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A new amplification strategy for ultrasensitive electrochemical aptasensor with network-like thiocyanuric acid/gold nanoparticles

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Abstract

An ultrasensitive and highly specific electrochemical aptasensor for detection of thrombin based on gold nanoparticles and thiocyanuric acid is presented. For this proposed aptasensor, aptamerI was immobilized on the magnetic nanoparticles, aptamerII was labeled with gold nanoparticles. The magnetic nanoparticle was used for separation and collection, and gold nanoparticle offered excellent electrochemical signal transduction. Through the specific recognition for thrombin, a sandwich format of magnetic nanoparticle/thrombin/gold nanoparticle was fabricated, and the signal amplification was further implemented by forming network-like thiocyanuric acid/gold nanoparticles. A significant sensitivity enhancement had been obtained, and the detection limit was down to 7.82 aM. The presence of other proteins such as BSA and lysozyme did not affect the detection of thrombin, which indicates a high specificity of thrombin detection could be achieved. This electrochemical aptasensor is expected to have wide applications in protein monitoring and disease diagnosis.

Keywords: Aptamers; Nanoparticles; Thrombin; Electrochemical aptasensor; Thiocyanuric acid

1. Introduction

With the increasing application of proteomic strategies, protein detection is of great importance for early clinical diagnosis of deadly diseases (Fields, 2001; Service, 2001). While antibody-based protein detection methods are enormously useful in research and medical diagnostics, they are not well adapted to rapid, sensitive protein detection (Merkoci et al., 2005). Now aptamers have been emerging as new protein recognition elements in a wide range of bioassays (Ellington and Szostak, 1990). Aptamers, the synthetic DNA/RNA oligonucleotides isolated for their ability to selectively bind to various biomolecules through SELEX (Tuerk and Goldberg, 1990; Robertson and Joyce, 1990; Bock and Griffin, 1992), have many advantages over antibodies, including simpler synthesis, easier storage, reproducibility, and wider applicability (German et al., 1998). Aptamer-based protein detections have been illustrated in connection to colorimetric (Stojanovic and Landry, 2002), fluorescence (Merino and Weeks, 2003), quartz crystal microbalance (Liss et al., 2002) and electrochemical detection (Kawde et al., 2005). Among various aptasensors, the electrochemical aptasensor has attracted particular attention because it provides a sensitive, simple, and miniaturized platform. To improve the sensitivity of electrochemical detection, several electrochemical methods using nanoparticles with high sensitivity and selectivity have been developed (Wang et al., 2001; Park et al., 2002).

Nanoparticle-based materials offer excellent prospects for protein detection because of its unique physical and chemical properties. Magnetic nanoparticle has been widely used in bioanalysis (Wang and Kawde, 2002; Wang et al., 2002) because it serves as both the solid support and the means of separation in the system, and it can also collect the sample by magnetic field to offer promise as sensitive sensors. Our group (Cheng et al., 2005) had developed a DNA biosensor based on magnetic nanoparticles to realize the detection of one base mismatch.

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Effective disease diagnosis is highly dependent on the development of sensitive method, so signal amplification may be very useful in DNA and protein recognition (Laios et al., 2001; Zhang et al., 2003). Most of the approaches used for the signal amplification of the biosensor have been accomplished by using nanoparticles substituting for traditional tags or enzymes for both protein and DNA recognition (Yin et al., 2005; Soto et al., 2006). Among nanoparticle tags, gold nanoparticles are widely used for electrochemical biosensors due to the splendiferous bioconjugation of biomolecules and their excellent electrochemical properties (Maxwell et al., 2002; Mirkin and Letsinger, 1996; Taton et al., 2000; Pumera et al., 2005; Zheng et al., 2006).

Herein, a new amplification strategy for ultrasensitive electrochemical detection of thrombin through the formation of the network-like thiocyanuric acid (TCA)/gold nanoparticles is presented. In this strategy, thrombin acted as a target protein, which includes two binding sites for aptamer (Pavlov et al., 2004). AptamerI, 15 bases DNA sequence, and aptamerII, 29 bases DNA sequence. The magnetic nanoparticle-immobilized aptamerI was used for capturing and separation. The gold nanoparticle-labeled aptamerII offered electrochemical signal transduction. Fig. 1 shows through the specific recognition for thrombin, a sandwich format of magnetic nanoparticle/thrombin/gold nanoparticle was fabricated (step a), and the signal amplification was further implemented by forming network-like TCA/gold nanoparticles to cap more gold nanoparticles for per sandwich format (step b). TCA, a star-shaped trithiol molecule, was found to be an effective capping agent for the formation of the network structure (Tan et al., 2002). The transducing aptamer recognition event to detectable electrochemical signal was greatly enhanced by the aggregation of network-like TCA/gold nanoparticles. The detection limit could go down to 7.82 aM of thrombin with a signal-to-background ratio of 3, one of the most sensitive assays concerning the detection of protein (Li et al., 2004; Gokulrangan et al., 2005). The quantitative assay of thrombin in plasma was accomplished using the proposed electrochemical aptasensor, and the reliability of the assay through extensive repeat analysis was investigated. The proposed electrochemical aptasensor offers a promise of a convenient method to ultrasensitively recognise target protein in complex biological samples.

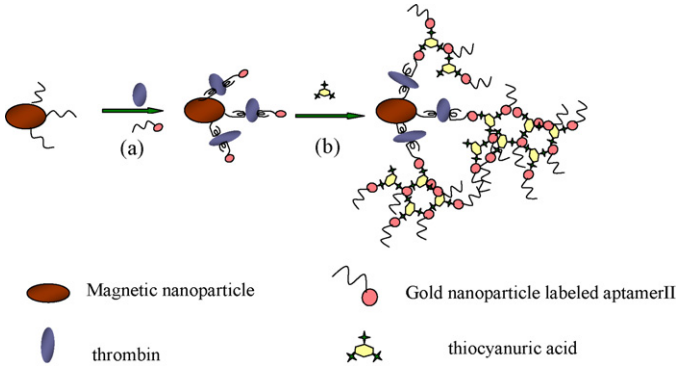

Fig. 1. Schematic representation of fabricating the thrombin aptasensor and the signal amplification. Step a, formation of the sandwich format of magnetic nanoparticle/thrombin/gold nanoparticle; step b, formation of the network-like TCA/gold nanoparticles.

2. Experimental

2.1. Reagents

The oligonucleotides used in this study were purchased from Invitrogen Biotechnology Inc. (Shanghai, China) with the following sequences: the oligonucleotide with amino group at its 5′ end (aptamerI): 5′ H_2N-$(CH_2)_6$-ATAGGTTGGTGTGGTTGG; the oligonucleotide with mercapto-group at 5′ end (aptamerII): 5′SH-$(CH_2)_6$-AGTCCGTGGTAGGGCAGGTTGGGGTGACT; the random oligonucleotide with mercapto-group at 5′ end: 5′SH-GAGCGGCGCAACATTTCAGGTCGA. Thrombin, bovine plasma albumin (BSA), lysozyme, and pancreatic enzyme were purchased from Sigma–Aldrich. Thiocyanuric acid was purchased from TCI Co. Ltd. (USA). Magnetic nanoparticle (100 mg/ml, carboxyl functionalized, average diameter 100 nm) was purchased from Chemicell Inc. (Berlin, Germany). Sodium citrate, $AuCl_3HCl{\cdot}4H_2O$ were purchased from Shanghai Chemical Reagent Inc. (Shanghai, China). Ethyl-3-(dimethylaminopropyl)-carbodiimide hydrochloride (EDAC) was purchased from Sigma–Aldrich. PBS, Tris–HCl buffer were purchased from Invitrogen Biotechnology Inc. (Shanghai, China). Ultrapure water was used from Aquapro system (specific resistance is 13 MΩ cm).

2.2. Instrumentation

Differential pulse voltammetry (DPV) measurements were performed using a CHI Instruments model 832 Electrochemical Analyzer (CHI Instrument Inc., USA). The electrochemical system comprised of a working electrode of glassy carbon electrode, a Ag/AgCl reference electrode, and a platinum wire counter electrode. The scanning electron microscope images were recorded using a JEOL JSM-6700F field emission scanning electron microscope (JEOL, Japan). UV-visible absorption spectra were recorded on a Varian Cary 50 UV-visible spectrophotometer (Varian, USA).

2.3. Preparation of gold nanoparticles

Gold nanoparticles were prepared according to the literature (Amihood et al., 1995). In brief, all glassware used in the following procedure were cleaned in a bath of freshly prepared 3:1 HNO_3–HCl, rinsed thoroughly in twice-distilled water and dried in air. One percent (w/w) $HAuCl_4$ and sodium citrate solution needed to be filtered through a 0.22 μm microporous membrane filter prior to use. Gold nanoparticles were prepared by adding 2.5 ml of 1% sodium citrate solution to 100 ml boiling aqueous solution containing 1 ml of 1% $HAuCl_4$ (w/w), and stirred for 30 min. Within the time, the color of the solution changed from grey, blue, purple, to wine red. The mixture continued to stir for 10 min after removal from the heater. Preparations were stored in dark glass bottles at 4 °C for further use. The average size of gold nanoparticles obtained was 10 nm.