) containing 0.1 M AA or 1 mg/mL trypsin as the supporting electrolyte. EIS was performed in 0.1 M KCl containing 5.0 mM $K_3[Fe(CN)_6]$-$K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe at an applied voltage of 5 mV over a frequency range from 0.1 Hz to 100 kHz.

2.3. preparation of Bio-APOAA

The APOAA and Bio-APOAA were prepared according to previous reports (Chen and Zhao, 2017; Yin et al., 2014) with minor modifications. The Bio-APOAA was prepared as follows: 1 mg/mL biotin-NHS solution (0.1 M PBS, pH 7.40) mixed with as-obtained APOAA solution (Chen and Zhao, 2017) (v/v: 4/1) and stirred for 3 h at room temperature. Then, a dialysis for 36 h was carried out to remove free biotin-NHS using the MD10 dialysis bag (Union Carbide Corporation, USA) with a molecular weight cutoff (MWCO) of 8 000–14 000 against 0.1 M PBS (pH 7.40). The dialysis process was repeated for three times. The prepared Bio-APOAA solution was stored at 4 °C for further use.

2.4. Preparation of the modified electrodes

The ITO slices were sonicated in acetone, deionized water, ethanol, deionized water and NaOH (1 M) in 1:1 (v/v) ethanol/water successively for about 20 min each, followed by washing copiously with deionized water, and then used as the working electrodes. To ensure the area of the ITO electrode, the detailed preparation process was as follows. The electrodes were cut to dimensions of 0.5 cm×6 cm. Then, the electrodes were affixed with adhesive tape, leaving with a fixed area of 0.5×0.5 cm^2 before modifying. Optimally, 20 μL CS (0.05%) and 25 μL CdSe (8 nM) were dropped onto the 0.25 cm^2 active area of the ITO electrode surface (ITO/CdSe). After drying in air, the ITO/CdSe was ringed with 1 M NaOH solution. Then 20 μL GLD (5%) was further dropped on the electrode surface and reacted with CS for 30 min, the ITO electrode was washed copiously with deionized water.

The modified electrodes were performed as follows then: the ITO/CdSe electrode was incubated with AFP (a certain concentration, 20 μL), which could assembled onto the electrode. It was placed at 4 °C more than 12 h to obtain ITO/CdSe/AFP. Then, the gained electrode was immersed into blocking solution of BSA (500 μL, 1%) for 1 h at 37 °C to eliminate nonspecific adsorption and block any possible remaining active sites. The modified electrode was marked as ITO/CdSe/AFP/BSA. Then the obtained electrode was incubated into Bio-anti-AFP (10 μL, 30 mg/mL) at 37 °C for 40 min, which was captured onto the surface of the electrode to form the antigen-antibody immunocomplex. The electrode was noted as ITO/CdSe/AFP/BSA/Bio-anti-AFP. The electrode was washed with PBS thoroughly after each step of modified process.

The above obtained electrode was further placed into 20 μL PBS (0.1 M, pH7.40) containing 1 mg/mL SA for 1 h. The SA conjugated onto the electrode surface through the specific interaction between biotin and avidin (the electrode was recorded as ITO/CdSe/AFP/BSA/Bio-anti-AFP/SA). Finally, the electrode was incubated in 20 μL of Bio-APOAA solution for 1 h, followed by washing with PBS. The modified electrode indicated as ITO/CdSe/AFP/BSA/Bio-anti-AFP/SA/Bio-APOAA was obtained. Ultimately, the obtained electrodes were stored at 4 °C for further measurement.