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A pseudo triple-enzyme electrochemical aptasensor based on the amplification of Pt–Pd nanowires and hemin/G-quadruplex

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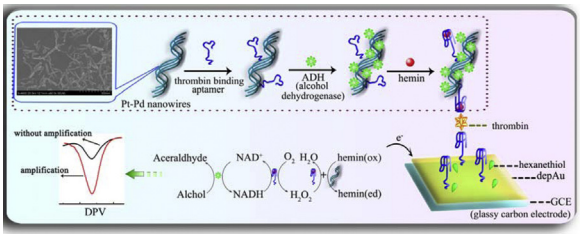
HIGHLIGHTS

- Pt–Pd nanowires were combined with pseudo triple-enzyme electrochemical aptasensor.
- Pt–Pd nanowires served as signal enhancer.
- Labeling process, deactivation and spatial distribution of enzymes were solved.

GRAPHICAL ABSTRACT



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ABSTRACT

Our present work aimed at developing a pseudo triple-enzyme cascade electrocatalytic electrochemical aptasensor for determination of thrombin with the amplification of alcohol dehydrogenase (ADH)-Pt–Pd nanowires bionanocomposite and hemin/G-quadruplex structure that simultaneously acted as NADH oxidase and HRP-mimicking DNAzyme. With the addition of ethanol to the electrolyte, the ADH immobilized on the Pt–Pd nanowires catalyzed ethanol to acetaldehyde accompanied by NAD^+ being converted to NADH. Then the hemin/G-quadruplex firstly served as NADH oxidase, converting the produced NADH to NAD^+ with the concomitant local formation of high concentration of H_2O_2. Subsequently, the hemin/G-quadruplex acted as HRP-mimicking DNAzyme, bioelectrocatalyzing the produced H_2O_2. At the same time, the Pt–Pd nanowires employed in our strategy not only provided a large surface area for immobilizing thrombin binding aptamer (TBA) and ADH, but also served as HRP-mimicking DNAzyme which rapidly bioelectrocatalyzed the reduction of the produced H_2O_2. Thus, such a pseudo triple-enzyme cascade electrochemical aptasensor could greatly promote the electron transfer of hemin and resulted in the dramatic enhancement of electrochemical signal. As a result, a wide dynamic concentration linear range from 0.2 pM to 20 nM with a low detection limit of 0.067 pM for thrombin (TB) determination was obtained. The excellent performance indicated that our strategy was a promising way for ultrasensitive assays in electrochemical aptasensors.

1. Introduction

Aptamer are nucleic acids that specifically bind to low molecular-weight substances or proteins [1,2]. Compared to traditional binding constants of antibodies to antigens, aptamers have many excellent properties such as relatively simple to synthesis, long-term storage, high specificity and wide applicability [3–8]. A large amount of

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materials have been developed for immobilizing aptamers, for example, nanoparticles [9,10], noble metals [11–15] and nanowires [16,17]. Particularly, Pt–Pd nanowires, a one-dimensional nanostructures metal, have been more extensively used as materials for immobilization of aptamers due to its unique properties such as huge surface-to-volume ration, superior conductivity, rich surface chemistry and its excellent electrocatalytic activity [18–24]. The excellent electrocatalytic activity in electrochemical performance of Pt–Pd nanowires can be attributed to two major factors: (1) bimetallic nanowires with nanoporous and dendritic structures provides high roughness of surface, thus inducing high electrocatalytic activity [25]; (2) through reduction of the interface resistance between nanobranches, the abundant interconnected nanobranches of multi-dimensional nanostructures provides facile way for transference of electron [26]. Recently, Xia's group [27] has discovered an oriented way to prepare Pt–Pd nanowires with high electrocatalytic activity, which provide a superior prospect for application of Pt–Pd nanowires.

Hemin/G-quadruplex, formed by interacting hemin into guanine-rich nucleic acid sequences, is a class of catalytic nucleic acid (DNAzyme) [28,29]. Compared to traditional protein enzymes, hemin/G-quadruplex has many excellent properties, for example, low cost, simple synthesis, high chemical stability, easy modification, and more inspiring, high electrocatalytic activity for oxidation of H_2O-mediated [30]. More importantly, Willner's group [31] demonstrated that not only the hemin/G-quadruplex acted as HRP-mimicking DNAzyme, but also as NADH oxidase and NADH peroxidase mimicking DNAzyme. Inspired by their report, our group previously constructed a pseudo triple-enzyme cascade electrocatalytic amplification system by using ADH and hemin/G-quadruplex [32]. Compared to previously monoenzyme and dobienzyme amplification system, the pseudo triple-enzyme cascade electrocatalytic amplification system has the following three major advantages: (1) the pseudo triple-enzyme electrochemical aptasensor could achieve higher sensitivity with its further amplified electrochemical signal; (2) since the hemin/G-quadruplex simultaneously acted as HRP-mimicking DNAzyme and NADH oxidase, a high catalytic efficiency can be obtained by in suit producing substrates such as H_2O_2 and NADH with high concentration; (3) the pseudo triple-enzyme electrochemical aptasensor is interesting to resolve the fussy labeling process, deactivation and spatial distribution of each enzyme.

Inspired by the previous report, our present work fabricated an electrochemical aptasensor by combined Pt–Pd nanowires with pseudo triple-enzyme cascade amplification system with using ADH and hemin/G-quadruplex structure that simultaneously acted as NADH oxidase and HRP-mimicking DNAzyme. Pt–Pd nanowires were used as materials to immobilize ADH and TBA for its large surface area and excellent electrocatalytic activity. Thrombin (TB), a serine protease involved in thrombin, was served as a target substance. Hemin was served as electron mediator. In an electrolyte containing NAD^+ and ethanol, the ADH firstly converted ethanol to acetaldehyde accompanied by NAD^+ being converted to NADH. Then the hemin/G-quadruplex firstly served as NADH oxidase, converting the produced NADH to NAD^+ with the concomitant local formation of high concentration of H_2O_2. Subsequently, the hemin/G-quadruplex acted as HRP-mimicking DNAzyme, rapidly catalyzing the produced H_2O_2. At the same time, the Pt–Pd nanowires also served as HRP-mimicking DNAzyme, rapidly bioelectrocatalyzing the produced H_2O_2 to H_2O. Thus, a dramatically amplified electrochemical signal could be obtained with fabricated pseudo triple-enzyme cascade electrocatalytic system. This work provided a promising way for TB detection with high sensitivity.

2. Experimental

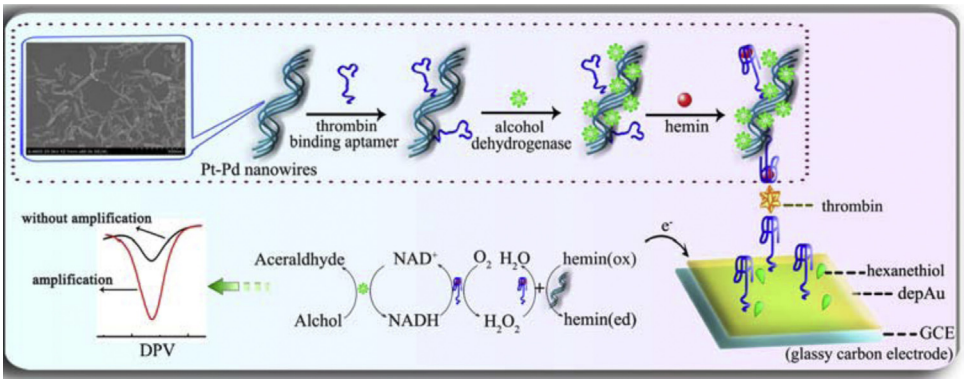
2.1. Chemicals and materials

Chloroplatinic acid (H_2PtCl_6), palladium potassium chloride (K_2PdCl_4), potassium hydroxide (KOH), ethylene glycol (EG), *N*,*N*-dimethylmethanamide (DMF), alcohol, alcohol dehydrogenase (ADH), hemin, gold chloride ($HAuCl_4$), hexanethiol (96%, HT), thrombin (TB), hemoglobin (HB), tris(hydroxymethyl)aminomethane hydrochloride (Tris) and nicotinamide adenine dinucleotide (NAD^+) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The thrombin binding aptamer was synthesized and purified from Sangon Biotech (Chongqin, China). Thrombin binding aptamer: 5′-SH-$(CH_2)_6$-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 buffer solution (PBS) (pH 7.0, 0.1 M) containing 10 mM KCl, 2 mM $MgCl_2$ was used as working buffer solution. All other chemicals were of analytical grade.

2.2. Instrumentation

The pH measurements were carried out with a pH meter (MP 230, Mettler-Toledo, Switzerland). Cyclic voltammetry (CV) and differential pulse voltammograms (DPV) were performed with a CHI 660 D electrochemical workstation (Shanghai Chenhua Instrument, China). A three-electrode system with a modified glassy carbon electrode (GCE, $\phi = 4$ mm) as a working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as a refeerence electrode. The scanning electron micrographs were taken with scanning electron microscope (SEM, S-4800, Hitachi, Japan). The transmission electron micrographs



Scheme 1. The schematic diagrams of fabricated pseudo triple-enzyme electrochemical aptasensor based on the electrochemical signal amplification of Pt–Pd nanowires and hemin/G-quadruplex.