



An electrochemical aptasensor for thrombin using synergetic catalysis of enzyme and porous Au@Pd core-shell nanostructures for signal amplification

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ARTICLE INFO

Article history:

Received 27 June 2014

Received in revised form

25 August 2014

Accepted 30 August 2014

Available online 16 September 2014

Keywords:

Electrochemical aptasensor

Thrombin

Glucose oxidase

Au@Pd core-shell nanostructures

Signal amplification

ABSTRACT

In this work, a sensitive electrochemical aptasensor for thrombin (TB) based on synergetic catalysis of enzyme and porous Au@Pd core-shell nanostructure has been constructed. With the advantages of large surface area and outstanding catalytic performance, porous Au@Pd core-shell nanostructures were firstly employed as the nanocarrier for the immobilization of electroactive toluidine blue (Tb), hemin/G-quadruplex formed by intercalating hemin into the TB aptamer (TBA) and glucose oxidase (GOx). As a certain amount of glucose was added into the detection cell, GOx rapidly catalyzed the oxidation of glucose, coupling with the local generation of H₂O₂ in the presence of dissolved O₂. Then, porous Au@Pd nanoparticles and hemin/G-quadruplex as the peroxidase mimics efficiently catalyzed the reduction of H₂O₂, amplifying the electrochemical signal and improving the sensitivity. Finally, a detection limit of 0.037 pM for TB was achieved. The excellent performance of the aptasensor indicated its promising prospect as a valuable tool in simple and cost-effective TB detection in clinical application.

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1. Introduction

Quantitative detection of thrombin (TB) is very important in clinical diagnosis, owing to the crucial role of TB in the cardiovascular diseases, regulation of inflammation processes, anti-clotting therapeutics, and so on (Tasset et al., 1997; Centi et al., 2007). In recent years, many approaches for detecting TB have been developed based on the specific binding between TB and the TB aptamer (TBA), such as electrochemiluminescence (Li et al., 2011), surface plasmon resonance (Bai et al., 2013), colorimetry (Wang et al., 2008), electrochemistry (Jiang et al., 2013) and fluorescence (Wang et al., 2011). Comparatively, electrochemical aptamer-based sensor (aptasensor) with the advantages of low cost, simple device portability and high sensitivity (Zhao et al., 2011; Liu et al., 2012), has attracted distinct attention in TB determination.

In order to improve the sensitivity of the analytical method, a large number of nanomaterials have been employed for sensitive biosensors, owing to their advantages of high surface areas, high loading of receptor molecules and unique electronic and catalytic properties (Huang et al., 2014). Particularly, noble metal-based nanoparticles, such as gold nanoparticles (AuNPs) with large specific surface area and excellent biocompatibility (Wen et al., 2014), Palladium nanoparticles (PdNPs) that possess excellent electrocatalytic performance (Qi et al., 2014), have been extensively used in the construction of biosensors (Wang et al., 2014a; Xia et al., 2013; Lan et al., 2014). Recently, as the result of the enhanced physical, chemical, and prominent catalytic properties over the corresponding monometallic components (He et al., 2012; Ferrer et al., 2007), bimetallic nanoparticles with core-shell structures have aroused much attention in various of biosensors (Gui and Jin, 2013; Miao et al., 2014; Chung et al., 2013). Besides, porous nanomaterials have also received particular interest in biosensing field due to their large specific surface area, numerous catalytic hot spots and high-index exposed facets (Wang et al., 2013a; Zhu et al., 2010). Especially, metallic nanocrystals with highly porous structures and exhibiting high catalytic performance (Lim and Xia, 2011), have been applied to numerous biosensors,

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which is very helpful to decrease noble-metal consumption (Wang et al., 2014b; Xu et al., 2014). However, bimetallic nanoparticles with porous and core-shell nanostructures, integrating the merits of the two types of structures, have gotten little attention in aptasensors. Based on these points, Huang and coworkers developed an efficient and simpler method for preparing porous Au@Pd core-shell nanostructures that conjugated the merits of AuNPs and PdNPs (Huang et al., 2013). In addition, brilliant catalytic performance of the porous Au@Pd core-shell nanostructures as mimicking natural peroxidases has been discovered by Ge's group (Ge et al., 2014), endowing its ability for developing sensitive electrochemical aptasensors.

Herein, an amplified electrochemical TB aptasensor based on synergetic catalysis of glucose oxidase (GOx), porous Au@Pd core-shell nanostructures and hemin/G-quadruplex as peroxidase mimics has been developed. Owing to the large surface area, porous Au@Pd core-shell nanostructures were firstly used as the nano-carrier for the immobilization of amino terminated TBA (NH₂-TBA) and electroactive toluidine blue (Tb), followed by the intercalation of hemin to form hemin/G-quadruplex with favorable peroxidase-mimicking properties (Stefan et al., 2011). Subsequently, GOx acted as the blocking reagent to block the remaining active sites on the porous Au@Pd core-shell nanostructures, achieving the secondary TB aptamer (Tb, GOx and hemin/G-quadruplex multi-labeled Au@Pd bioconjugates). Through "sandwich" reaction, a detectable electrochemical signal was obtained. The signal amplification of the aptasensor was accomplished by the following manner: As the addition of glucose, GOx efficiently catalyzed the oxidation of glucose into gluconolactone, coupling with the local formation of H₂O₂, whose reduction was immediately catalyzed by the peroxidase mimics, which including porous Au@Pd core-shell nanostructures and hemin/G-quadruplex. Ultimately, the electron transfer of Tb was promoted, resulting in a conspicuously enhanced electrochemical signal. Furthermore, the developed aptasensor shows good sensitivity, stability and satisfactory reproducibility, indicating its potential application in diagnostics.

2. Experimental

2.1. Materials and reagents

Thrombin (TB), bovine serum albumin (BSA), hemoglobin (Hb), gold chloride (HAuCl₄), potassium chloropalladate (K₂PdCl₄), L-ascorbic acid (AA), toluidine blue (Tb), hemin and glucose oxidase (GOx) were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). Human IgG, Carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) were ordered from Biocell Company (Zhengzhou, China). L-cysteine (L-cys) and L-arginine (L-arg) were purchased

from Kangan Amino Acid Company (Shanghai, China). Hexadecylpyridinium chloride monohydrate (C₅H₅N(Cl)(CH₂)₁₅CH₃•H₂O, HDPC, ≥ 99.0) was bought from Aladdin Industrial Corporation. Serum specimens were provided by Xinqiao Hospital of Third Military Medical University (Chongqing, China). Trishydroxymethylaminomethane hydrochloride (Tris-HCl) was supplied by Roche (Switzerland). Amino-terminated thrombin aptamer (NH₂-TBA): 5'-NH₂-(CH₂)₆-GGTGGTGTGGTGG-3' was obtained from Sangon Biotech (Shanghai) Co., Ltd.

0.1 M phosphate-buffered solution (PBS, pH 7.0) as the working buffer solution was prepared with 10 mM Na₂HPO₄, 10 mM NaH₂PO₄ and 2 mM MgCl₂. 0.2 M Tris-HCl buffer (pH 7.4) as the binding buffer solution was prepared using 140 mM NaCl, 5 mM KCl, 1 mM CaCl₂ and 1 mM MgCl₂. Double distilled water was used throughout the study.

2.2. Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS) were conducted with a CHI 660D electrochemical workstation (Shanghai CH Instruments, China) containing a conventional three-electrode system: a platinum wire as counter electrode, a saturated calomel reference electrode (SCE) and a bare or modified glassy carbon electrode (GCE, $\Phi=4$ mm) as working electrode. The morphology of porous Au@Pd nanoparticles was tracked by a scanning electron microscope (SEM, S-4800, Hitachi Instrument, Japan).

2.3. Preparation of porous Au@Pd core-shell nanostructures

The porous Au@Pd nanostructures were synthesized according to the literature (Ge et al., 2013) with a slight modification. Briefly, K₂PdCl₄ solution (10 mM, 2 mL) and HAuCl₄ (10 mM, 0.5 mL), and 0.1 g HDPC were added into 25 mL H₂O, followed by sonication to form a homogeneous mixture. Subsequently, freshly prepared AA (0.1 M, 1.5 mL) aqueous solution was added quickly into the mixture under gentle shaking. The resulting mixture was kept at 35 °C for 3 h without interruption. Finally, the blank product was centrifuged, washed with ethanol, double distilled water for several times and redispersed in 10 mL water under mild sonication. The obtained porous Au@Pd core-shell nanostructures were stored at 4 °C for further use. The morphology of the as prepared porous Au@Pd core-shell nanostructures was characterized by SEM, and the results were displayed in Fig. 1(A).

2.4. Preparation of Tb, GOx and hemin/G-quadruplex multi-labeled Au@Pd bioconjugates (secondary TB aptamer)

The preparation procedure of Tb, GOx and hemin/G-quadruplex multi-labeled Au@Pd bioconjugates (secondary TB aptamer) was

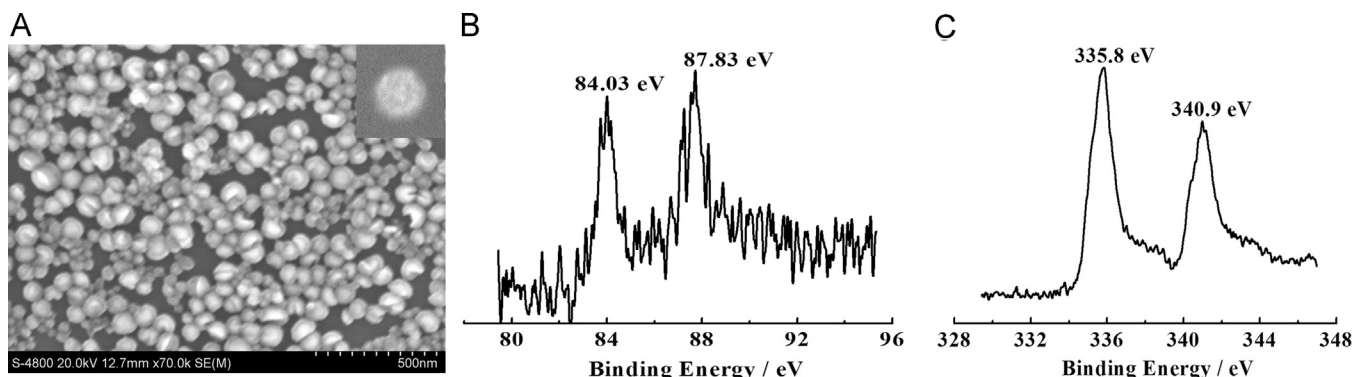


Fig. 1. (A) SEM image of the porous Au@Pd core-shell nanostructures and XPS spectra of (B) Au, (C) Pd for the porous Au@Pd core-shell nanostructures.

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