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Redox and catalysis 'all-in-one' infinite coordination polymer for electrochemical immunosensor of tumor markers

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ABSTRACT

Prostate-specific antigen (PSA), as a glycoprotein enzyme encoded in humans by the *KLK3* gene, is one of the most important biomarkers for the diagnosis and prognosis of prostate cancer. Herein, a new electrochemical immunosensor for sensitive determination of PSA was designed by using redox and catalysis 'all-in-one' infinite coordination polymer (PtNP@ICP) as signal tag on the polyamidoamine dendrimers modified electrode interface. To construct such 'all-in-one' PtNP@ICP nanostructures, the coordination polymerization was fully carried between metal ions and polydentate bridging ligands, and the PtNP was encapsulated into the ICP in the process of polymerization. The prepared PtNP@ICP nanocatalyst was characterized by transmission electron microscope (TEM), energy dispersive X-ray spectrometry (EDX), ultraviolet and visible (UV–vis) spectrophotometry and Fourier transform infrared spectroscope (FTIR). And the synthesized PtNP@ICP was utilized as signal tag for the label of PSA. With a sandwich-type immunoassay format, the conjugated signal tag on the transducer increased with the increasing PSA concentration in the sample thus enhancing the signal of the electrochemical immunosensor due to the catalytic reduction toward H_2O_2 of the enveloped PtNP. Under optimal conditions, the current was proportional to the logarithm of PSA concentration ranging from 0.001 to 60 ng/mL. The detection limit (LOD) was 0.3 pg/mL at $3s_B$. The immunosensor displayed an acceptable reproducibility, stability and selectivity. In addition, the methodology was evaluated with human serum specimens receiving good correlation with results from commercialized enzyme-linked immunosorbent assay (ELISA) method.

1. Introduction

Sensitive and accurate determination of tumor markers is of great importance for the clinical cancer screening, disease diagnosis and monitoring (Lehr et al., 2014; Park et al., 2013; Jie et al., 2010). Immunoassays, based on the highly biospecific recognition interactions of antibody–antigen, are widely applied in bioanalysis and clinical chemistry because of their high sensitivity, high selectivity, rapid detection, and possible analysis of difficult matrices without extensive pretreatment. Compared with other immunoassay techniques, e.g. fluorescence (Long et al., 2014), surface-enhanced Raman scattering spectroscopy (SERS) (Wang et al., 2013a, 2013b), enzyme-linked immunosorbent assay (ELISA) (Hsu et al., 2014), electrochemiluminescence (Deng et al., 2013), and chemiluminescence (Zhang et al., 2014), electrochemical immunosensor has gained considerable interest and been extensively used to determine tumor markers due to the intrinsic advantages of electroanalytical method such as good portability, low cost, low power requirements, high sensitivity, and high compatibility with advance micromachining technologies (Das et al., 2006).

As early cancer diagnosis requires highly sensitive methods to accurately determine specific protein biomarkers at ultralow levels, great efforts are currently focusing on the development of effective signal amplification strategies for achieving ultrasensitive immunoassays. Early researches on this subject had concentrated on the development of enzyme labels containing horse radish peroxidase (HRP), glucose oxidase (GOx), alkaline phosphatase (ALP) (Yu et al., 2006; Lai et al., 2014; Jeong et al., 2013). However, the activity of these enzymes could be easily affected by temperature, solution pH, humidity, and toxic chemicals, which is a great obstructive for the development of the stability of the immunosensor. Recently, multifarious nanomaterials especially noble metals such as Pt, Au, and Pd are mostly used to fabricate enzymeless immunosensors. Our team had developed some enzyme-free electrochemical immunoassay with catalytic reduction of p-nitrophenol and recycling of

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p-aminophenol using gold nanoparticles-coated carbon nanotubes or platinum–cerium oxide hybrid as nanocatalysts (Tang et al., 2011, 2013). Among those, Pt nanoparticle (PtNP) has good electrocatalytic activity for the reduction of hydrogen peroxide (H_2O_2), since the over-potential occurred at unmodified electrodes was decreased a lot (Li et al., 2014; Wu et al., 2013).

To improve the biocompatibility between PtNP and protein, a great number of organic or inorganic reagents are employed for fabricating nanostructured Pt materials with optimum analytical properties (Wang et al., 2013a, 2013b; Nagaiah et al., 2013). Some research developed Au–Pt alloy electrodeposited onto the electrode surface to increase the amount of biomolecule/antibody (Liu et al., 2014). However, PtNP could be endowed other additional properties by some organic crosslinking reagent. Infinite coordination polymer (ICP), as a new class of emerging micro- and nanoscaled functional materials formed by coordination polymerization between metallic nodes and polydentate bridging ligands repeatedly, has attracted enormous research interest in chemistry and materials science because of their unique properties including size- and morphology-dependent structural tailorability and potential applications in many fields, such as sensing, catalysis, drug delivery, gas sorption, ion exchange, and bio-imaging (Lu et al., 2014; Spokoyny et al., 2009; Oh and Mirkin, 2005, 2006). More interestingly, the rational inclusion and adaptive encapsulation of functional species into self-supported ICP networks during their self-assembly processes open up a new avenue for the multifunctional and bioactive ICP materials. This unique property of ICPs inspired us to synthesize bio-catalytic active ICP nanostructures for electrochemical immunosensing through efficiently encapsulating metal catalytic elements into a single ICP nanoparticle during the coordination polymerization process. Mao and colleagues had synthesized bioelectrochemically functional infinite coordination polymer nanoparticles for highly sensitive and selective in vivo electrochemical monitoring (Lu et al., 2013; Huang et al., 2011).

In this study, we explored a redox and catalysis 'all-in-one' infinite coordination polymer to construct the sensitive electrochemical immunosensor for detection of low-abundance protein (PSA as a model). We co-confined the PtNP and ferrocenedicarboxylic acid (FcDA) into the mixed solution and exposed it to the natural light to trigger the coordination polymerization process. Initially, FcDA was decomposed into deprotonated FcDA and Fe^{II} under the natural light, and then the dissociated Fe^{II} species was further oxidized into Fe^{III} by O_2, following that Fe^{III} coordinates with the deprotonated FcDA to form ICP. Compared with enzyme-assisted polymerization (Wu et al., 2011), the processes of ICP is simple without any other adminicle such as enzyme only need natural light, which could effectively avoid contamination and improve efficiency. Meanwhile, the catalytic element PtNP was adaptively included and encapsulated into each single nanoparticle during the self-assembling process of ICP to form the redox and catalysis 'all-in-one' PtNP@ICP nanoparticles. It was found that the resultant PtNP@ICP could crosslink with the antibody as the label. Based on the sandwich-type immunoassay format, the protein was subsequently quantified by voltammetry in the sample. The aim of this work is to explore a novel, in situ amplified electrochemical immunoassay method for ultrasensitive detection of disease-related proteins.

2. Experimental

2.1. Materials and reagents

Mouse anti-human monoclonal prostate-specific antibody (anti-PSA, designated as Ab, 0.1 mg/mL) was purchased from Amyjet Scientific Inc. (Abcam product, Wuhan, China). PSA standards with various concentrations were obtained from Biocell Biotechnol. Co., Ltd. (Zhengzhou, China). Chloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), sodium borohydride ($NaBH_4$), 1,1′-ferrocenedicarboxylic acid (FcDA), polyamidoamine dendrimers (PAMAM), *N*-Hydroxysulfosuccinimide sodium salt (NHS), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) were achieved from Alfa Aesar®. All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system ($\geq$ 18 MΩ, Milli-Q) was used in all runs. Phosphate-buffered saline (PBS, 0.1 M) solution was prepared by mixing 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and 0.1 M KCl was added as the supporting electrolyte. Clinical serum samples were made available by Fujian Provincial Hospital, China.

2.2. Preparation of platinum nanoparticle (PtNP)

Initially, small platinum nanoparticle with a diameter of 5 nm was prepared according to the previous literature (Kim et al. 2011). To accomplish this, 3 mL of a 0.2% solution of $H_2PtCl_6 \cdot 6H_2O$ was added to 39 mL of boiling deionized water. After 1 min, 0.92 mL of 1% sodium citrate was added, followed by rapid injection of 0.46 mL of freshly prepared 0.08% solution of sodium borohydride containing 1% sodium citrate 30 s later. During this process, the Pt^{IV} was reduced to zero-valent Pt^0. After 10 min, the sol solution was cooled to room temperature, and then the synthesized PtNP was preserved in freezer at 4 °C for further use.

2.3. Preparation of redox and catalysis 'all-in-one' infinite coordination polymer (PtNP@ICP)

Synthesis of bioelectrochemically active 'all-in-one' PtNP@ICP was performed according to the early report (Lu et al., 2013). Firstly, ferrocenedicarboxylic acid (FcDA) was dissolved in methanol to give an orange solution. To form precipitate, the solution was then exposed to natural light for a certain amount of time. Typically, when 5 mM FcDA methanol solution containing PtNP was exposed to natural light for up to 2 h, the solution color turned to taupe, and taupe precipitate was formed at the same time. Then, the precipitate was centrifuged and washed with methanol at least three times, collected, and redispersed in the same amount of methanol.

Next, as-prepared PtNPs@ICP was employed for the labeling of anti-PSA antibody (Ab_2) by using the classical carbodiimide coupling method. Initially, 11 mg of NHS and 15 mg of EDC were dissolved into 1 mL of PtNP@ICP suspension, followed by continuous stirring for 45 min at room temperature (RT, 25 ± 1.0 °C). Afterward, 500 μL of Ab_2 (0.1 mg/mL) was added into the mixture, and gently stirred for 12 h at 150 rpm at 4 °C. The excess chemicals and antibodies were removed by centrifugation. Finally, the as-prepared PtNPs@ICP-Ab_2 was dispersed into 1.0 mL PBS (0.1 M, pH 7.4) containing 1.0 wt% BSA and stored at 4 °C for further usage for the detection of PSA.

2.4. Electrochemical measurement

A glassy carbon electrode (GCE, 3 mm in diameter) was polished repeatedly with 0.3 and 0.05 μm alumina slurry, followed by successive sonication in bi-distilled water and ethanol for 5 min and dried in air. Following that, 5 μL of 1.0 wt% PAMAM solution was thrown on the glassy carbon electrode and then removed and parched under an infrared light for 20 min. Subsequently, the PAMAM-coated electrode (PAMAM-GCE) was washed and kept in EDC and NHS mixed solution for 1 h. Then, 5 μL of anti-PSA antibody (Ab_1) was dropped on the surface of PAMAM-GCE, and