Sensors and Actuators B 266 (2018) 9–18

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Rolling circle amplification triggered poly adenine-gold nanoparticles production for label-free electrochemical detection of thrombin

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ARTICLE INFO

Article history:
Received 14 October 2017
Received in revised form 12 March 2018
Accepted 18 March 2018
Available online 20 March 2018

Keywords:
Label-free
Adenine
Electrocatalytic
Thrombin

ABSTRACT

We developed a novel label-free strategy for electrochemical detection thrombin based on rolling circle amplification (RCA) triggering poly adenine production for adsorption gold nanoparticles (AuNPs). In this assay, the aptamer 1 was first immobilized on the gold electrode (GE) surface as the capture probe via poly adenine-Au. Subsequently, the thrombin and the aptamer 2 were deposited on the electrode surface. The aptamer 2 fragment on the sensor surface as a primer hybridized with the RCA template to initiate the RCA process, which generated massive long DNA sequences that contained many adenines. Subsequently, the AuNPs was absorbed on the long-repeated adenines of RCA product, resulting in the multiplication of AuNPs on the electrode surface, which were used for subsequent electrocatalytic reduction of H_2O_2. In this sensing system, the electrochemical signals with the concentration of thrombin over a range from 0.1 pM to 10 nM were detected and the detection limit obtained was 35 fM, and have high selectivity toward its target protein. In addition, due to the high affinity between poly adenine and GE or AuNPs, the detection can be carried out without any fussy modification process, thus had a promising application in clinical diagnosis.

1. Introduction

Thrombin, a kind of serine protease, plays a significant role in blood coagulation. It can directly transform soluble fibrinogen into insoluble fibrin that subsequently forms the fibrin gel for clots [1–3]. Changes in thrombin concentration levels in the blood are known to be associated with various coagulation abnormalities and it is considered as a biomarker for tumor diagnosis [4,5]. Therefore, highly sensitive detection of thrombin is of immense importance for early diagnosis, clinical practice and following disease recurrence [6,7].

The conventional diagnostic method is enzyme-linked immunosorbent assay (ELISA) based on the presence of specific antibodies of bacterium and flagellar antigen. The method is used as standards, but the antibodies have the disadvantages such as low affinity, poor chemical stability, complicated preparation and high cost. In these regards, it has been of great interest to replace antibodies with aptamers in ELISA. Aptamers are a class of nucleic acids that can selectively bind, with high affinity, to a wide array of target molecules [8–12]. Various techniques and methods based aptamer-protein detection, such as fluorescence [13,14], colorimetric [15–19], electrochemiluminescence [20–22], chromatography [23], Raman [24]; surface plasmon resonance [25], photoelectrochemical [26,27] and electrochemical [28–30], have been designed for ultrasensitive detection of thrombin. Among these methods, the electrochemical aptasensors are widely applied and play an important role in future research due to their high sensitivity, inherent simplicity, portability and low cost [31–33].

To achieve a highly efficient electrochemical aptasensors, one major challenge is to amplify the detectable signal during the measurement [34,35]. To realize this issue, the enhanced detection signals via the use of nanomaterial or enzyme molecules have been exploited to enhance the sensitivity of the electrochemical aptasensors. In many aptamer assays, enzyme molecules (e.g., horseradish peroxidase and alkaline phosphatase) [4,36] or nanomaterial (e.g., metal nanoparticles and carbon materials) [37–40] were usually attached on the aptamer or antibodies through antibody-antigen interaction, biotin-streptavidin (avidin) interaction, or chemical cross linking. However, this approach commonly involved in the fussy labeling process and might result in the deactivation of

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https://doi.org/10.1016/j.snb.2018.03.112
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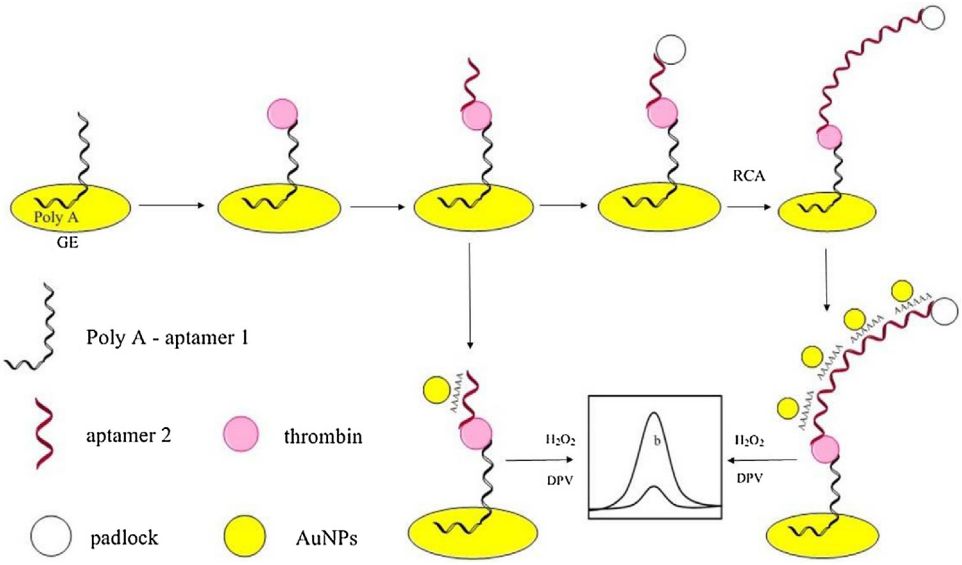

Scheme 1. Schematic representation of label-free electrochemical detection of TB based on RCA triggering poly adenine production for adsorption AuNPs.

aptamer. Therefore, the label-free electrochemical aptasensing systems have received increasing interest, due to the simplicity of these sensing approaches without the involvement of any enzymes or modifications to the aptamers to generate the electrochemical signal output.

Recently, poly adenine (Poly A)-mediated self-assembly strategies have been developed as a novel and promising means for modulating the DNA distribution on the surface of AuNPs or AgNPs in a quantitative manner [41,42]. Notably, the whole poly A blocks consisted of multiple consecutive adenines fully covered the gold surface with high intrinsic affinity, distinctly different from thiolated DNA that had only one contact point for gold surface [43,44]. Thus, poly A can serve as a high-efficacy anchoring block, enabling systematic modulation of lateral spacing and surface density of DNA on surface of AuNPs [45]. Inspired by these unique work, herein, we developed a novel method for assay of thrombin (TB) based on rolling circle amplification (RCA) triggering the combination of poly adenine with gold nanoparticles. In typical RCA, a long linear concatenated DNA product containing thousands of tandem repeats complementary to the circular template can be produced within 1–2 h under constant temperature. As illustrated in Scheme 1, the aptamer 1 (Apt1) was first immobilized on the gold electrode (GE) surface via polyA-Au as the capture probe. Subsequently, the thrombin and the Apt2 was deposited on the electrode surface. The Apt2 fragment on the sensor surface is subject to in situ RCA to generate massive long DNA sequences that contained many adenines. Subsequently, AuNPs as signal tag were deposited on the electrode surface. Many AuNPs were adsorbed on the electrode surface by polyA, which exploiting polyA-Au interactions. The performance of the resulting electrochemical aptasensor was tested by recording the AuNPs electrocatalytic reduction toward H_2O_2. The results demonstrated that the aptasensor based on this amplification strategy has a wide linear range and a low detection limit for TB. It thus shows great promise for application in biomedical research and clinical diagnosis.

2. Material and methods

2.1. Reagents and materials

Bovine serum albumin (BSA), IgG, hemoglobin (Hb) and TB were purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd. Phi29 DNA polymerase, T4 DNA ligase, ATP and deoxyribonucleoside, 5′-triphosphates mixture (dNTPs) were purchased from Sangon (Shanghai, China). 6-*mer*capto-L-hexanol (MCH), and $HAuCl_4$ was purchased from alladin. All other reagents were obtained from Sinopharm Chemical Reagent Company, China. Various buffer solutions were prepared from analytical grade chemicals without further purification and were prepared with ultrapure water (Milli-Q A10 system, Millipore, USA). DNA hybridization buffer was phosphatebuffered saline (137 mM NaCl, 2.5 mM Mg^{2+}, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4, pH 7.4). Storage buffer used for oligonucleotides was 10 mM pH 8.0 tris-(hydroxymethyl) aminomethane (Tris-HCl) containing 1 mM ethylenediaminetetraacetic acid (EDTA). Phosphate-buffered saline (PBS, 0.1 M) of various pH was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. The washing buffer was PBS (0.1 M, pH 7.4) containing 0.05% (w/v) Tween-20. 40.0 mM Tris–HCl buffer (pH 7.5), 50.0 mM KCl, 10.0 mM $MgCl_2$, 5.0 mM $(NH_4)_2SO_4$, and 4.0 mM DTT formed RCA reaction buffer. The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. The detailed sequence of different oligonucleotides probes were as follows: Apt1: 5′-AAAAAAAAA**GGTTGGTGTGGTTGG**-3′, Apt2: 5′-**AGTCCGTGGTAGGGCA GGTTGGGGTGACT**GTGTG AAAAAA GTGTG −3′, Padlock: 5′-P- TTT CACAC CACCA CCA CCA CCA CCA CCA CCA CCA CCA CCA CACAC TTT −3′.

2.2. Preparation of AuNPs

A 20 mL aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ and 2.5×10^{-4} M trisodium citrate was prepared in a conical flask. Next, 0.6 mL of ice-cold, freshly prepared 0.1 M $NaBH_4$ solution was added to the solution while stirring. The solution turned pink immediately after adding $NaBH_4$, indicating particle formation. Here, citrate serves only as a capping agent since it cannot reduce the gold salt at room temperature (25 °C) [46].

2.3. Immobilization of the sandwich structure

The gold electrode was immersed in piranha solution (H_2SO_4: 30% H_2O_2 = 3:1) for 5 min to remove the adsorbed organic matter, and then rinsed with pure water. After that, the electrode was suc-