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Electrochemiluminescent immunosensing of prostate-specific antigen based on silver nanoparticles-doped Pb (II) metal-organic framework

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ABSTRACT

In this work, silver nanoparticles-doped Pb (II) metal-organic framework (Ag-MOF) was prepared and exploited as a luminescence probe for the development of label-free electrochemiluminescence (ECL) immunosensing scheme for prostate-specific antigen (PSA). The β-cyclodextrin based MOF, Pb-β-cyclodextrin (Pb(II)-β-CD) shows excellent ECL behavior and unexpected reducing capacity towards silver ions. Silver nanoparticles could massively form on the surface of Pb(II)-β-CD (Ag@Pb(II)-β-CD) without use any additional reducing agent, while the ECL behavior of Pb(II)-β-CD still was well retained. The Ag@Pb(II)-β-CD was used as a substrate material to modify glass carbon electrodes and formed a sensing platform for the fabricating ECL immunosensor. The presence of silver nanoparticles enables the facile immobilization of capturing antibody of PSA. The specific binding of PSA onto the electrode surface induces the decrease of ECL signals. A linear range of 0.001–50 ng mL^{-1} with a detection limit of 0.34 pg mL^{-1} ($S/N=3$) was obtained after the optimization of experimental conditions. This simply fabricated immunosensor exhibits good stability, accuracy and acceptable reproducibility, which suggesting its potential applications in clinical diagnostics.

1. Introduction

Metal-organic frameworks (MOFs) have attracted extensive research interests, especially in the fields of ion exchange, gas storage and separation (Ma et al., 2009), sensing probe and catalysis (Bai et al., 2013; Rebilly et al., 2009).As a special type of porous materials, cyclodextrins (CDs)-based MOFs has recently attracted considerable attention attributing to its special structure and properties. CDs have a large number of glycosidic oxygen atoms and can provide plenty of coordination sites to chelate metal ions (Wei et al., 2012). Moreover, the water solubility and biocompatibility of CDs (Liu et al., 2007) make CDs-based MOFs good candidates for application in biological sensing field. In the present work, a novel type of MOFs has been prepared from β-cyclodextrin and lead ions (Pb(II)-β-CD). Here, we report for the first time the electrochemiluminescence (ECL) of Pb(II)-β-CD using $K_2S_2O_8$ as a coreactant. Pb(II)-β-CD shows excellent ECL behavior and unexpected reducing capacity towards silver ions. Silver nanoparticle-doped Pb(II)-β-CD (Ag@Pb(II)-β-CD) was prepared by a simple method and used to fabricate ECL immunosensor. Ag nanoparticles can enhance the ECL intensity of Pb(II)-β-CD and improve the sensitivity of ECL immunosensor (Li et al., 2013).

Immunosensors, based on the highly specific molecular recognition between antigen and antibody, are the most widely used biosensors in clinical examinations (Skottrup et al., 2008), biochemical analyses of environmental pollutants (Jiang et al., 2008) and food quality control (Chen et al., 2010). In immunoassay, the determination of cancer markers associated with certain tumors plays an important role in diagnosing cancer diseases (Kim et al., 2010). The 5-year survival rate of cancer patient can be elevated with early detection and active therapy. Therefore, development of simple method to detect cancer markers is very necessary and important. Prostate-specific antigen (PSA) is the best serum marker which is currently available for the detection of prostate and breast cancer (Jang et al., 2015). It is well known that the PSA concentration for normal person ranges from 0 to 4 ng mL^{-1} (Yang et al., 2010).

There are two types of PSA immunosensors: sandwich-type immunosensors and label-free immunosensors. Label-free detection is particularly attractive for monitoring of biomolecular interactions. But sandwich-type immunosensors can alter the binding affinity of biomolecules, increase the analysis cost and complicate the assay procedure (Fan et al., 2008). In this work, a new type label-free ECL immunosensor for detecting PSA was developed using the as-prepared Ag@Pb(II)-β-CD as a substrate material. The detection mechanism was based on the specific

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binding between antibody and antigen. The detailed process was that firstly, antibody of PSA was immobilized on the surface of Ag@Pb(II)-β-CD through adsorption ability of Ag towards proteins (Brondani et al., 2013; Jin et al., 2013). Therefore, only PSA antigen can combine the modified electrode surface through specific binding with PSA antibody. The ECL mechanism was investigated and the analytical performance of this immunosensor was studied. This work extends the applications of CDs-based MOFs and provides a versatile avenue for selective and sensitive detection of PSA.

2. Experimental section

2.1. Materials

PSA and PSA antibody (anti-PSA) were obtained from Beijing Biosynthesis Biotechnology Co. Ltd., (Beijing, China). $AgNO_3$, β-CD, $PbCl_2$, β-cyclohexanol and triethylaminewere purchased from Shanghai Chemical Reagent Co. Ltd., (Shanghai, China). Chitosan and BSA were obtained from Sigma-Aldrich. Phosphate buffer saline (PBS) was prepared by using 1/15 mol L^{-1} Na_2HPO_4 and 1/15 mol L^{-1} KH_2PO_4 solution. All aqueous solutions were prepared using ultrapure water. Chitosan was dissolved in 1% acetic acid.

2.2. Apparatus

Transmission electron microscope (TEM) images were obtained from an H-800 microscope (Hitachi, Japan). Scanning electron microscope (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) were recorded by JEOL JSM-6700F microscope (Japan). X-ray photoelectron spectroscopy (XPS) spectra were recorded with an Escalab MK II (VG Company, UK). The ECL measurements were performed with a MPI-F flow-injection chemiluminescence detector (Xi'an remax Electronic Science Tech. Co. Ltd., China) and electrochemical measurements were carried out on CHI760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd, China) using a three-electrode system consisted of a platinum wire as an auxiliary electrode, an Ag/AgCl electrode as reference electrode, and a glassy carbon electrode (GCE, 4 mm in diameter) as working electrode.

2.3. Preparation of Ag@Pb(II)-β-CD

Pb(II)-β-CD was prepared as described previously with some slight modifications (Wei et al., 2012). 23 mg β-CD and 45 mg $PbCl_2$ were dispersed in 6 mL ultrapure water. The mixture was stirred at 80 °C until no further precipitate is produced. After removing precipitate, the mixture was transformed to reaction kettle, then 6 mL mixed solventof cyclohexanol and triethylamine (v/v=1:1) was slowly added. The reaction kettle was then sealed and heated to 110 °C for 3 days. The finally product was washed with ultrapure water and dried in air to obtain Pb(II)-β-CD. The prepared Pb(II)-β-CD was heated at 160 °C for 0.5 h before use.

Ag@Pb(II)-β-CD was synthesized as follows: 55 mg Pb(II)-β-CD was dispersed in 50 mL ultrapure water by ultrasonication for 4 h. Then, 100 mg $AgNO_3$ was added in the above solution and stirred until the color changed to dark red, followed by centrifuging to remove unreacted Ag^+. The finally product was vacuum dried at 35 °C for 12 h. The obtain Ag@Pb(II)-β-CD and it was dispersed in 0.5% chitosan until use.

2.4. Fabrication of the ECL immunosensor

Fig. 1 shows the fabrication procedure of the immunosensor. Firstly, GCE was polished to a mirror-like finish with 1.0, 0.3 and 0.05 μm alumina powder and then thoroughly cleaned with ultrapure water and dried in air. Ag@Pb(II)-β-CD was dropped on the surface of well-polished GCE and dried at 4 °C. Anti-PSA was dropped on the electrode surface and after drying, the modified electrode was washed with ultrapure water. Then, 5 μL of 10 mg mL^{-1} BSA solution for 1 h to block nonspecific binding. When the electrode was dried, it was washed with ultrapure water and casted 6 μL of different concentrations PSA on the surface of electrode and dried at 4 °C. After washing, the prepared electrode was stored at 4 °C until use.

2.5. ECL detection of PSA

The resulting electrode was inserted into the ECL cell, which included 10 mL PBS (pH 8.0) containing 0.1 M KCl and 0.12 M $K_2S_2O_8$. The relating ECL signal was recorded while the electrode potential was scanned from −2.0 to 0 V at the scan rate of 0.1 V s^{-1}. The photomultiplier tube (PMT) was set at 800 V in the process of detection.

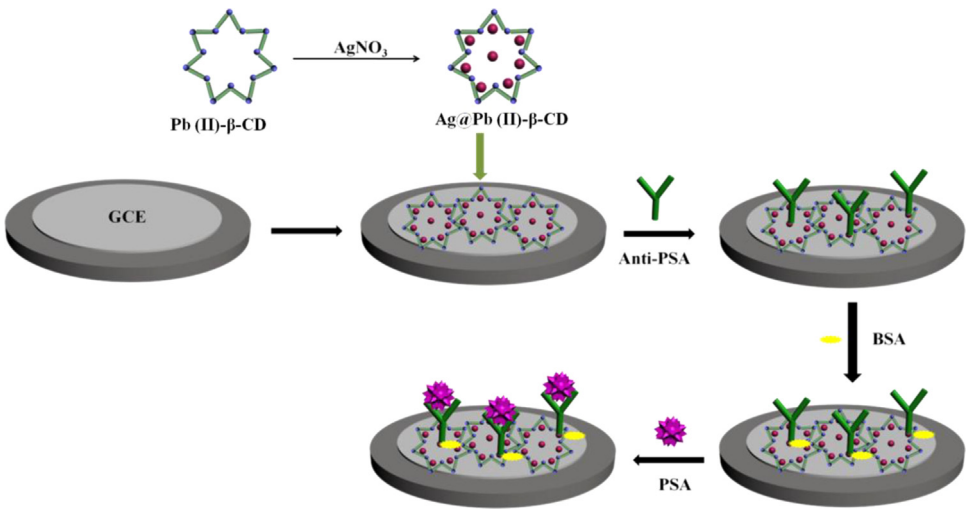

Fig. 1. Schematic diagram for the fabrication of the immunosensor.