Biosensors and Bioelectronics 86 (2016) 156–163

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Pt NPs and DNAzyme functionalized polymer nanospheres as triple signal amplification strategy for highly sensitive electrochemical immunosensor of tumour marker

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ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form
15 June 2016
Accepted 15 June 2016
Available online 16 June 2016

Keywords:
Electrochemical immunosensor
Polymer nanospheres
Pt NPs
DNAzyme
Alpha fetal protein

ABSTRACT

Highly sensitive determination of tumour markers is the key for early diagnosis of cancer. Herein, triple signal amplification strategy resulting from polymer nanospheres, Pt NPs, and DNAzyme was proposed in the developed electrochemical immunosensor. First, electroactive polymer nanospheres were synthesized by infinite coordination polymerization of ferrocenedicarboxylic acid, which could generate strong electrochemical signals due to plentiful ferrocene molecules. Further, the polymer nanospheres were functionalized by Pt NPs and DNAzyme (hemin/G-quadruplex) with the ability of catalyzing H_2O_2, which contributes to enhance the electrochemical signals. The prepared conjugations were characterized by transmission electron microscope (TEM) and energy dispersive X-ray spectroscopy (EDX). And the process of preparation was monitored by zeta potential. Based on the sandwich-type immunoassay, the electrochemical immunosensor was constructed employing the conjugations as signal tags. Under optimal conditions, the DPV peak increased with the increasing of alpha fetal protein (AFP) concentration, and the linear range was from 0.1 pg mL^{-1} to 100 ng mL^{-1} with low detection limit of 0.086 pg mL^{-1}. Meanwhile, the designed immunosensor exhibited excellent selectivity and anti-interference property, good reproducibility and stability. More importantly, there were no significant differences in analyzing real clinical samples between designed immunosensor and commercial ELISA.

1. Introduction

Cancer incidence and mortality have been increasing all over the world and become the major public health problem in the country (Chen et al., 2016). Tumour markers found in blood, urine, or body tissues could be elevated due to the presence of one or more types of cancer (Lu et al., 2008). To realize the early diagnosis of cancer, the sensitive determination of tumour markers is of great importance. Immunoassay based on antigen and antibody specific binding has been widely utilized in the detection of tumour markers. An amount of different approaches *e.g.* fluorescence (Zhang and Du, 2016b), electrogenerated chemiluminescence (Habtamu et al., 2015), colorimetric (Bui et al., 2015), photoelectrochemical (Shu et al., 2016), surface-enhanced raman scattering (Wang et al., 2015), electrochemical (Qin et al., 2016) and so on have been intensively developed. However, electrochemical immunosensors with unique advantages including high sensitivity, low-cost, microminiaturization and easy-combination with other instruments have attracted a growing interest in recent years.

For electrochemical immunosensor, current signals mainly result from electron mediator, which triggers redox reaction within a certain range of potential and is closely related to the immunoreaction of antigen and antibody. Common electronic mediators contain thionine (Gayathri et al., 2016), ferrocene (Feng et al., 2016), $Fe(CN)_6^{3-/4-}$ (Li et al., 2015), methylene blue (Wang et al., 2016), alizarin red (Zhang and Ding, 2016a) and so on. Increasing the quantity of electronic mediators in the immunosensor is an effective way to enhance the current response. A great deal of technologies including self-assembly, electrolytic deposition, adsorption, and package have been applied to fix more electron mediator (He et al., 2015; Zhou et al., 2013; Zhang et al., 2013a, 2013b; Lin et al., 2015). Among them, coordination polymerization plays an important role by incorporating a myriad of monomer molecules in the process of polymerization, which could greatly enhance the current strength because of the participation of each monomer molecules. In recent years, coordination polymers have attracted more and more attention in chemistry, catalysis, molecular sensing, material and medical science due to their unique morphology and highly tailorable characteristics as well as

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http://dx.doi.org/10.1016/j.bios.2016.06.048
0956-5663/

promising applications (Yadav et al., 2016; Pakhira et al., 2015; Sun et al., 2015). For example, Li et al. prepared monodisperse selenium-platinum coordination dendrimers, which showed controlled anticancer activity by themselves without loading additional drugs (Li et al., 2016). For the electrochemical biosensor, Zhang et al. synthesized electroactive infinite coordination polymer and exploited its application in bioelectrochemistry (Zhang et al., 2013a, 2013b).

Sensitivity is one of the most important indexes for assessing the performance of electrochemical immunosensor. A variety of signal amplification methods including enzyme catalysis, nanomaterials, and biological amplification have been excavated (Zhou et al., 2016; Dutta et al., 2015; Song et al., 2014), wherein enzyme catalysis is always ahead of the curve due to its highly catalytic efficiency for substrate. However, bio-enzyme is easily affected by environment factors *e.g.* temperature, pH, humidity. Peroxidase mimetics nanomaterials exhibit enormous advantages based on its stability and activity. Except for metal nanoparticles, metallic oxide as well as carbon nanomaterials also possessed the activity of peroxidase (Chen et al., 2012; Sun et al., 2014; Xia et al., 2015). For example, Gao et al. reported Au@PtNHs exhibited highly catalytic activity, which was utilized as peroxidase mimic to construct colorimetric immunoassay (Gao et al., 2015). Meanwhile, DNAzyme are DNA oligonucleotides that are capable of catalyzing specific chemical reactions, similar to the action of other biological enzymes. Hemin/G-quadruplex is one kind of DNAzyme which exhibits horseradish peroxidase-like activity. For example, Trifonov et al. employed hemin/G-quadruplex units as a secondary amplification path for the detection of DNA through an electrocatalyzed reduction of H_2O_2 (Trifonov et al., 2016).

In this study, based on ferrocenedicarboxylic acid molecules, electroactive polymer nanospheres were synthesized by infinite coordination polymerization under sunshine. Then Pt NPs and DNAzyme were decorated onto the surface of polymer nanospheres to enhance the DPV response through catalysis reaction of H_2O_2. Polyclonal AFP antibody-Pt NPs@PS-DNA conjugates were utilized as signal tags to construct electrochemical immunosensor. Although each of elements of Pt NPs, DNAzyme, and polymer nanospheres also could be employed as labels in the immunosensors, there is still huge development space to enhance the sensitivity-one of the most important characteristics for electrochemical immunosensor-by the ingenious combination of those three elements. According to sandwich-type immunoassay, electrochemical signals of DPV resulting from signal tags were obtained and enhanced due to the synergistic effect of polymer nanosphere, Pt NPs and DNAzyme. By measuring the change of DPV, the concentration of tumour marker in sample was quantificationally detected.

2. Experimental

2.1. Chemicals

Alpha fetal protein antigen (AFP) with various concentrations (5 ng mL^{-1}, 10 ng mL^{-1}, 50 ng mL^{-1}, 100 ng mL^{-1}) and monoclonal AFP antibody (designed as Ab_1) were purchased from Biocell Biotech. Co., Ltd. (Zhengzhou, China). Polyclonal AFP antibody (designed as Ab_2) was purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). Oligonucleotide designed in this experiment was synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China), which was purified by high-performance liquid chromatography and confirmed by mass spectrometry. The sequence of the hemin-based aptamer was designed as follows according to the previous report (Hou et al., 2014): *5′-SH-GGG TAG GGC GGG TTG GGT-3′*. *1,1′*-Ferrocenedicarboxylic acid (Fc-COOH) was obtained from Energy Chemical Co., Ltd. (Beijing, China). Potassium chloroplatinate, polyethylenimine (PEI), sodium citrate, sodium borohydride ($NaBH_4$), chloroauric acid, bovine serum albumin (BSA), hydrogen peroxide (H_2O_2, 30 wt%) and hemin were purchased from Aladdin Reagent Company (Shanghai, China). All the other chemicals were of analytical reagents grade and used without further purification. The 0.2 M phosphate buffer solutions (PBS) at various pH values were prepared by mixing the stock solutions of 0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4 and 0.2 M KCl with different proportion. Clinical serum samples were made available by Shanxi Tumour Hospital, China.

2.2. Preparation of polymer nanosphere (PS)

The polymer nanosphere was synthesized by infinite coordination polymerization according to the previous literature (Zhang et al., 2015). Briefly, *1,1′*-ferrocenedicarboxylic acid (Fc-COOH, 5 mg) was dissolved in 5 mL methanol, then the solution was exposed to sunlight for 2 h. The decomposition appeared automatically due to solar radiation, which generated deprotonated Fc-COO^- and Fe^{3+}, at the same time, the polymerization between deprotonated Fc-COO^- and Fe^{3+} toke place. And the color of solution changed from yellow to taupe. Subsequently, the precipitate was centrifuged and washed with methanol at least three times. Finally, the polymer nanoparticles were saved in PBS (pH 7.0) for the further use.

2.3. Decoration of polymer nanosphere with Pt nanoparticles (Pt NPs@PS)

Initially, Pt nanoparticles were prepared for the next experimental procedure. 42 mL deionized water containing K_2PtCl_6 (3 mL, 0.2%) was boiled and kept for 1 min, and 0.92 mL sodium citrate (1%) was added. Then 0.46 mL freshly prepared sodium borohydride (0.08%) was injected rapidly. After reaction of 10 min, the solution was cooled down to room temperature and Pt nanoparticles (Pt NPs) were obtained. Subsequently, the prepared Pt NPs were used as accessories to modify polymer nanosphere. Firstly, positively polymer nanosphere was synthesized by mixing 2 mL PS solution with 1 mL PEI under shaking for 2 h. After centrifugation, 1 mL suspension solution of Pt NPs was added into the positively polymer nanosphere solution and shaked for 2 h to form the stable structure of Pt NPs@PS by electrostatic attraction. Finally, the prepared Pt NPs@PS was centrifuged and washed with deionized water at least three times, and then the precipitate was re-dispersed into PBS solution (pH 7.0).

2.4. Conjugation of Pt NPs@PS with Ab_2 and DNAzyme (Ab_2-Pt NPs@PS-DNA)

After 250 μL of Ab_2 (0.01 mg mL^{-1}) and 250 μL oligonucleotide (1 μM) were added into the above Pt NPs@PS solution and reacted for 5 min at room temperature. Then, to fully conjugate Ab_2 and oligonucleotide with Pt NPs@PS, the mixed solution was transferred to refrigerator and reacted overnight under shaking. Finally, the solutions were centrifuged and washed at least three times, and the precipitate was collected. Then 500 μL hemin (0.2 mM) was added into the conjugates and reacted for 30 min to form Hemin/G-quadruqlex as DNAzyme (designed as Ab_2-Pt NPs@PS-DNA). The conjugation of Ab_2-Pt NPs@PS-DNA was obtained by centrifugation and was preserved at 4 °C for further use.

2.5. Fabrication of the immunosensor

A glassy carbon electrode (GCE, 3 mm in diameter) was polished repeatedly with 1.0 and 0.3 nm alumina slurry, followed by