Biosensors and Bioelectronics 26 (2010) 881–885

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

A signal-on electrochemical probe-label-free aptasensor using gold–platinum alloy and stearic acid as enhancers

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ARTICLE INFO

Article history:
Received 16 May 2010
Received in revised form 7 July 2010
Accepted 11 July 2010
Available online 16 July 2010

Keywords:
Target-induced strand displacement
Electrochemical probe-label-free
Electrocatalytic
Stearic acid
Thrombin (TB)
Aptasensor

ABSTRACT

In the present study, a new electrochemical probe-label-free aptasensor for thrombin (TB) based on the amplification of gold–platinum alloy nanoparticles (Au–PtNPs) and stearic acid was reported. Nafion@multiwalled carbon nanotubes (MWCNTs) coated electrode was firstly modified with electrochemical probe of methylene blue (MB). Then, Au–PtNPs were electrodeposited onto the electrode surface for the immobilization of aptamer and further hybridization of stearic acid labeled thrombin aptamer (TBA). In the presence of TB, the TBA bound with TB and released from the self-assembled duplex on the electrode into solution, decreasing the steric hindrance of the aptasensor and facilitating catalytic efficiency of Au–PtNPs in the presence of MB toward H_2O_2 with an enhanced electrochemical signal. With the enhanced effect of Au–PtNPs/MB modified electrode and stearic acid, a detection limit as low as 3 pM for TB was achieved. The aptasensor also exhibited good selectivity and reproducibility.

1. Introduction

Aptamers are molecule recognition elements made of single-stranded DNA or RNA oligonucleotides, which are elicited by the systematic evolution of ligands by the exponential enrichment (SELEX) procedure (Tuerk and Gold, 1990; Ellington and Szostak, 1990), capable of specifically binding a variety of molecules. Because of their relative ease of isolation and modification, tailored binding affinity, and resistance against denaturizing, aptamers can rival antibodies for molecular recognition and detection (Huang et al., 2008; Deng et al., 2009a). Various aptasensors have been developed for detecting molecules based on different technologies, such as electrochemistry (EC) (Zuo et al., 2009; Rahman et al., 2009; Zhang et al., 2009; Wang et al., 2009a), capillary electrophoresis (CE) (Zhang et al., 2008a), surface-enhanced Raman spectroscopy (SERS) (Hu et al., 2009a), mass spectrometry (Zhao et al., 2009), fluorescence (Lao et al., 2009; Xiang et al., 2009), electrochemiluminescence (ECL) (Wang et al., 2009b; Qi et al., 2009; Hu et al., 2009b; Yin et al., 2009), and colorimetry (Higuchi et al., 2008). Among them, EC-based approach has attracted particular attention because it provides a simple, sensitive and selective platform for molecular detection.

As one of strategies in the design of electrochemical aptasensors, target-induced strand displacement has been proven as one of the most important approach for molecules. In the target-induced strand displacement strategy, the complementary DNA or aptamer duplex are assembled to a solid support. After binding to their target molecules, the aptamers or its complementary DNA are displaced from the solid support, leading to a significant change of electrochemical signal. Thanks to no need of prior knowledge of aptamers' secondary or tertiary structure, the strategy of target-induced strand displacement is easy to carry out, and it should be suited for the development of electrochemical aptasensors (Nutiu and Li, 2003). Nevertheless, the preparation of electrochemical probe-tagged aptamer or its complementary sequence takes a risk of loss in redox activity and complexity in synthesis process. Meanwhile, a low sensitivity may be obtained because only one electrochemical probe could be tagged to one aptamer (Du et al., 2009). As a result, increasingly attractive attention has been placed toward a probe-label-free aptasensor, based on the target-induced strand displacement strategy with simplicity and high sensitivity for the determination of proteins (Peng et al., 2009; Du et al., 2009; Zhang et al., 2008b).

The coupling of enzymes as biocatalytic amplifying labels is a general paradigm advantageous for amplifying electrochemical signals of aptasensors, yet disadvantageous because it is complicated, expensive, and their rigorous detection conditions (Deng et al., 2009b). Therefore, aptamer-functionalized metal nanoparticles for biocatalytic amplifying electrochemical detection have been employed. Willner et al. have introduced thrombin aptasensors (TBAs) using aptamer-functionalized Pt nanoparticles (PtNPs) as biocatalytic labels for the amplified electrochemical detection

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doi:10.1016/j.bios.2010.07.035

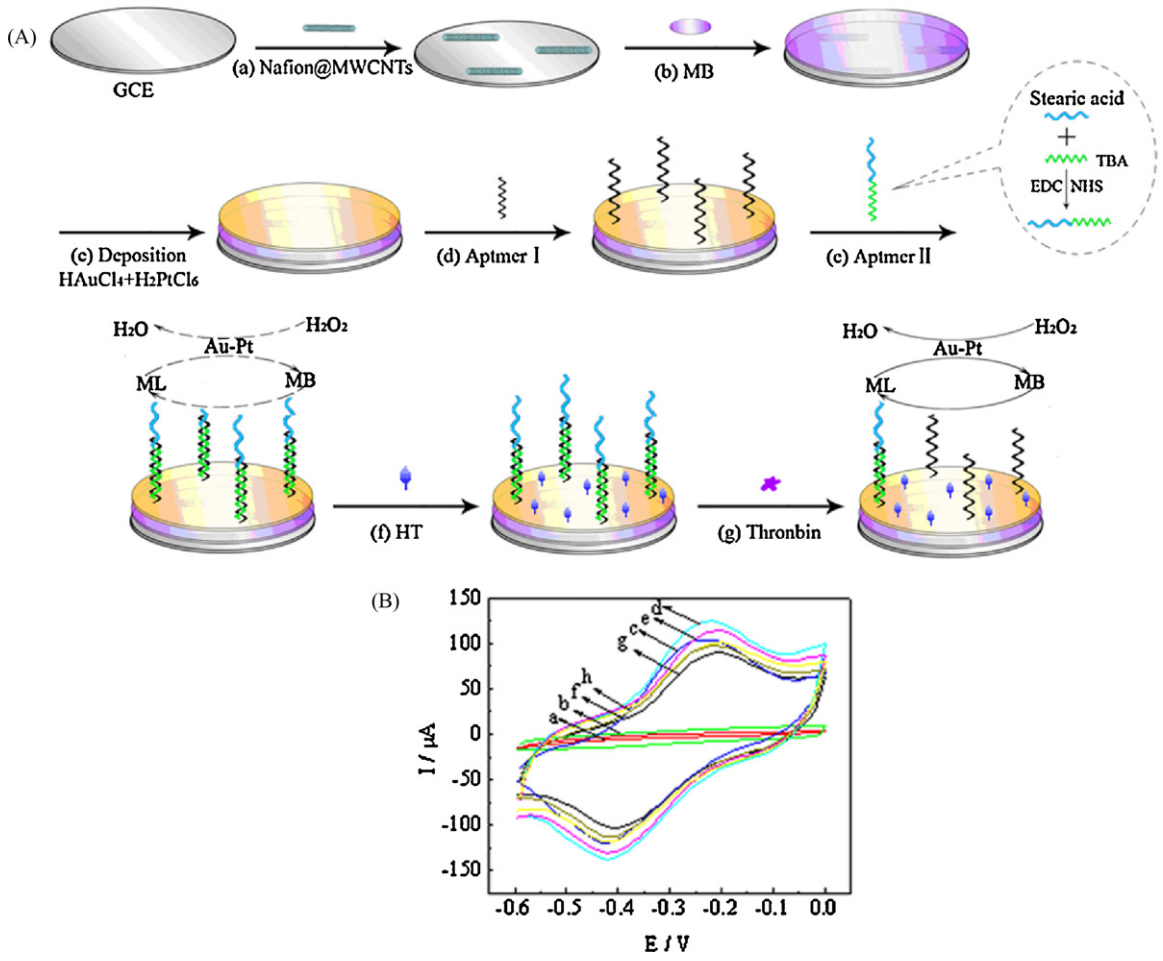

Fig. 1. (A) Schematic representation of fabrication of electrochemical aptasensor. (B) The CVs of different electrodes obtained in PBS (pH 7.0): (b) Nafion@MWCNTs/GCE; (c) MB/Nafion@MWCNTs/GCE; (d) Au–Pt/MB/Nafion @MWCNTs/GCE; (e) aptamer I/Au–Pt/MB/Nafion@MWCNTs/GCE; (f) aptamer II/aptamer I/Au–Pt/MB/Nafion@MWCNTs/GCE; (g) HT/aptamer II/aptamer I/Au–Pt/MB/Nafion@MWCNTs/GCE, and (h) the as-prepared aptasensor after the incubation with 20 nM TB. The scan rate was 50 mV/s.

(Polsky et al., 2006; Golub et al., 2009). Additionally, the association of inert substance to enlarge steric hindrance is also an effective method for amplifying electrochemical signals of aptasensors. Dong et al. reported a kind of amplified impedimetric aptasensor using Au nanoparticles (AuNPs) as a signal enhancer (Li et al., 2007). Chen et al. have introduced an impedimetric aptasensor with enlarged surface-charged gold nanoparticles for signal amplification (Deng et al., 2009b). They all might overcome some of the problems associated with the thermal and environmental instability inherent in biological materials such as enzymes.

Based on the analogical method of signal amplification mentioned above, we report a simple signal-on electrochemical aptasensor for thrombin (TB) based on the stearic acid and gold–platinum alloy nanoparticles (Au–PtNPs) for signal amplification. Au–PtNPs, which exhibit better catalytic properties than their monometallic do (Lang et al., 2004; Kang et al., 2007), were indirectly electrodeposited on MB/Nafion@multiwalled carbon nanotubes (MWCNTs) modified electrode. With amounts of MB redox probes modified on electrode surface, the Au–PtNPs electrocatalyzed the reduction of H_2O_2 effectively, which provided an extreme amplified electrochemical signal. After the self-assembling duplexes consisting of inert organic substance stearic acid labeled TBA and its complementary DNA on Au–PtNPs, the steric hindrance of the aptasensor was increased, which led to a decrease in the catalytic efficiency of Au–PtNPs toward H_2O_2. However, the introduction of target TB could induce the displacement of stearic acid labeled TBA from the self-assembled duplex on the electrode, decreasing the steric hindrance with an increase of electrochemical signal. As a result, a detection limit as low as 3 pM for TB was achieved. Details of the preparation, optimal conditions and characterization of the aptasensor are discussed as follows.

2. Experimental

2.1. Chemicals and material

The MWCNTs (>95% purity) were purchased from Chengdu Organic Chemicals Co. Ltd (Chengdu, China). N-hydroxy succinimide (NHS) and N-(3-dimethylaminopropyl)-N,-ethylcarbodiimidehydrochloride (EDC) were purchased from Shanghai Medpep Co. Ltd (Shanghai, China). TB, bovine serum albumin (BSA), hemoglobin (Hb), hexanethiol (96%, HT), nafion, MB, gold chloride ($HAuCl_4$), chloro platinic acid (H_2PtCl_6) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tris–hydroxymethylaminomethane hydrochloride (tris) was purchased from Roche (Switzerland). Aptamer I: 5′-NH_2-$(CH2)_6$-CCA ACC ACA CCA ACC-3′, TBA: 5′-NH_2-$(CH2)_6$-GGT TGG TGT GGT TGG-3′, aptamer III: 5′-GGT TGG TGT GGT TGG-3′ were purchased from TaKaRa (Dalian. China). All other chemicals were of reagent grade and used as received. Nafion@MWCNTs was prepared according to literature (Su et al., 2009; Shobha Jeykumari and Sriman Narayman, 2008). The preparation of stearic acid labeled aptamer (aptamer II) was presented in S1 (see Supplementary material).