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Short communication

Binding-induced internal-displacement of inverted aptamer beacon: Toward a novel electrochemical detection platform

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ABSTRACT

In this paper, we reported a novel electrochemical aptasensor based on an inverted aptamer beacon (IAB). The IAB architecture with a hairpin structure consisted of an aptamer arm and a ferrocene (Fc)-labeling signal arm, and was inverted on the electrode through a thiol-linker on the middle site of its loop. The inverted immobilization strategy prevented the end-labeling of ferrocene tag from approaching the electrode, thus resulting in a weak electrochemical signal. In the presence of thrombin, binding of target with the aptamer arm displaced the signaling arm, producing a flexible single-stranded element, *i.e.* internal-displacement which allowed Fc tag to collide with the electrode, thereby producing a relatively strong electrochemical signal. By monitoring the increased signal, thrombin could be readily detected in the signal-on mode with a detection limit (LOD) of 0.21 nM. Inspiringly, the IAB-based strategy required no auxiliary strands and did not depend on structure-switching of specific aptamer/target couple, thus presenting as a generalized method for fabrication of other electrochemical aptasensors.

1. Introduction

Nucleic acid aptamers are isolated from random-sequence DNA or RNA libraries by an *in vitro* selection process termed the systematic evolution of ligands by exponential enrichment (SELEX) [1], which has specific binding affinity to various targets, ranging from small inorganic and organic substances to even a protein or cell [2–4]. Due to their inherent advantages over other recognition elements in terms of repeatable synthesis, easy modification, long-term stability and less immunogenicity, aptamers have been greatly focused in the area of biosensors, bringing about a new branch as aptasensors [5–9]. Among various methods, electrochemical aptasensors have attracted significant interest because they are simple, rapid, environment-insensitive and low-cost [10].

The key issue for the development of electrochemical aptasensors is how to convert target recognition and binding into a measurable electrochemical signal. A number of electrochemical aptasensors have been reported based on a large conformational change of the aptamer which upon target binding influences the electrochemical response of a redox tag [11,12]. Xiao et al. utilized the target binding induced G-quadruplex formation of anti-thrombin aptamer and the corresponding change in electrochemical signal to achieve sensitive detection of thrombin [13]. Although elegant, such an allosteric-based detection strategy is restricted to aptamer/target couples able to induce sufficiently high conformational change [14,15]. Adoption of molecular beacon (MB) strategies still suffers from the same problem, and most of them are "signal-off" ones (target binding reduces the electrochemical signal, leading to poor signal gain and low sensitivity) [16,17]. An important alternative approach was proposed by employed auxiliary DNA strands to prefabricate the sensor architectures [18,19]. Zuo et al. engineered the anti-ATP aptamer with partial duplex structure by introduction an auxiliary complementary strand, which was responsive to ATP through binding-induced displacement of the auxiliary strand [20]. Unfortunately, as these architectures are constructed by hybridization, the auxiliary strands may potentially lose when the sensor is washed or stored for long durations, leading to false positives [21]. Importantly, the design of efficient DNA sensor architectures can provide a new approach for studying protein sensor or DNA-protein interaction [22–24]. Thus, there is still a need for exploiting new sensor architectures to achieve the generalization of the electrochemical aptasensors. Herein, we report a novel signal-on electrochemical aptasensor platform for sensitive thrombin detection by employing an internal-displacement, inverted aptamer beacon.

2. Experimental

Human α-thrombin, 6-mercaptohexanol (MCH), *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-(dimethyl) aminopropyl) carbodiimide hydrochloride (EDC), were purchased from Sigma-Aldrich. All the other chemicals were of analytical grade, and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in

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all runs. The ferrocene (Fc) modified aptamer beacon was synthesized and purified through HPLC by Sangon Biotech. Co., Ltd (Shanghai, China), with a sequence as follows: 5′-**AGT CCG TGG TAG GGC AGG TTG GGG TG**A CTG CAA AAA AAT($(CH_2)_6$-NH_2)-5′-CCT CAG CAG TCA CCC TT-ferrocene-3′ (the bold letters are the sequence of anti-thrombin aptamer, the underlined letters are the stem sequence. A NH_2-moiety was attached on T base in the middle of loop through a $(CH_2)_6$ linker).

The thiol-linker in the middle site of loop was synthesized by conjugating 3-mercaptopropionic acid molecule (3-MPA) with the NH_2-moiety using the succinimide coupling (EDC-NHS) method. Briefly, 100 μL of 10 μM aptamer beacon was mixed with 100 μL of 10 mM PBS (pH 7.4) containing 0.1 mM of 3-MPA, 1 mM EDC, and 5 mM Sulfo-NHS, and incubated at 37 °C for 4 h. The product was then dialyzed against 10 mM PBS (50 mL) at 4 °C for 24 h to remove excessive 3-MPA. The structure of thiol-linker was shown in the insert of Fig. 1A.

Gold electrode (2 mm diameter) was polished with 0.3 and 0.05 μm alumina powder, and ultrasonicated in ethanol and water for 5 min respectively. Then it was electrochemically activated in 0.5 M H_2SO_4, washed with ultrapure water and dried under nitrogen stream. 5 μL of 0.5 μM aptamer beacon in 10 mM PBS buffer (pH 7.4) containing 0.5 M NaCl and 0.2 mM $MgCl_2$ was dropped onto the electrode and kept for 2 h at room temperature. Subsequently, nonspecific absorption probe was removed by rinsing with buffer solution thoroughly. Dried in nitrogen, the probe modified electrode was immediately incubated in 1 mM MCH for 1 h to block the uncovered surface. When finishing assembly, the modified electrode was thoroughly rinsed, dried, and then incubated in 1 M $NaClO_4$ before electrochemical measurements.

For thrombin detection, the modified electrode was incubated in a thrombin solution with different concentration diluted by 10 mM PBS, pH 7.4 with 150 mM NaCl, 20 mM $MgCl_2$ and 20 mM KCl at 37 °C for 1.5 h. Then the electrode was rinsed with 10 mM PBS to remove nonspecific adsorption of thrombin. Electrochemical measurements were performed using alternating current voltammetry (ACV, CHI 630D Electrochemical Workstation) with a step potential of 4 mV, an amplitude of 25 mV and a frequency of 100 Hz since ACV is a powerful electroanalytical technique for quantitative monitoring of electroactive species [25]. Aptamer beacon modified gold electrodes, platinum wire electrode and Ag/AgCl electrode served as working electrodes, counter electrode and reference electrode, respectively. Electrochemical measurement was conducted in nitrogen-purged 0.1 M $NaClO_4$ solution. SPR measurements were conducted on a single-channel AutoLab ESPR (Eco Chemie, The Netherlands).

3. Results and discussion

As shown in Fig. 1A, the aptamer beacon possesses an asymmetric structure, the longer arm of which the anti-thrombin aptamer partially hybridizes with the signaling strand (the shorter arm with Fc tag at its 3′-end) to form the stem of hairpin. Unlike the traditional method in which MB probe is immobilized on electrode through a terminal linker, the developed beacon was anchored onto gold electrode surface through a thiol-linker on the middle site of loop, thus was in an inverted form. The inverted immobilization form prevented the end-labeling of ferrocene tag from approaching the electrode surface, suppressing Faradaic currents. In the presence of thrombin, the binding of thrombin with aptamer arm displaced the signaling arm, producing a much more flexible single-stranded element. This, in turn, allowed the Fc tag to collide with the electrode surface, producing a readily detectable Faradaic current. As shown in Fig. 1B, in the absence of target, only small peak (at 0.257 V, *vs.* Ag/AgCl) was observed in the ACV curve of inverted aptamer beacon (IAB) modified gold electrode (curve 'a'). When challenged the sensor architecture with thrombin, a dramatic increase in the peak current occurred (curve 'b'). To further clarify this issue, binding kinetics of thrombin/aptamer beacon was measured both by SPR binding-curve and ACV response signal (Fig. 1C). As observed, the SPR

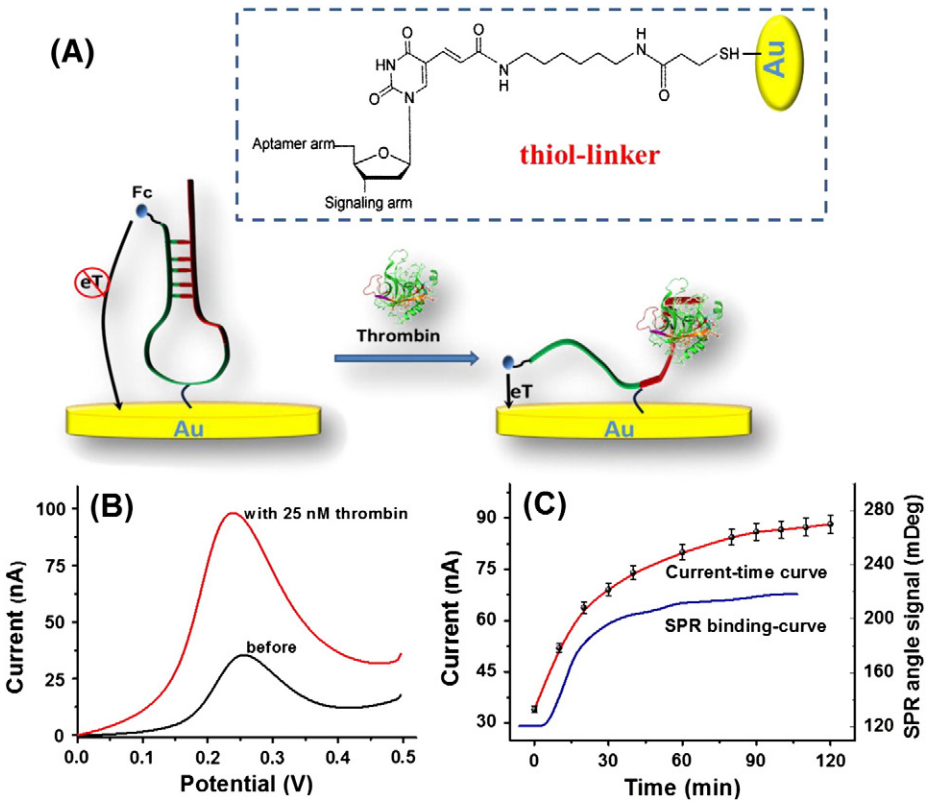

Fig. 1. (A) Schematic illustration of the signal-on aptasensor for thrombin detection based on the internal-displacement of inverted aptamer beacon, (B) ACV responses of the aptasensor before and after incubation with 25 nM thrombin, and (C) binding kinetics of thrombin with IAB probe measured by SPR and ACV, respectively (25 nM thrombin used in this case).