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Ultrasensitive electrochemical aptasensor for the detection of thrombin based on dual signal amplification strategy of Au@GS and DNA-CoPd NPs conjugates

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ABSTRACT

In this work, an ultrasensitive electrochemical aptasensor for the detection of thrombin was developed based on Au nanoparticles decorated graphene sheet (Au@GS) and CoPd binary nanoparticles (CoPd NPs). A sulfydryl-labeled thrombin capture probe (Apt1) and a biotin-labeled thrombin reporter probe (Apt2) were designed to achieve a sandwich-type strategy. Au@GS was used as a sensing platform for the facile immobilization of Apt1 through Au–S bond, forming a sensing interface for thrombin. The specific recognition of thrombin induced the attachment of Apt2–CoPd NPs to the electrode. The labeled CoPd NPs showed good catalytic properties toward the reduction of H_2O_2, resulting in an amperometric signal. The amperometric response was correlated to the thrombin concentration in sample solutions. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) confirmed the successful fabrication of the aptasensor. A linear response to thrombin in the range of 0.01–2.00 ng mL^{-1} with a low detection limit (5 pg mL^{-1}) was achieved. The proposed aptasensor showed good selectivity, good reproducibility and acceptable stability. This proposed strategy may find many potential applications in the detection of other biomolecules.

1. Introduction

Thrombin, a kind of proteinase generated from prothrombin precursor, can transform fibrinogen into fibrin and promote the blood coagulation, which is beneficial for topical stanch of capillary bleeding as well as tissue healing after surgery (Chen et al., 2014). Meanwhile, it is also one of the biomarkers for cardiovascular disease and a crucial tumor marker for the diagnosis of pulmonary metastasis (Nierodzik and Karpatkin, 2006; Sinha et al., 2014; Yoon et al., 2013). Under normal conditions, the concentration of thrombin in blood during the coagulation progress varies from nM to low μM levels (Lu et al., 2015; Wang et al., 2011). If the content of thrombin in human body was at abnormal level, it would lead to thrombotic diseases or even death. Therefore, the concentration of thrombin can be applied as a significant index for coagulation mechanism, as well as for the early diagnosis and prognosis of some kinds of diseases (Fan et al., 2016; Yang et al., 2015).

Aptasensor is a kind of biosensor which applies aptamer as sensitive recognition element. Aptamer, a specific sequence of gene, is selected by the technique called systematic evolution of ligands by exponential enrichment (SELEX) (Gao et al., 2012). It can be single-stranded DNA, double-stranded DNA as well as RNA oligonucleotides (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Aptamer can combine with proteins or other small molecules specifically, such as enzymes, antibodies, growth factors, gene regulation factor, plant lectins, intact viral particles, etc. It can be screened out *in vitro* and synthesized by chemical process. The chemical property of aptamer is stable and can recover even after high temperature (~70 °C) (Bruno and Kiel, 1999; Du et al., 2010; Friedman et al., 2015; Shao et al., 2011). Electrochemical aptasensor combining electrochemical methods and aptasensor can transform the biological signals between aptamer and target molecule into electrochemical signal. It has many advantages such as rapid, sensitive, simple, nontoxic, and lowcost. Therefore, it has gained extensive attention from biologists and chemists in the areas of clinical pathologic diagnosis, drug analysis, and

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environment monitoring (Fu et al., 2013; Hayat and Marty, 2014; Liu et al., 2013; Roushani and Shahdost-fard, 2015; Zhao et al., 2014).

As is known to all, the sensitivity of electrochemical aptasensor is closely related to the electrode materials and the used labels. Graphene sheet (GS), a one-atom-thick planar sheet with carbon atoms densely packed in a honeycomb crystal lattice, has showed promising signs for the fabrication of aptasensor due to its large specific surface area and excellent electron transfer ability (Chen et al., 2013; Feng et al., 2011; Wang et al., 2014). Meanwhile, noble metal nanoparticles, especially Au nanoparticles, have attracted more and more attention in fabricating aptasensor in recent years due to its good electrical conductivity and fine biocompatibility (Bruno and Kiel, 1999; Ma et al., 2015; Miao et al., 2016; Niazov-Elkan et al., 2014; Shao et al., 2011; Shukla et al., 2005; Zhang et al., 2013). Au@GS can possess the superiorities of Au nanoparticles and GS, which undoubtedly improve the electron transferability and then improve the sensitivity of electrochemical aptasensor.

The labels used to fabricate the electrochemical aptasensor are another key point for improving the sensitivity. Noble metal nanoparticles also are the ideal materials for labeling thrombin reporter probe (Apt2). For instance, Xu et al. (2015) reported an electrochemical aptasensor for detecting thrombin by using Au@Pd core–shell nanostructures as one signal amplification strategy. Fan et al. (2015) constructed a photoelectrochemical aptasensor based on SiO_2@Au for the enhancement of signal. Yuan et al. (Jing et al., 2014) reported a sensitive and selective electrochemical aptasensor for thrombin detection by using Fe_3O_4–Au nanocomposites with glucose oxidase and peroxide-mimicking enzyme activity as the signal enhancers. To the best of our knowledge, CoPd nanoparticles (CoPd NPs) have not been employed as labels up to now.

In this study, Au@GS was utilized to modify the electrode surface which played a vital role for increasing the specific surface area and improving the electron transfer efficiency on the electrode surface. Simultaneously, CoPd NPs were applied as the labels of Apt2 which was on account of its good electrical conductivity and excellent catalytic properties toward H_2O_2. A sandwich-type strategy was employed to fabricate the electrochemical aptasensor. The thrombin capture probe (Apt1) was immobilized onto the Au@GS modified electrode. Subsequently, the thrombin and Apt2–CoPd NPs were modified onto the electrode surface in turn. Eventually, the performance of the electrochemical aptasensor was tested by recording the current of electrocatalytic reduction toward H_2O_2. The obtained results indicated that the proposed aptasensor has quite a good performance for the detection of thrombin and provides a new promising platform for determination of other biomolecules.

2. Materials and methods

2.1. Materials and reagents

All oligonucleotides were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China), their sequences are as follows: the thrombin capture probe (Apt1) 5′-TTTTTTTTTTGGTTGGTGTGGTTGG-SH-3′ and the thrombin reporter probe (Apt2) 5′-TTTTTAGTCCGTGGTAGGGCAGGTTGGGGTGACT-bio-3′. Thrombin was purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). Bovine serum albumin (BSA, 96–99%) was purchased from Sigma (USA). 6-mercapto-1-hexanol, oleylamine, trioctylphosphine, cobalt acetylacetonate and palladous bromide were purchased from Shanghai Civi Chemical Technology Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade, and all chemicals were used without further purification. Phosphate buffered solutions (PBS, pH 7.4) were prepared by using 0.067 mol L^{-1} KH_2PO_4 and 0.067 mol L^{-1} Na_2HPO_4. The ultrapure water (resistivity = 18.25 MΩ cm) was used throughout the experiments.

2.2. Apparatus

All electrochemical measurements were carried out using a CHI 760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China). Electrochemical impedance spectroscopy (EIS) was obtained from the IM6e impedance measurement unit (Zahner, Germany). High resolution transmission electron microscope (HRTEM) images and energy dispersive X-Ray spectroscopy (EDX) were obtained from TECNAI20U-TWIN microscope (Philips, Holland). Scanning electron microscope (SEM) images were obtained by using field emission SEM (Zeiss, Germany).

2.3. Preparation of Au@GS

Firstly, graphite oxide (GO) was synthesized by using a modified Hummers method according to the previous reports (Hummers and Offeman, 1958; Marcano et al., 2010). Then graphene sheets (GS) was obtained from GO through a thermal exfoliation method (McAllister et al., 2007; Wang et al., 2014).

Au@GS composites were synthesized via a simple chemical strategy. Briefly, GS was mixed with $HAuCl_4$ (1:1, M:M) in aqueous solution for 24 h. After centrifugation, the supernatant was discarded and the obtained solid was dispersed in aqueous solution again. Then the solution was adjusted to pH < 2 by using HCl and stirred for at least 30 min. Subsequently, 50 mmol L^{-1} of $NaBH_4$ was added into above solution dropwise until the $HAuCl_4$ was all reduced. Eventually, the solution was centrifuged, washed three times with ultrapure water, and dried in vacuum.

2.4. Preparation of Pd NPs and CoPd NPs

Pd NPs were obtained according to the previous report (Lim et al., 2009). CoPd NPs were synthesized as follows: 0.35 mmol of cobalt acetylacetonate and 0.30 mmol palladous bromide were dissolved in 18 mL of oleylamine. Then the mixture was stirred and heated to 60 °C under nitrogen atmosphere. Afterwards, 0.5 mL of trioctylphosphine was added into the mixture, and the color of the mixture turned from pink to dark green. The solution was heated to 260 °C at a rate of 5 °C min^{-1} and maintained at this temperature for 2 h. Subsequently, the solution was cooled down to room temperature. After addition of 40 mL of ethanol, the CoPd NPs could be obtained by centrifugation at 8000 r min^{-1}. The obtained CoPd NPs were washed with 20 mL of hexane and 30 mL of ethanol twice. Finally, the obtained precipitate was redispersed in hexane and dried with nitrogen.

2.5. Preparation of Apt2–CoPd NPs

The synthesized CoPd NPs (4 mg) were dispersed in 2 mL of PBS (pH = 7.4) which contains 0.0364 g of cetyltrimethylammonium bromide (CTAB) by ultrasonication for 4 h. Then the solution was centrifuged at 4000 r min^{-1} for 10 min and washed three times by using PBS (pH = 7.4). The obtained solid was redispersed in 2 mL of PBS (pH = 7.4). Afterwards, 100 μL of 1 mg mL^{-1} avidin was added into the above solution. The mixture was stirred at 37 °C for 2 h, followed by centrifugation at 4000 r min^{-1} for 10 min, and redispersed in 2 mL of pH 7.4 PBS. Then, 100 μL of 10 μg mL^{-1} Apt2 was added into the solution. The mixture was allowed to react at 37 °C under stirring for 1 h followed by centrifugation, and was then dispersed in 2 mL of pH 7.4 PBS. Apt2 could be immobilized on CoPd NPs through the