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A multi-amplification aptasensor for highly sensitive detection of thrombin based on high-quality hollow CoPt nanoparticles decorated graphene

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ABSTRACT

In this work, we have successfully demonstrated a facile strategy to incorporate high-quality hollow CoPt bimetal alloy nanoparticles (HCoPt) onto reduced graphene oxide sheet (HCoPt-RGs). An advanced sandwich-type electrochemical aptasensor for thrombin was proposed by using the HCoPt-RGs conjugates as secondary label. The formed conjugates provided large surface area for loading plentiful redox probe thionine (Thi), horseradish peroxidase (HRP) and secondary aptamer (Apt II) with good stability and friendly biocompatibility, indicating their superior properties in electroactive mediator enrichment and biomolecule immobilization. Furthermore, activated by glutaraldehyde (GA), the chitosan-hollow CoPt alloy nanoparticle (CS-HCoPt) film can greatly facilitate the capture of primary aptamer (Apt I) and dramatically reduce the nonspecific binding. Excellent sensitivity was obtained by detecting the conspicuously enhanced electrochemical signal of Thi, which was amplified by HCoPt alloy nanoparticles and HRP toward the catalytic reduction of H_2O_2. The aptasensor displayed excellent performance for thrombin with a wide linearity in the range from 1.0×10^{-12} to 5.0×10^{-8} M and a relatively low detection limit of 3.4×10^{-13} M. Moreover, the resulted aptasensor also exhibited good specificity, acceptable reproducibility and stability, indicating that the present strategy could pave a promising way for the wide application of graphene in clinical research.

1. Introduction

Thrombin is a trypsin-like serine protease that has played significant roles in many life processes and relates to a multitude of diseases (Ishii et al., 1993; Shuman, 1986; Fenton et al., 1988). Therefore, sensitive detection of thrombin is critical to many areas of biomedical research and diagnostics. Increasing efforts have been recently devoted to design electrochemical aptamer-based detection systems (Liu et al., 2009), since they often achieve high sensitivity due to different signal amplification platforms (Yigit et al., 2008; Willner and Willner, 2010; Zheng et al., 2007). Commonly, three approaches have been employed for signal amplification to increase the sensitivity of electrochemical biosensors: (i) multifunctional nanomaterials, such as aptamer-functionalized Au nanoparticles (Pavlov et al., 2004) and ruthenium complex labeled single-walled carbon-nanotubes (Li et al., 2010), have been used to increase electrochemical signal for amplification, since they possess high surface-to-volume ratio and are extremely sensitive to electronic perturbations in the surrounding environment; (ii) enzyme labeling, based on glucose oxidase (Zhuo et al., 2009), DNAzyme (Pelossof et al., 2010) and other enzymes (Xiang et al., 2010), has been proved to be a significant approach to introduce further signal amplification based on the enzymatic catalytic reaction. Using HRP as the label enzyme, Yang et al. (2010) had built up an sensitive immunosensor through detecting the enhanced electrochemical signal amplified by HRP toward the catalytic reduction of H_2O_2 in the presence of Thi; (iii) sandwich-type assays, adopting functional nanomaterials and enzymes as labels for signal amplification (Lee et al., 2011; Centi et al., 2007), have also been developed to realize ultrasensitive detection, owning to the superiority of low detection limits and high sensitivity. Zhao et al. (2011) reported a simple and ultrasensitive sandwiched aptasensor for thrombin based on an amplification mechanism resulting from HRP and AuNP dual label aptamer probe, a 30 fM level detection limit had been achieved through signal amplification by AuNPs and the oxidation of HQ catalyzed by HRP.

Since its discovery by Novoselov in 2004 (Novoselov et al., 2004), graphene, a single layer of carbon atoms densely packed in a honeycomb two-dimensional (2D) lattice, has triggered a gold rush to exploit its possible applications in both experimental and theoretical studies (Gulbakan et al., 2010; Zhu et al., 2010). It has a large theoretical specific surface area ($2630\ m^2\ g^{-1}$), high intrinsic mobility ($200{,}000\ cm^2\ V^{-1}\ s^{-1}$; Balandin et al., 2008), high thermal conductivity ($\sim 5000\ W\ m^{-1}\ K^{-1}$) and good electrical conductivity

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merit (Meyer et al., 2007; Geim and Novoselov, 2007; Xia et al., 2010). These unique nanostructure and properties drive the dreams of applying graphene in various biosensors, such as the direct electron transfer of redox enzymes promoted by graphene (Zhang et al., 2010), amplified assay based on aptamer–graphene complex (Lu et al., 2010), and catalyst support for electron transfers and signal amplification (Du et al., 2011). However, the poor biocompatibility and irreversible agglomerates obstruct its further application (Su et al., 2009; Li et al., 2008). Therefore, covalent or noncovalent functionalization methods, such as TiO_2 chemically converted graphene (Williams et al., 2008), polyvinylpyrrolidone protected graphene (Shan et al., 2009) and Pd (Jin et al., 2010), Pt (Yoo et al., 2009), Au (Günes et al., 2010), Ag (Tang et al., 2011) decorated graphene have been employed to improve its biocompatibility, dispersibility, and conductivity. Recently, it has been reported that metal alloy nanoparticles showed better electrocatalytic performance than their single-metal components (Vasquez et al., 2005). Thus, hollow CoPt bimetal alloy nanoparticles, may potentially pave an effective way to expand the application of graphene, owning to their high surface to volume ratio as well as other useful properties such as unique catalysis, excellent conductivity and friendly biocompatibility (Vasquez et al., 2005; Zhai et al., 2008). Besides, the surface of the hollow CoPt alloy nanoparticles can be readily functionalized with signal species and targeting probes, facilitating the signal amplification for ultrasensitive detection of protein. Moreover, instead of using the conventional in situ reduction of metallic compound on graphene sheet, a two step method to incorporate high-quality HCoPt alloy nanoparticles onto RGs surface will be more efficient, since it can produce graphene with efficient nanoparticle loading, perfect large surface area and excellent biocompatibility. Therefore, the integration of graphene with hollow CoPt alloy nanoparticles might potentially provide excellent opportunity for signal amplification in biosensor.

In this study, we presented an effective two step method to prepare high-quality hollow CoPt alloy nanoparticles converted graphene. Since the electrochemical signals of Thi could be amplified by HCoPt alloy nanoparticles and HRP toward the catalytic reduction of H_2O_2, the resulted HCoPt-RGs composites were modified with redox mediator Thi to label Apt II to fabricate electrochemical sandwich aptasensor. Employed as a label platform, this novel conjugates exhibited the following advantages: first of all, the HCoPt can dramatically enlarge the surface area of RGs for loading large amount of Thi, which was important for highly sensitive aptasensor. Meanwhile, the conjugates can be an ideal material to provide a biocompatible microenvironment for the immobilization of aptamer and HRP while still preserving their native bioactivity. Furthermore, the existence of HCoPt on RGs not only promoted the dispersion of RGs in aqueous solution but also enhanced electrochemical signal toward the catalytic reduction of H_2O_2. In addition, the HCoPt were also adopted to enhance the conductivity of chitosan (CS) film, which were further used as a platform to capture large amount of primary aptamer (Apt I) and reduce the nonspecific binding. Based on the advantages mentioned above, the resulted aptasensor was extraordinarily sensitive to the thrombin detection with high specificity, and it will make the new sensor capable of detecting a variety of proteins for in vitro diagnostics.

2. Experimental

2.1. Reagents and apparatus

Graphene oxide (GO) was obtained from Nanjing xianfeng nano Co. (Nanjing, China). Poly(ethylene imine) (PEI), thrombin, horseradish peroxidase (HRP), hemoglobin (Hb), thionine (Thi), chloroplatinic acid (H_2PtCl_6) and hexanethiol (96%, HT), chitosan (CS), bovine serum albumin (BSA) were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). Poly(vinylpyrrolidone) (PVP, MW = 40,000), $CoCl_2{\cdot}6H_2O$, $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, glutaraldehyde (GA) were purchased from Beijing Chemical Reagent Co. (Beijing, China). Tris-hydroxymethylaminomethane hydrochloride (Tris) was purchased from Roche (Switzerland). Thrombin aptamer was obtained from TaKaRa (Dalian, China), and the sequences of Apt I and Apt II were the same, the oligonucleotides were as follows: 5′-NH_2-$(CH_2)_6$-GGT TGG TGT GGT TGG-3′.

20 mM Tris–HCl buffer (pH 7.4) containing 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ was used as a binding buffer. Phosphate-buffered solution (PBS) (pH 7.4, 0.1 M) was used as working buffer solution. All other chemicals were of analytical grade and used as received.

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and amperometric measurements were performed on CHI 660D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China). A conventional three-electrode system was used for all electrochemical measurements: a glassy carbon electrode (GCE, 4 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode. The morphology of RGs and HCoPt-RGs was estimated from transmission electron microscopy (TEM) (H600, Hitachi Instrument, Japan). The pH measurements were made with a pH meter (MP 230, Mettler-Toledo, Switzerland).

2.2. Electrochemical measurements

All electrochemical experiments were carried out in a conventional electrochemical cell containing a three-electrode arrangement. CVs of the electrode fabrication were performed in 3 mL 5 mM $K_3[Fe(CN)_6]$ solution containing 0.2 M KCl, scanning from −0.6 to 0.2 V at a scan rate of 100 mV/s. CV and DPV were performed in 3 mL 0.1 M PBS (pH 7.4) containing 10 mM KCl, 2 mM $MgCl_2$ and 1.7 mM H_2O_2 to investigate the performance of the aptasensor. The DPV parameters applied were: 20 mV pulse amplitude, 50 ms pulse width, 0.2 s pulse period and voltage range from −0.45 to 0.05 V. Amperometric measurements were carried out in 3 mL 0.1 M PBS (pH 7.4) under continuous stirring.

2.3. Synthesis of the hollow CoPt alloy nanoparticles incorporated reduced graphene oxide sheet

Firstly, the reduced graphene sheet (RGs) was prepared from graphene oxide according to the literature (Cao et al., 2010) with a minor change. In a typical experiment, a stable dispersion of exfoliated graphene oxide sheets (0.5 mg/mL, 30 mL) was mixed with PEI (3%, 1.5 mL) and heated under reflux at 135 °C for about 3.5 h. The obtained black dispersion was washed several times by ultrapure water, and collected by centrifugation. The obtained product was characterized by TEM in Fig. S1 (see Supplementary material), the thin flake-like mono-layer sheets could be observed in most of regions, suggesting the successful preparation of RGs for further functionalization.

Hollow CoPt alloy nanoparticles incorporated reduced graphene oxide sheet was prepared as follows according to the literature (Vasquez et al., 2005): briefly, 5.6 mg $CoCl_2{\cdot}6H_2O$ and 100 mg PVP were dissolved fully in 50 mL double distilled water. After purged with N_2 for 15 min, a freshly prepared $NaBH_4$ (15 mM) and H_2PtCl_6 (12 mL, 0.1%) were added dropwise into the solution under vigorous stirring, respectively. The color of the solution became blackish brown gradually, which showed the successful synthesis of HCoPt. Then, the obtained RGs was added to the above solution and stirred for 8 h. Finally, the black dispersion was collected by centrifugation and washed several times by ultrapure water. And then the product was redispersed in 5 mL PBS at pH 7.4. Inset of Scheme 1