Analytica Chimica Acta 899 (2015) 85–90

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Label-free aptamer biosensor for selective detection of thrombin

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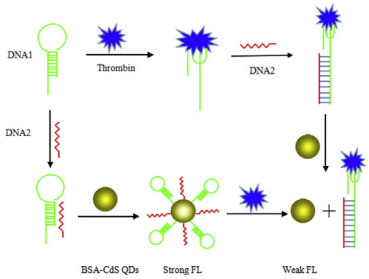
HIGHLIGHTS

- A novel strategy for the detection of thrombin was established based on BSA-CdS QDs.
- DNA could serve as the co-ligands to stabilize CdS QDs and enhance the fluorescence intensity.
- Thrombin could change the structure of DNA1 and quench the fluorescence of CdS QDs.
- Thrombin in real sample was detected with satisfactory results.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form
22 September 2015
Accepted 24 September 2015
Available online 8 October 2015

Keywords:
Thrombin
Thrombin aptamer
Fluorescence
CdS quantum dots

ABSTRACT

We fabricated a novel fluorescence biosensor for the selective detection of thrombin by using bovine serum albumin-capped CdS quantum dots (BSA-CdS QDs). Two kinds of designed DNA (DNA1 and DNA2) could bind to CdS QDs through the electrostatic interaction between DNA and Cd^{2+} on the surface of CdS QDs. The obtained DNA/BSA-CdS QDs kept stable in the solution with the fluorescence intensity obviously enhanced. Hairpin structure of DNA1contained two domains, one is the aptamer sequence of thrombin and the other is the complementary sequence of DNA2. When thrombin was added, it would bind to DNA1 and induce the hairpin structure of DNA1 changed into G-quadplex structure. Meanwhile, DNA2 would transfer from the surface of CdS QDs to DNA1 via hybridization, which resulted in the removal of DNA1 and DNA2 from the surface of CdS QDs, and led to the fluorescence intensity of CdS QDs reduced. Thus, the determination of thrombin could be achieved by monitoring the change of the fluorescence intensity of CdS QDs. The present method is simple and fast, and exhibits good selectivity for thrombin over other proteins. We have successfully detected thrombin in human serum samples with satisfactory results.

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]. Thrombin is not present in the blood of healthy individuals. However, during the coagulation process, the concentration in blood varies considerable even from nM to mM [4,5]. The high or low concentration of thrombin in blood is dangerous to patients. Excessive thrombin will induce thrombosis while low content of thrombin will induce an excessive bleeding. Moreover, it is considered as a useful tumor marker in the regulation of tumor growth, apoptosis, metastasis [6–8]. Therefore, it is significant to detect thrombin by a sensitive and selective method.

An aptamer, a single-stranded DNA or RNA, can especially recognize and bind to the targets with high affinity, good stability

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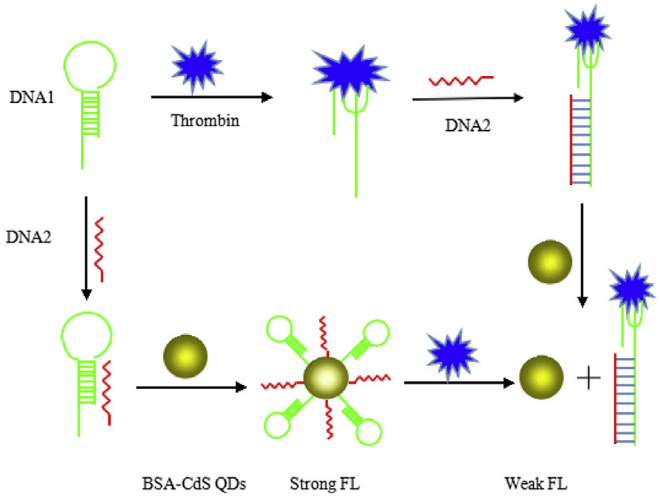
http://dx.doi.org/10.1016/j.aca.2015.09.051
0003-2670/

and storage properties [9–11]. Two well-known thrombin-binding aptamers for thrombin with 15 (Apt15) and 29 (Apt 29) bases have been specifically selected that bind to its two electropositive exosites, namely fibrinogen and heparin sites, situated on the sides of the active site [12,13]. Since the thrombin-binding aptamer was selected, numerous aptasensors have been fabricated based on the conformational changes induced by target binding. The key issue in the development of aptasensors is how to convert target recognition and binding into measurable signals. Up to now, many methods have been used for this purpose, including colormetric [14], fluorescence [15,16], electrochemiluminescence [17,18], surface plasmon resonance [19,20] and so on. For example, Li. et al. developed a colorimetric assay for thrombin. Apt 15 labeled paramagnetic particles and Apt 15 labeled Ag nanoparticles, can recognize the fibrinogen and heparin sites of thrombin, would be hold together by thrombin to form a sandwich-type complex. The sandwich-type complex has the catalytic ability. The colorimetric detection was achieved through the reduction of dye catalyzed by the sandwich-type complex [21]. He. et al. developed a novel thrombin detection strategy based on dual-hairpin DNA structure. First, DNA S1 was stabilized on the gold electrode and formed a hairpin DNA structure due to the designed five pairs of base matches in the stem region. DNA S2, modified with methylene blue (MB), who could be captured by S1 via forming Dual-Hairpin structure. Upon the addition of thrombin, the dual-hairpin structure was changed, and the S2 would divorce from the S1 modified electrode. Because the S2 that contained the signal molecules MB was far away from the electrode, the current would drop sharply [22]. These sensor platforms exhibited a broad dynamic range, high sensitivity. Among these methods, fluorescence methods have attracted significant interest due to its rapidness, cost-effectiveness, simplicity, and nondestructive. In fluorescence methods, QDs always be used for fabricating the sensing system owing to their ideal optical properties. A series of QDs-based fluorescence sensors have been designed for various protein detection, such as lysozyme detection [23,24]. However, only few sensors were designed for the assay of thrombin.

In this work, we constructed a new label-free biosensor for thrombin detection by employing a conformation change-based hybridization reaction (Scheme 1). We designed two kinds of DNA. The first one is a hairpin structured DNA, defined as DNA1, contained two domains termed as I and II according to their different functions. Region I is the sequence of Apt 15 and region II is the complementary sequence of DNA2. The other one is a 27-mer DNA, defined as DNA2, could hybridize with region II of DNA1. In the absence of thrombin, DNA1 and DNA2 could combine with the BSA-CdS QDs to passivate its surface defects, and the fluorescence of the CdS QDs would be enhanced. In the presence of thrombin, Region I of DNA1 would recognize and combine with the thrombin, the hairpin structure will be opened upon the interaction between DNA1 and thrombin to form a stable G-quadruplex structure, then the region II sequence of DNA1 and DNA2 would bind together via complementary base pairing. The fluorescence intensity of CdS QDs would be reduced since the removal of DNA1 and DNA2 from the surface of CdS QDs. The fluorescence intensity of CdS QDs would gradually decrease with the increasing of the thrombin concentration.



Scheme 1. Schematic representation the fluorescent detection of thrombin based on BSA-CdS QDs.

2. Experimental section

2.1. Materials

All reagents were of at least analytical grade. The water used in all experiments had a resistivity higher than 18 MΩ cm^{-1}. Cadmium (II) chloride ($CdCl_2$), sodium hydroxide (NaOH), Sodiumsulfide nonahydrate ($Na_2S·9H_2O$), trihydroxymethyl aminomethane (Tris) and hydrochloric acid were purchased from Shanghai Qingxi Technology Co., Ltd. Thrombin and Bovine serum albumin were purchased from Sigma–Aldrich Corporation. The oligonucleotides with the following sequences were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. DNA1 (5′-GGT TGG TGT GGT TGG CAT CAA AAA AAT CCT CAG ATG CCA ACC-3′), DNA2 (5′-GGT TGG CAT CAG AGG ATT TTT TTG ATG-3′). The 0.1 mol/L Tris–HCl buffered solution (pH 7.4) was used as the medium for detection process.

2.2. Apparatus

The fluorescence spectra were obtained by using a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a xenon lamp using right-angle geometry. UV–vis absorption spectra were obtained by a Varian GBC Cintra 10e UV–vis spectrometer. In both experiments, a 1 cm path-length quartz cuvette was used. All pH measurements were made with a PHS-3C pH meter (Tuopu Co., Hangzhou, China). Transmission electron microscopy (TEM) experiments were performed on a Philips Tecnai F20 TEM operating at 200 KV acceleration voltage.

2.3. Preparation of BSA-CdS QDs

BSA-CdS QDs were synthesized in aqueous solution. 400 μL 0.5 mmol/L BSA solution, 120 μL 50 mmol/L $CdCl_2$ solution and 150 μL 0.1 mol/L Tris–HCl buffer (pH 7.0, 150 μL) were added into a 2 mL calibrated centrifuge tube and shaken thoroughly for 10 min. After that, 90 μL 30 mmol/L Na_2S solution was added into the centrifuge tube and diluted to 1500 μL with deionized water followed by the thoroughly shaking and equilibrated for 15 min 50 μL BSA-CdS QDs was diluted to 1.5 mL with deionized water. The fluorescence spectra were recorded from 405 nm to 650 nm with the excitation wavelength of 340 nm. The slit widths of excitation and emission were both 10 nm. The fluorescence quantum yield of BSA-CdS QDs was 23.46%.

2.4. Preparation of DNA/BSA-CdS QDs

The BSA-CdS QDs solution (50 μL), 150 μL 0.1 mol/L Tris–HCl buffer (pH 7.0, 150 μL), 12 nmol/L DNA1 and 12 nmol/L DNA2 was