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Short communication

DNA aptamer-based QDs electrochemiluminescence biosensor for the detection of thrombin

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ABSTRACT

A novel biosensor for the detection of thrombin was developed by using QDs electrochemiluminescence (ECL) technique. The thiol-terminated aptamer with 15 nucleotides (probe I) was first immobilized on Au electrode, and then thrombin was imported to form the aptamer–thrombin bioaffinity complexes. Another 5′-biotin modified aptamer (29 nucleotides, probe II) was next hybridized with the combined thrombin to form a sandwich type structure. Streptavidin modified QDs (avidin–QDs) were bound to probe II via the biotin–avidin-system. The QDs ECL signal was responsive to the amount of probe II, which was indirect proportional to the combined thrombin. The ECL intensity of the biosensor increased with the increase of thrombin concentration in the range of 0–20 $\mu g\,mL^{-1}$. In addition, the biosensor exhibited the excellent selectivity responses and good stability toward the target analyte.

1. Introduction

Aptamer, as a new class of single-stranded DNA or RNA oligonucleotides, is obtained by the method called "systematic evolution of ligands by exponential enrichment (SELEX)" from random RNA or DNA libraries (Ellington and Szostac, 1990). It can specifically bind to a broad range of target compounds from metal ions, small organic molecules, and complex proteins to entire organism (Gold et al., 1995; Hesselberth et al., 2000; Smith et al., 2007; Zhang et al., 2008; Lu et al., 2008). As a new molecular recognition device, aptamer has received a great deal of attention. In addition to having selectivity comparable to those of monoclonal antibodies (Xu and Ellington, 1996), aptamers possess other advantages over traditional antibody-based reagents (Jayasena, 1999). Aptamers could be reproducibly synthesized and modified with certain functional groups. Now, aptamers-based biosensor is considered as a useful tool for recognizing and quantitating protein expression. The key issue to develop aptamer-based analytical methods is to convert target recognition into a measurable signal, and many techniques like HPLC (Michaud et al., 2003), electrochemical (Lu et al., 2008), and luminescent (Wang et al., 2005) assay are used for this purpose.

Since Bard's group found that QDs could generate efficient and stable ECL during the potential cycling or pulsing (Ding et al., 2002; Myung et al., 2004), highly luminescent QDs gained increasing attention for the applications in bioconjugates and optical biosensors (Wang et al., 2002; Choi et al., 2006; Feng et al., 2007; Jie et al., 2007; Jie et al., 2008).

This research developed a novel QD-based ECL biosensor for the detection of α-human thrombin via the "sandwich type" assay. In order to fabricate this "sandwich type" biosensor, two different types of anti-thrombin aptamers were employed. The single-stranded DNA oligonucleotide with 15 nucleotides (probe I) was the first aptamer selected in vitro that could specifically bind to a protein with nucleic acids-binding properties (Bock et al., 1992). A guanine-quartet based quadruplex structure, named "G-quartet structure", was formed when this aptamer binds to thrombin (Macaya et al., 1993; Smirnov and Shafer, 2000). Another anti-thrombin aptamer (probe II), a 29-nucleotide single-stranded DNA, has also been developed for combining with thrombin by binding to the heparin-binding exosite (Tasset et al., 1997). With the help of two aptamers for the same target, the sandwich structure among probe I, thrombin and probe II (Sonia et al., 2007; Apon et al., 2008) could be formatted. Different from those reports for the detection of aptamer–protein with the electrochemistry (Radi et al., 2005; Li et al., 2008a,b) or fluorescence (Cao and Tan, 2005), this aptasensor is based on the QDs ECL property in aqueous solution. To the authors' knowledge, this is the first report about the application of QDs ECL in aptasensor field via the sandwich method. It provided an alternative convenient, low-cost and specific method for protein detection. What is more, the development of QDs for aptamer biosensors may intrigue researchers into gaining a new interest in the investigation of the QDs ECL and promote the exploitation in the bioapplications.

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2. Experimental

2.1. Chemicals and materials

Labeled DNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of these two oligomers employed are given below:

- The 5′-thiol modified aptamer I:

  5′ – HS – $(CH_2)_6$ – GGT TGG TGT GGT TGG – 3′

- The 5′-biotin modified aptamer II:

  5′ – biotin – AGT CCG TGG TAG GGC AGG TTG GGG TGA CT – 3′

Bovine serum albumin (BSA), 6-mercapto-1-hexanol (MCH), lysozyme (from hen egg white), α-thrombin (from human plasma, MW = 36,700 g mol^{-1}) and adenosine 5′-triphosphate (ATP) were purchased from Sigma–Aldrich. Avidin–QDs (CdSe/ZnS core–shell structure about 10 nm) were obtained from Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China) with a prime concentration of 1.10 μM. All other reagents were of analytical reagent grade and used without further purification. The buffer solution contained 100 mM Na_2HPO_4 and NaH_2PO_4, 5 mM $MgCl_2$. The electrolyte for ECL measurements was 5 mL PBS^+ solution containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl. EIS (electrochemical impedance spectroscopy) was recorded in solution of 0.1 M KCl and 2 mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$. Millipore ultrapure water (resistivity ≥ 18.2 MΩ) was used throughout the experiment.

2.2. Apparatus

The ECL emission was detected using a model MPI-A electrochemiluminescence analyzer (Xi'an Remex Analysis Instrument Co., Ltd., Xi'An, China). The spectral width of the photomultiplier tube (PMT) was 300–650 nm, and the voltage of the PMT was set at 650 V during the detection process. The EIS analyses were performed on an Autolab PGSTAT12 (Ecochemie, BV, The Netherlands) and controlled by GPES 4.9 and FRA 4.9 softwares with the frequency range of 0.1–1.0 × 10^5 Hz. In all electrochemical experiments, the conventional three-electrode system was employed with a modified Au electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a Pt wire as the counter electrode.

2.3. Preparation procedure

Prior to use, the Au electrode was cleaned with freshly made piranha solution (98% H_2SO_4:30% H_2O_2 = 7:3, v/v) for 10 min twice (*CAUTION: piranha solution should be handled with great care*), and all the oligonucleotides solutions in PBS^+ were heat-treated in 90 °C for 3 min and then cooled in ice for 10 min.

For the preparation of thrombin biosensor, 20 μL of 5 μM aptamer I solution was first spread on the pre-cleaned Au electrode surface for 12 h at 37 °C in the 100% humidity. After being washed with the PBS^+, the electrode was immerged in 1 mM MCH for 2 h to remove the nonspecific DNA adsorption. Next, this electrode was immersed in thrombin solution for 1 h, washed with wash buffer (Tween-20 in PBS^+) to remove the nonspecifically bound thrombin, then rinsed with PBS^+ and dried with a stream of N_2. Afterward, the electrode was immediately covered with 20 μL probe II solution (5 μM in PBS^+) for another 1 h in 100% humidity. At last, 20 μL of diluted avidin–QDs liquid was dropped to the electrode surface. Before use, 1 μL obtained avidin–QDs solution was dissolved in 100 μL bovine serum albumin (BSA) solution (1 mg mL^{-1} in PBS^+) under shocking condition for 1 h for the purpose of blocking the active sites of the QDs. All these above incubation steps were carried out at 37 °C. Prior to ECL measurement the biosensor was washed with wash buffer solution. The electrode above was in contact with ECL electrolyte and scanned from 0 to −1.5 V to measure the ECL signals. The whole preparation process was outlined in Fig. 1.

3. Results and discussion

EIS was reported as an effective technique to monitor the surface feature and understand the chemical transformation (Bard and Faulkner, 1980). In the experiments, EIS characterization was used to check the whole modification processes. Fig. 2A is the

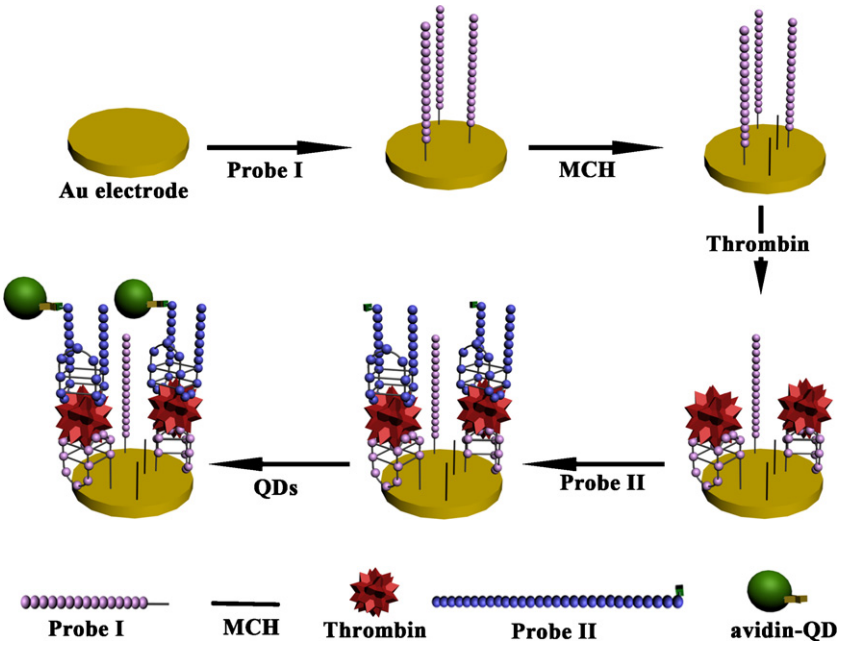

Fig. 1. Schematic diagram for the biosensor fabrication.

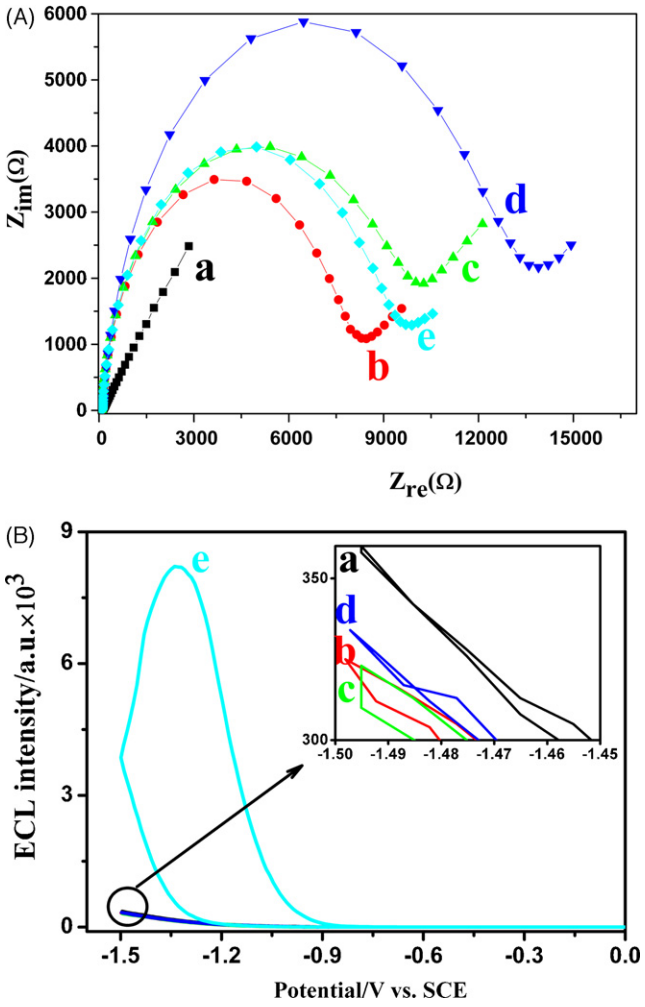

Fig. 2. Impedance spectra (Nyquist plots, *Zim* imaginary impedance, *Zre* real impedance) (A) and ECL intensity (B) of modified Au electrode at different stages. (a) bare Au electrode, (b) probe I/Au electrode, (c) thrombin/probe I/Au electrode, (d) probe II/thrombin/probe I/Au electrode, (e) QDs/probe II/thrombin/probe I/Au electrode. The insert in B is the amplifier of the circle section. The electrolyte for EIS detection: 0.1 M KCl + 2 mM $[Fe(CN_6)]^{3-}/[Fe(CN)_6]^{4-}$. The frequency range is $0.1–1.0 \times 10^5$ Hz. The electrolyte for ECL measurement: 5 mL pH 7.4 PBS^+ solution containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl. The voltage of the photomultiplier tube: 650 V. Scan rate: 100 mV s^{-1}.

Nyquist plots of EIS at different modified steps. Compared with the resistance of bare Au electrode (curve a in Fig. 2A), the probe I-modified Au electrode shows a larger *eT* resistance (curve b in Fig. 2A). This enlargement was resulted from the electrostatic repulsion between negative charges of the DNA aptamer backbone and the electroactive redox $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ probe. This *eT* resistance was further enlarged after the probe I-modified electrode was immersed into the thrombin solution for the formation of aptamer–thrombin bioaffinity complex between probe I and thrombin (curve c in Fig. 2A). This resistance enlargement could be attributed to the fact that the resistive hydrophobic layer of thrombin molecules insulates the conductive support and perturbs the interfacial electron transfer between the electrode and the electroactive species in solution (Cai et al., 2006). When probe II solution was dispread onto the above electrode, a largest resistance value (curve d in Fig. 2A) was observed. And the *eT* resistance decreased after QDs were successfully bond to the modified electrode (curve e in Fig. 2A).

According to previous reports (Ding et al., 2002; Myung et al., 2002), electrochemically reduced or oxidized QDs can react with coreactants to produce ECL. In this case, upon the negative potential scan, the QDs immobilized on the electrode were reduced to nanocrystal species ($QD^{\bullet -}$) by charge injection, and the coreactant $S_2O_8^{2-}$ was reduced to the strong oxidant $SO_4^{\bullet -}$. When $QD^{\bullet -}$ react with $SO_4^{\bullet -}$, excited state QD* was produced and light was emitted as the excited state QD* went back to ground state QD in the

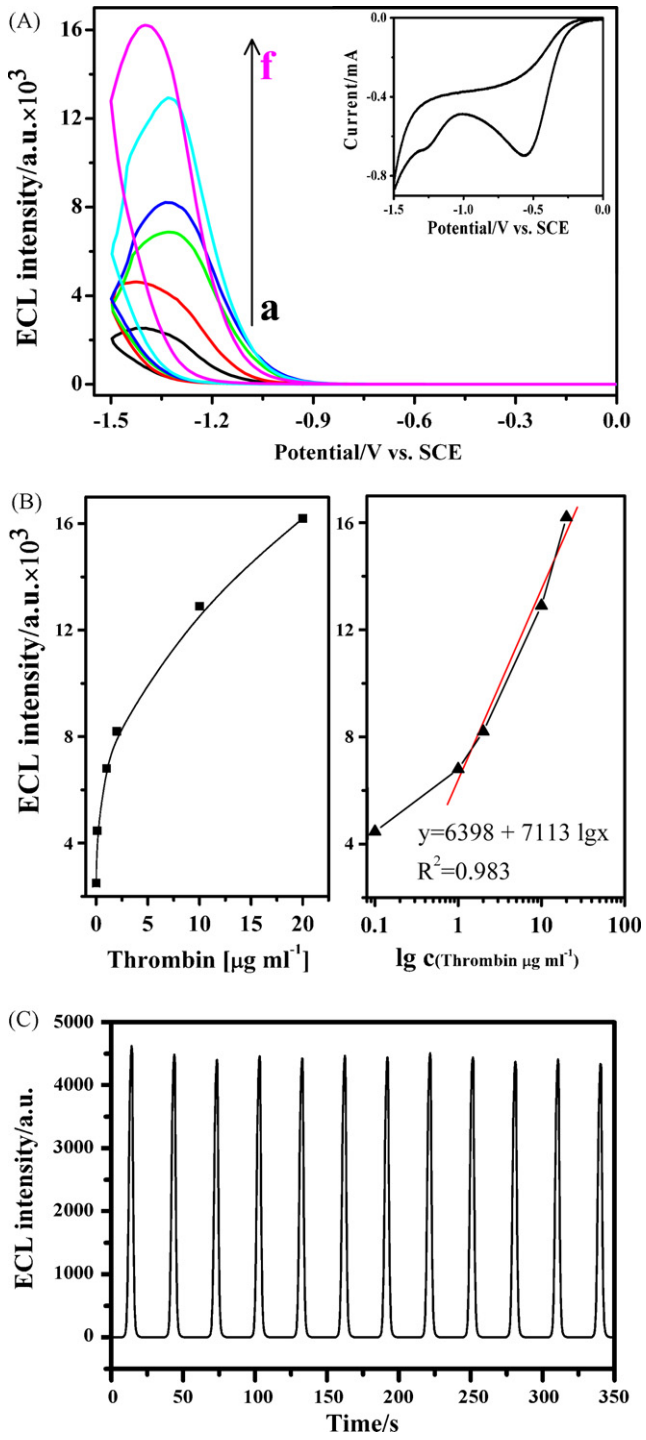

Fig. 3. (A) The ECL intensity of the biosensor with different thrombin concentrations (μg mL^{-1}). Curve a is ECL signal of the QDs/probe II/probeI modified Au electrode in the absence of thrombin. Curve b–f is drawn from the ECL signal of biosensor incubated with different concentrations of thrombin (from b to f, 0.1, 1.0, 2.0, 10.0, 20.0 μg mL^{-1}, respectively). The insert shows the CV curve during the ECL measurement. (B) The ECL intensity–thrombin concentration curve that drawn from (A). (C) ECL-time curve of QDs/probe II/thrombin/probe I/Au electrode under continuous cyclic voltammetry scan with thrombin concentration of 0.1 μg mL^{-1}. Other conditions for ECL measurement are the same as Fig. 2B.

aqueous solution. The detailed principle and process have been discussed in the previous report (Jie et al., 2007). Here, the ECL signal is responsive to the amount of QDs bonded to the probe II oligonucleotides, which was indirectly proportional to the combined thrombin.

In order to confirm that the ECL signal was caused by QDs, the ECL measurements were carried out after each modification steps. As could be seen in Fig. 2B, before avidin–QDs were bound, the ECL signals of the modified electrodes were very low, even negligible. This proved that the QDs played the decisive role in the ECL system. The influence of QDs incubation time on the ECL responses was investigated. After the incubation time for the avidin–QDs binding to 5′-biotin modified probe II was changed from 1 h to 3 h during the ECL measurement, no obvious ECL intensity change was observed. The influence of the $K_2S_2O_8$ concentration on the ECL intensity was also discussed. Three different $K_2S_2O_8$ concentrations (0.05 M, 0.1 M and 0.2 M) were employed and the intensity remains the same. This indicated that the coreactant is adequate for the ECL consumption during the measurement. Therefore, 1 h of incubation time and 0.1 M coreactant $K_2S_2O_8$ were selected in the thrombin detection.

In this system, the ECL signal is responsive to the amount of QDs that bonded to the probe II oligonucleotides, while the aptamer II were hybridized with the combined thrombin to form the sandwich structure. Therefore, the quantity of thrombin is proportional to the intensity of ECL signal. Fig. 3A shows the ECL signals with different concentration of thrombin, and the insert curve is the CV during the ECL measurement. Shown in Fig. 3B is the calibration curve that drawn from Fig. 3A. It was observed that the ECL intensity increased with the increase of thrombin concentration in the range of 0–20 $\mu g\,mL^{-1}$ (545 nM). The ECL intensity is proportional to the logarithm of thrombin concentration from 27.2 nM to 545 nM. The minimum detectable concentration is 2.72 nM, and this concentration is comparable with the electrochemical method (Radi et al., 2005, 2 nM) and fluorescent method (Wang et al., 2008, 2.7 nM).

As the ECL signal of the sensor mainly lies on the formation of the sandwich structure, while the formation of sandwich structure is relied on the conformational change of aptamers caused by the target protein binding-induced combination. By employing two other analytes, lysozyme and ATP, for the probe–target combination, the specificity of the thrombin biosensor was studied. When 10 $\mu g\,mL^{-1}$ lysozyme or ATP were incubated, the ECL intensity are almost the same as the background response of the fresh-prepared electrode (curve a in Fig. 3A), while the incubation into the same concentration of thrombin produced a clear increase in the ECL signal. This comparison suggests that the biosensor was specific for thrombin determination. Fig. 3C shows the ECL intensity of the biosensor in the presence of 0.1 $\mu g\,mL^{-1}$ thrombin under continuously scans for 12 cycles. When the biosensor was continuously scanned, stable and high ECL signals could be still observed, which meant that the biosensor possessed potential cycling stability. What's more, the ECL method is simple and effective. It avoids the label of target protein. The QDs show high ECL intensity, good biocompatibility and conductivity. Via the aptamer–target combination and avidin–biotin system, the components could be immobilized firmly onto the electrode.

4. Conclusion

This work described a novel QDs ECL assay for the detection of thrombin. A sandwich type of bioaffinity complex was constructed among two anti-thrombin aptamers and thrombin, and avidin–QD was bound tightly to the probe II. The ECL signal of the biosensor increased with the increase of thrombin concentration. In addition, the biosensor exhibited the good selectivity responses and potential cycling stability toward the target. It expands the application of QDs ECL in aptamer field.

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