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An aptamer–SWNT biosensor for sensitive detection of protein via mediated signal transduction

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ABSTRACT

An aptamer–SWNT based electrochemical biosensor is developed for sensitive detection of thrombin via mediated signal transduction. To realize this purpose, a dense monolayer of 16-mercaptohexadecanoic acid was modified on the gold electrode. In the presence of thrombin, SWNTs were controllably assembled on this insulating monolayer, which could mediate efficient electron transfer between the electrode and eletroactive species to generate a larger redox current. Through detecting the redox signal mediated by SWNTs, this strategy could present significant signal amplification and a detection limit of 50 pM thrombin was achieved. Such an aptamer–SWNT based biosensor opens a rapid, selective and sensitive route for thrombin detection and offers a promising strategy for specific protein detection.

1. Introduction

Aptamers are single-stranded DNA/RNA oligonucleotides selected from systematic evolution of ligands by exponential enrichment process in vitro that bind to their target molecules with high affinity, including proteins, carbohydrates, or lipids [1]. These artificial nucleic acid ligands offer several advantages over antibodies owing to their relative ease of isolation, modification, tailored binding affinity and storage [2]. Research focused on aptamers has exhibited promising potentials in various fields such as pharmaceutics and diagnostics [3].

Thrombin, a highly specific serine protease, plays an important role in cardiovascular diseases and acts as tumor marker in the diagnosis of pulmonary metastasis [4]. Therefore, recognition and quantification of thrombin are crucial in fundamental research and clinical practice. Until now, many aptamer-based detection systems for thrombin have been developed such as surface-enhanced Raman spectroscopy, colorimetry, fluorescence and electrochemistry [5–8]. Among them, electrochemistry holds great potential as the next generation detection strategy because of its high sensitivity, simple instrumentation and excellent compatibility with miniaturization. For example, the electrochemical sensors based on aptamer were fabricated by tethering a redox active substance to the terminal nucleic acid of aptamer immobilized on an electrode and the oligonucleotide undergoes a conformational change after binding the target molecule [8,9]. Considering that aptamer usually needs to be modified which might lead to the loss of affinity and specificity [10], development of label-free aptamer based electrochemical biosensors would be important.

Carbon nanotubes (CNTs), owing to great chemical stability, excellent electrical conductivity and high electro-catalytic activity, become very bright in the development of advanced electrochemical biosensors [11,12]. The CNTs modification affords an efficient platform for facilitating electron transfer between redox labels and the electrodes to improve biosensing sensitivity [13,14]. CNTs can also function as the carriers of conventional electrochemical labels [15], offering a substantial signal amplification. Selective assembly of CNTs on the electrode to mediate signal transduction would be attractive to ensure a highly sensitive response, which has rarely been applied for the development of aptamer biosensors.

Here we report a novel label-free electrochemical biosensing strategy for sensitive detection of thrombin based on aptamer–SWNT complex. Without any covalent modification, this protocol is simple and both the activity and specificity of aptamer and electrochemical property of SWNTs can be well preserved. In the presence of target protein, the SWNTs are controllably adsorbed on the isolating MHA self-assembled monolayer modified electrode. With a low background current, this SWNT assembly can mediate efficient electron transfer between the electrode and electroactive species, generating a high redox current due to the signal amplification, which ensures a very sensitive and specific biosensing for thrombin.

2. Experimental

2.1. Materials

The SWNTs were purchased from Carbon Nanotechnologies, Inc. (Houston, USA). Thrombin, bovine serum albumin (BSA) and cytochrome c were purchased from Aldrich. The aptamer was

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obtained from Sangon Corp. (Shanghai) and the sequence is 5′-AGCCGTGGTAGGGCAGGTTGGGGTGACT-3′. DNA, thrombin, BSA and cytochrome c were all prepared in 10 mM Tris–HCl buffer at pH 7.4. Milli-Q water (>18 MΩ) was used throughout the experiments.

2.2. Preparation of aptamer–SWNT complex

The SWNTs (0.5 mg) were mixed with aptamer (0.15 mg) in Tris–HCl buffer (10 mM, pH 7.4) under sonication with ice bath for 4 h. The resulting suspension was centrifuged at 12000 rpm for 1.5 h to remove possible SWNT aggregation. The SWNTs in the supernatant were then collected and filter through a Pall centrifugal filter with the molecule cutoff of 30 kDa to remove excessive oligonucleotides not wrapping around the SWNT. After washing with buffer for five times, the aptamer–SWNT complex was resuspended in Tris–HCl buffer (10 mM, pH 7.4) and stored at 4 °C when not in use.

2.3. Gold electrode modification

Gold disk electrodes were treated with pirahna solution (H_2O_2/H_2SO_4 1:3 in volume) for 5 min, then polished with 1, 0.3 and 0.05 μm alumina powder sequentially and washed with ultrasonication, respectively. The clean gold electrodes were dried and immersed into an ethanol solution of MHA (10 mM) for 24 h to allow the formation of a compact SAM. The MHA modified electrodes were then thoroughly rinsed with ethanol to remove physical adsorbed MHA and dried under mild nitrogen steam.

2.4. Electrochemical measurements

A 2 μL of aptamer–SWNT complex was added into 20 μL of mixture containing 10 mM Tris–HCl (pH 7.4), 100 mM NaCl, 10 mM KCl, 10 mM $MgCl_2$ and protein sample of a given concentration. The mixture was incubated at 37 °C for 60 min. Then the resulting solution was dropped onto the surface of the MHA modified electrode and incubated for 30 min at room temperature. Subsequently, the electrodes were thoroughly rinsed with ultrapure water to remove weakly adsorbed SWNTs on the electrodes.

Cyclic voltammetry (CV), square wave voltammetry (SWV) and quartz crystal microbalance (QCM) were performed with a CHI 660 C or CHI 440A workstation (Chenhua Instrument Co. Shanghai, China). Electrochemical impedance spectroscopy (EIS) was carried out with a μAutoLab III potentiostat (Eco Chemie, Utrecht, Netherland). A three-electrode system was employed for the electrochemical detection, while a saturated calomel reference electrode (SCE) served as the reference and a platinum wire served as the counter electrode. All electrochemical measurements were performed in 5 mM $Fe[(CN)_6]^{3-}$ solution containing 0.1 M KCl and repeated three times except as otherwise stated.

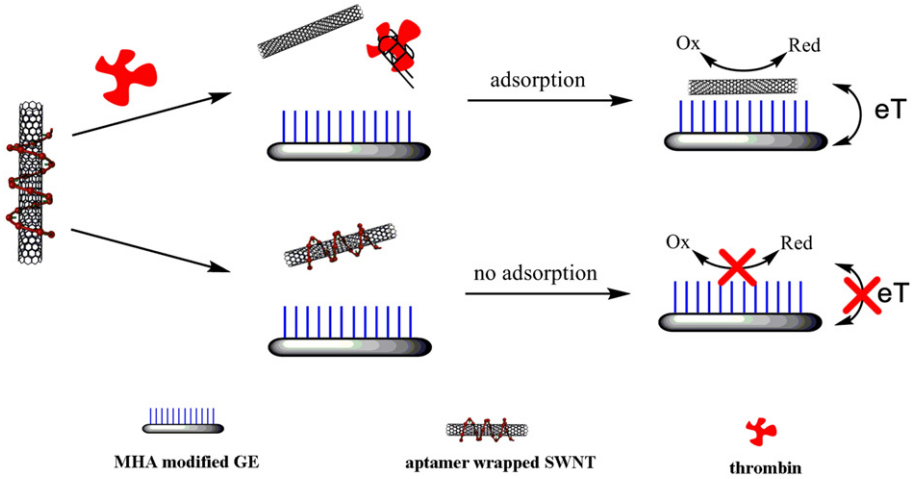
3. Results and discussion

3.1. Aptamer–SWNT complex based biosensing strategy

Scheme 1 illustrates the electrochemical response strategy of the aptamer–SWNT based biosensor for detection of target proteins. In this design, the gold electrodes are modified with a dense self-assembled monolayer (SAM) of MHA. This hydrophobic SAM can isolate the electrode from electroactive species in aqueous solution, which blocks the electron transfer between the electrode surface and redox probes. The isolation property of long alkanethiol monolayer modified electrode can be modulated by the binding between SAM and SWNTs [16], which can be used to evoke the signal transduction. And then SWNT is wrapped around by aptamer through aromatic interactions between nucleotide bases and SWNT sidewall [17], forming an aptamer–SWNT complex which stably disperses in the aqueous solution. In the presence of target protein, aptamer molecules can be peeled off from SWNT. Then the naked SWNTs will be precipitated from the solution and assembled on the MHA monolayer by the van der Waals and hydrophobic interactions between the long alkyl chains and SWNT sidewall [18]. The absorbed SWNTs on the isolating SAM can mediate efficient electron transfer between the electrode and electroactive species such as $[Fe(CN)_6]^{3-/4-}$, which generates a large redox current due to the signal amplification from SWNT to electroactive molecules. As the electrochemical current increases with the increasing concentration of target protein, this biosensor indeed offers a general and quantitative approach for sensitive detection of proteins.

3.2. Electrochemical characteristics of biosensor system

The electrochemical biosensing was characterized by cyclic voltammogram, electrochemical impedance spectroscopy and quartz crystal microbalance. Fig. 1A shows typical CV responses of thrombin assay. No remarkable peak was obtained for $[Fe(CN)_6]^{3-/4-}$at MHA modified electrode. With the addition of aptamer–SWNT on the electrode, little effect was observed on CV responses, and no significant increase of the redox current was observed. This observation showed that the adsorption of aptamer–SWNT on the MHA SAM was precluded because of strong electrostatic and hydration repulsion between aptamer–SWNT and MHA assembled surface [19]. However, in the presence of both aptamer–SWNT and thrombin, a couple of well-defined redox peaks



Scheme 1. Electrochemical biosensor strategy for thrombin using aptamer-wrapped SWNT as electrochemical labels.