Analytica Chimica Acta 860 (2015) 77–82

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Ultrasensitive electrochemical detection for thrombin using hybridization chain reaction with enzyme-amplification

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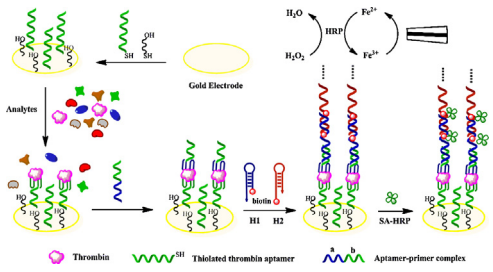
HIGHLIGHTS

- Ultrasensitive electrochemical detection for thrombin was obtained with aptasensor.
- Hybridization chain reaction was employed for amplifying signal.
- The proposed strategy is successfully applied in real sample assay.
- Scanning electrochemical microscopy was adopted to visualize captured thrombin.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 October 2014
Received in revised form 13 December 2014
Accepted 15 December 2014
Available online 17 December 2014

Keywords:
Aptasensor
Thrombin
Hybridization chain reaction
Topographic imaging

ABSTRACT

In this work, a new electrochemical aptasensor using hybridization chain reaction (HCR) for signal amplification was developed for highly sensitive detection of thrombin. The sandwich system of aptamer/thrombin/aptamer–primer complex was fabricated as the sensing platform. As the initiator strands, aptamer–primer complex could propagate a chain reaction of hybridization events between the two hairpin probes, and whether long nicked DNA polymers could be formed on the modified electrode. Then the biotin-labeled dsDNA polymers could introduce numerous avidin-labeled horseradish peroxidase (HRP), resulting in significantly amplified electrochemical signal through the electrocatalysis of HRP. On the basis of the enzymatic oxidization of Fe^{2+} by H_2O_2 to yield Fe^{3+}, the imaging of thrombin was detected by the reduction current of Fe^{3+} with the scanning electrochemical microscopic tip. The electrochemical signals had a good linear with logarithm of thrombin concentration in the range from 1.0 fM to 100 fM, reaching a detection limit of thrombin as low as 0.04 fM. In addition, the proposed strategy exhibited excellent specificity and was successfully applied in real sample assay which demonstrated the potential application in clinical diagnostics.

1. Introduction

DNA nanoassembly is a simple and effective approach for signal enhancement via target-probe hybridization. After designing properly, a wide variety of well-defined DNA nanostructures in one, two, and three dimensions have been created [1]. The concept of hybridization chain reaction (HCR) and detailed discussion of the process were first introduced by Dirks and Pierce [2]. Based on chain reaction of recognition and hybridization events between a pair of complementary, kinetically trapped hairpins, HCR technique offered an enzyme-free alternative for amplifying short sequences of oligonucleotides [3,4]. Besides, the HCR can be achieved at mild conditions. With these advantages, HCR has become a favorite strategy alternative to the polymerase chain

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http://dx.doi.org/10.1016/j.aca.2014.12.029
0003-2670/

reaction (PCR) and rolling circle amplification (RCA) for signal amplification in biomolecular analysis. Very recently, many self-assembled DNA nanostructures have been fabricated [5–10] and applied in the DNA detection system. Zhang et al. [11] designed an electrochemical aptasensor that was based on HCR and combined with hemin/G-quadruolex pseudobienzyme electrocatalytic recycling amplification to detect thrombin. Zhou et al. [12] described an ultrasensitive electronic detection protein system which was designed by using gold nanoparticle based bio-bar codes and an *in situ* amplified DNA-based hybridization chain reaction.

Scanning electrochemical microscopic (SECM), a noninvasive scanning technique, has found widespread applications in the study of biological processes and immobilized biomolecules in recent years. It offers a unique means to visualize surfaces by monitoring the limiting current at a microelectrode tip that is positioned within a few micrometers of a surface [13–17]. The nature of this electrochemical communication provides information about the topography and chemical reactivity of the surface with spatial resolution of the microelectrode [18].

In this contribution, a highly sensitive electrochemical detection for thrombin was presented by using hybridization chain reaction with enzyme-amplification reaction. Thrombin was captured on the electrode surface which was fabricated with thiolated aptamer via the self assembly. The aptamer–primer complex was localized as the initiator stand for hybridization chain reaction. HRP could attach to the double-stranded DNA via the interaction between biotin and avidin, resulting in amplified electrochemical response of HRP. In the presence of H_2O_2, Fe^{2+} was oxidized to Fe^{3+} through the HRP-catalyzed reaction at the modified substrate surface. The reduction current was monitored using the SECM tip ultramicroelectrode, and the topography was imaged simultaneously. Moreover, this method was successfully applied to detect thrombin in diluted human serum samples. This strategy held great potential to be used as a sensitive, selective and universal platform for the detection of other proteins with their aptamers.

2. Experimental

2.1. Materials and reagents

All oligonucleotides used in the present study were synthesized and HPLC purified by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). Their sequences are listed in Table S1. $K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$ were purchased from Tianjin Ruijinte Chemical Co., Ltd. (Tianjin, China). Streptavidin-HRP was obtained from Boster Bio-Engineering Co., Ltd. (Wuhan, China). H_2O_2 was supported by Tianjin Bodi Chemical Holding Co., Ltd. (Tianjin, China). Double-distilled, deionized water was used throughout the experiments. All reagents were of analytical grade and used without further purification.

2.2. Apparatus

Electrochemical and SECM experiments were carried out using a CHI 900C electrochemical working station (Shanghai CH Instrument, China). The SECM instrument consists of piezo inchworms, a stage, a controller that can move the tip in three dimensions, and a bipotentiostat. A gold electrode was served as the substrate for thrombin capture. A 10-μm diameter Pt ultramicroelectrode was used as an amperometric SECM tip. The reference electrode was Ag/AgCl/3 M KCl, and the counter electrode was a Pt wire. The electrochemical impedance spectroscopy (EIS) and differential pulse voltammograms (DPV) were performed in a polytetrafluoroethylene electrochemical cell at room temperature.

2.3. Fabrication of the electrochemical aptasensor

Prior to modification, the gold electrode was polished carefully with 0.3 and 0.05 μm alumina slurry and ultrasonically washed in ethanol and double distilled water. It was electrochemically cleaned in 0.1 M H_2SO_4 via potential scanning between −0.2 and 0.6 V until a remarkable voltammetric peak was obtained. After cleaning and being dried with nitrogen airflow, the gold electrode was soaked in 100 nM thiolated thrombin aptamer (TBA) solution to prepare aptamer-modified electrode for 16 h. After treatment with 1 mM 6-mercapto-1-hexanol (MCH) for 2 h, the unmodified region of the electrode was blocked. The resulting Au/TBA/MCH electrode was incubated for 2 h with various concentrations of thrombin to obtain the Au/TBA/MCH/thrombin electrode. Subsequently, the aptamer–primer complex (AP) was coated on the modified electrode, and interaction was kept for 2 h to obtain a sandwich sensing system.

2.4. Hybridization chain reaction

Prior to use, all the hairpin oligonucleotides were heated to 95 °C for 2 min and cooled to room temperature for 1 h. After washing with pH 7.4 phosphate buffer solution (PBS), the prepared electrode was incubated with a mixture of 5 μL hairpin probe biotin-H_1 (1 μM) and 5 μL biotin-H_1 (1 μM) in the reaction buffer for 2 h. During this process, the hybridization chain reaction was triggered and progressed to form the long nicked DNA polymers on the modified electrode. Then, 10 μL of 100 nM streptavidin-HRP was added and incubated at 37 °C for 1 h. The HRP-labeled double helix was obtained with the special recognition.

2.5. Electrochemical measurement

After rinsing thoroughly with pH 7.4 PBS to remove the unconjugated HRP, the electrochemical characteristics of the resulting sensors, including EIS, DPV, and cyclic voltammetry (CV), were preformed with a CHI 900C electrochemical working station at room temperature in a conventional three-electrode system. In this imaging experiment, the tip approached the gold electrode gradually and stopped at 75% of a steady-state current in detection cell containing 1.0 mM $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$. The current signals related to the thrombin concentrations could be measured by employing the substrate generation/tip collection (SG/TC) mode.

2.6. Application of the thrombin aptasensor in the biological assay

Human whole blood was provided by the local hospital. After the natural coagulation, the blood was centrifugated and the supernatant was obtained to be used. The actual samples were prepared by mixing different concentrations of thrombin. All the experiment conditions were the same as the target detection.

3. Results and discussion

3.1. Design strategy of the sensor

A novel strategy for thrombin assay using aptamer-triggering hybridization chain reaction amplification was successfully designed. As shown in Scheme 1, the thiolated thrombin–aptamer (TBA) and MCH were initially immobilized on the gold electrode surface by an Au–S affinity binding. Then the aptamer was used to capture the analyte thrombin in sample solution, obtaining the Au/TBA/MCH/thrombin electrode. The aptamer–primer complex (AP) was subsequently bound to the surface-captured thrombin, forming a sandwich system. The aptamer–primer complex