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An electrochemical aptasensor for thrombin detection based on the recycling of exonuclease III and double-stranded DNA-templated copper nanoparticles assisted signal amplification

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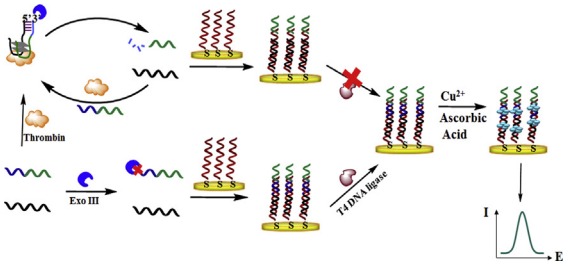
HIGHLIGHTS

- An improved electrochemical aptasensor has been proposed for thrombin detection.
- Both Exo III and dsDNA-templated CuNPs are used for signal amplification.
- The aptasensor can detect protein in a broad linear range with low detection limit.
- The aptasensor can easily distinguish thrombin in both buffer and complex sample.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, we report an improved electrochemical aptasensor based on exonuclease III and double-stranded DNA (dsDNA)-templated copper nanoparticles (CuNPs) assisted signal amplification. In this sensor, duplex DNA from the hybridization of ligated thrombin-binding aptamer (TBA) subunits and probe DNA can act as an effective template for the formation of CuNPs on the electrode surface, so copper ions released from acid-dissolution of CuNPs may catalyze the oxidation of o-phenylenediamine to produce an amplified electrochemical response. In the presence of thrombin, a short duplex domain with four complementary base pairs can be stabilized by the binding of TBA subunits with thrombin, in which TBA subunit 2 can be partially digested from 3′ terminal with the cycle of exonuclease III, so the ligation of TBA subunits and the subsequent formation of CuNPs can be inhibited. By electrochemical characterization of dsDNA-templated CuNPs on the electrode surface, our aptasensor can display excellent performances for the detection of thrombin in a broad linear range from 100 fM to 1 nM with a low detection limit of 20.3 fM, which can also specially distinguish thrombin in both PBS and serum samples. Therefore, our aptasensor might have great potential for clinical diagnosis of biomarkers in the future.

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1. Introduction

Aptamer, which is usually artificial DNA or RNA isolated by an in vitro systematic evolution of ligands by exponential enrichment (SELEX) process, can especially recognize and bind to the target molecules with high affinity, including ions, small molecules, proteins and cells [1,2]. Compared with the traditional recognition element antibody, aptamer is much easier for synthesis and preservation due to the simple structure and high stability [3]. Therefore, aptamer has been increasingly used as an appealing recognition element for the development of new biosensor as an alternative to the antibody-used immunosensor, which is named as aptasensor [4–6]. Among them, benefiting from the unique advantages of electrochemical techniques, the electrochemical aptasensor is the most attractive one and has aroused great attention for the advantages of low background, simple operation, rapid response, high sensitivity and specificity, etc. [7–11].

In order to continuously improve the sensitivity of biological detection, signal amplification strategies have been extensively involved in the design and construction of aptasensors. For example, nucleases that are vital tool in molecular biology have become compelling to assist DNA-based signal amplification for the improvement of the aptasensors [12–15]. So, exonuclease III (Exo III) that can selectively catalyze the stepwise removal of mononucleotides from 3′-terminal of duplex DNA has been the most frequently-used nuclease as it has no requirement for DNA sequences, which has been known to allow one target to interact with multiple DNA probes and thus lead to ultra-high sensitivity in the detection of biomolecules [16–18]. Meanwhile, nanoparticle with particular nano-scale size-dependent properties has been considered as another popular choice for the introduction of signal amplification into the fabrication of aptasensors [19–21]. Besides signal amplification from the large surface-to-volume ratio and well catalytic properties, the nanoparticles with metal components can also be signal tags to induce amplified responses in the electrochemical detection, such as gold nanoparticles, silver nanoparticles and quantum dots. In this case, large amounts of metal ions can be released from the dissolution of these nanoparticles, which can be accurately traced by electrochemical techniques with high sensitivity [22–25].

Recently, a selective formation of copper nanoparticles (CuNPs) by using double-stranded DNA (dsDNA) as templates has been proposed, whereas single-stranded DNA (ssDNA) cannot serve as an effective template to support the formation [26]. Since it was reported by Mokhir et al. in 2010, dsDNA-templated CuNPs have attracted great attention in fluorescence analysis of biomolecules for its facile synthesis and excellent optical properties [26–29]. In this paper, we have employed electrochemical technique as a substitution of fluorescent technique to characterize the formation of dsDNA-templated CuNPs for the development of an improved new aptasensor. Thrombin-binding aptamer (TBA) subunits after ligation reaction can hybridize with probe DNA to be the template for the formation of CuNPs on the electrode surface, while the presence of thrombin can induce Exo III-catalyzed cyclic degradation to inhibit the ligation of TBA subunits. Therefore, highly sensitive and selective detection of target protein can be realized by tracing the amplified electrochemical responses from dsDNA-templated CuNPs on the electrode surface when combined with copper ions-catalyzed oxidation of o-phenylenediamine (OPD).

2. Experimental

2.1. Materials and reagents

OPD, thrombin, ascorbic acid were purchased from Sigma. Exo III and T4 DNA ligase were purchased from New England Biolabs. Bovine Serum Albumin (BSA), ovalbumin (OVA) and hemoglobin (Hb) were purchased from Dingguo biotech. All other chemicals used were analytical grade. Milli-Q water (>18.0 MΩ) was used in all experiments, which was purified by a Milli-Q ultrapure water system (Millipore purification pack).

DNA oligonucleotides were synthesized by Sangon (Shanghai, China) and the sequences are as follows.

TBA subunit 1: 5′-P-AGTCCGTGGTAGGGC-3′;

TBA subunit 2: 5′-TAGGTTGGGGTGACT-3′;

Probe DNA: 5′-SH-TTTTTTCACGGACTAGTCACCCC-3′.

2.2. The preparation of probe DNA modified electrode

The substrate gold electrode (3 mm diameter) was first polished on sand paper and then silk with alumina oxide powder (particle sizes are about 1.0, 0.3 μm and 0.05 μm in sequence). Then, the residual organics were removed by ultrasonicating the electrode in both ethanol and double-distilled water for 5 min, respectively. Finally, the electrode was electrochemically cleaned in 0.5 M H_2SO_4 by scanning from 0 V to 1.6 V. Afterward, the gold electrode was immediately incubated with a solution containing 1 μM probe DNA for 16 h at room temperature and then treated with 1 mM 6-mercapto-1-hexanol (MCH) for 1 h. After being rinsed by double-distilled water thoroughly, probe DNA modified electrode was prepared for use.

2.3. Exo III-catalyzed degradation and dsDNA-templated formation of CuNPs

100 nM TBA subunit 1 and TBA subunit 2 were firstly incubated with desired concentration of thrombin at 37 °C for 1 h to help TBA correctly bind to thrombin. Then, TBA subunits was incubated with 0.025 U μL^{-1} Exo III at 37 °C for 20 min in NEB 1 buffer solution (10 mM Bis Tris Propane–HCl, 10 mM $MgCl_2$, pH 7.0), which was terminated by heating at 70 °C for 20 min. Afterward, probe DNA modified electrode was immersed into the resulting solution and incubated at 16 °C for 30 min after the addition of 1 mM ATP and 3.2 U μL^{-1} ligase for the ligation of TBA subunits on the electrode surface. After being thoroughly rinsed by double-distilled water, the electrode was immersed into a solution containing 200 μM Cu^{2+} and 100 mM ascorbic acid at the room temperature for 15 min to form CuNPs, which was followed by being thoroughly rinsed with ethylene diamine tetraacetic acid (EDTA) buffer to remove nonspecific adsorbed Cu^{2+}.

2.4. Electrochemical analysis

For the electrochemical measurement, CuNPs-coated electrode was firstly dipped in a 0.5 mM HNO_3 solution at room temperature for 1 h to dissolve CuNPs, and the resulting solution was then incubated with 1 mg mL^{-1} OPD at 80 °C water bath for 15 min to oxidize OPD. Differential pulse voltammetry (DPV) and chronocoulometry (CC) were performed on a model 660 C electrochemical analyzer with a three-electrode electrochemical system at room temperature. The three-electrode system consists of a working electrode, a saturated calomel reference electrode (SCE), and a platinum wire as the counter electrode. In order to maintain the solution anaerobic, all the electrolytes were thoroughly deoxygenated by bubbling high-purity nitrogen for at least 10 min, and a stream of nitrogen was blown gently across the surface of the solution throughout all the measurements.