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Electrochemiluminescence biosensor based on CdSe quantum dots for the detection of thrombin

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ABSTRACT

A novel QDs electrochemiluminescence (ECL) biosensor for the determination of thrombin was described. The CdSe QDs solution was dripped onto the clear surface of the ITO and then immersed in PBS which contained EDC and NHS as a coupling agent to activate the carboxyl-terminated surface of the CdSe QDs. The ITO electrode was immersed in the PBS containing 0.4 μM aptamer, followed by rinsing with PBS and dried with N_2 again, then dipped in the BSA solution for 30 min to decrease the non-specific binding. After that, the aptamer modified ITO was soaked in PBS to remove unbound aptamer. Under optimal conditions, the linear range was obtained from 0 to 64 μg mL^{-1} with a correlation coefficient of 0.9986 (n = 16). The control experiment was also carried out by using BSA, lysozyme and IgG in the absence of thrombin. The results showed that the aptasensor had good specificity, stability and reproducibility to the thrombin. Moreover, the aptasensor could be used for detection of real sample with consistent results in comparison with those obtained by electrochemical method which could provide a promising platform for fabrication of aptamer based biosensors.

1. Introduction

Aptamer is a single-stranded oligonucleotide which has been designed through an in vitro selection process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) [1,2]. It can recognize its targets, such as small molecules, proteins, viruses and even cells [3,4]. Comparing with the antibodies, the aptamers show a large number of advantages, such as simple synthesis, easy labeling, good stability and reproducibility, low cost and wide applicability [3].

Thrombin is a kind of serine protease, which plays an important role in the physiological and pathological process [5]. It is usually regarded as a tumor marker in the diagnosis of pulmonary metastasis and the high or low concentration of thrombin in blood is associated with coagulation abnormalities [6,7]. Therefore, the specific recognition and quantitative detection of thrombin is extremely important in both clinical practice and diagnostic application. Because the thrombin itself has two exosites: the fibrinogen-recognition and the heparin-binding exosite [7], it can easily bind with one or two kinds of aptamers, proteins or other materials at one time.

In recent years, a large number of methods have been used to detect the aptasensors, such as high performance liquid chromatography (HPLC) [8], chromatographic assays [9], fluorescence detection [10], surface plasmon resonance (SPR) [11], electrochemistry [12], electrogenerated chemiluminescence (ECL) [13]. Among them, the ECL technique has become an important and promising method which is a chemiluminescent reaction of species generated electrochemically at electrode surface. ECL method has been receiving more attention in recent years because of its simplicity, high sensitivity, rapidity and easy controllability, as well as high versatility [14–17].

At present, various strategies have been developed to improve the performance of the ECL aptamer biosensor [18–20]. Wang et al. used the tri(2,2′-bipyridyl)ruthenium(II)-doped silica nanoparticles ($Ru(bpy)_3^{2+}$-doped SNPs) as DNA tags for detection of the thrombin, which was based on the target protein-induced strand displacement of the DNA probe [21]. A minireview was reported to demonstrate the electrochemiluminescence based on quantum dots (QDs) and their analytical application [22]. Huang et al. designed a biosensor to detect the thrombin by using the CdSe/ZnS QDs ECL technique. They immobililized a thiol-terminated aptamer with 15 nucleotides. Then the thrombin was imported to form the aptamer-thrombin bioaffinity complexes. Subsequently, another 5′-biotin modified aptamer with 29 nucleotides was hybridized with the combined thrombin to form a sandwich type structure. By this way, the detection range of thrombin from 0 to 20 μg mL^{-1} was obtained. However, the obtained linear range was also not wide enough [13]. DNA aptasensors for the detection of ATP [23] and lysozyme [24] based on QDs electrochemiluminescence were

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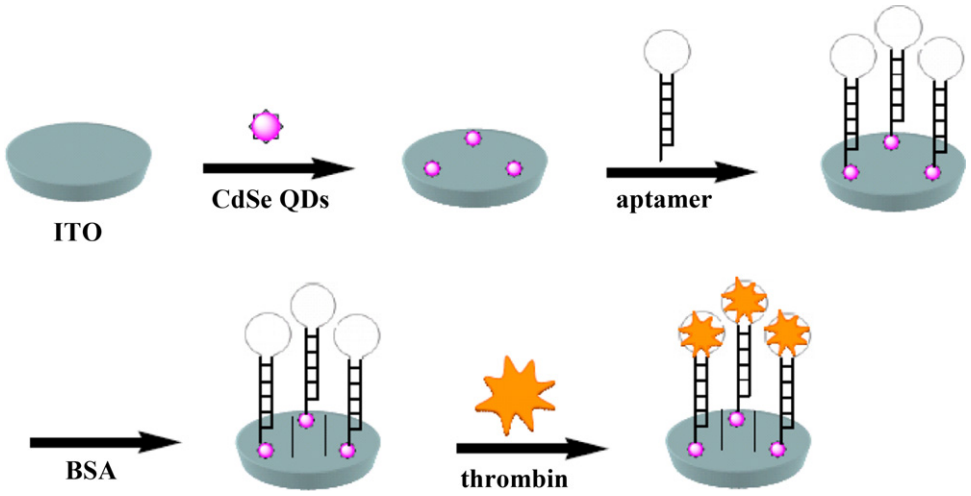

Scheme 1. The modification process of the electrode.

reported. The 5′-thiol-modified anti-target aptamers were first immobilized onto the Au electrode through an Au-S bond. The aptamer-target bioaffinity complexes were formed after the above electrode was immersed into a target solution. The free aptamers were hybridized with 5′-biotin-modified complementary DNA (cDNA) oligonucleotides to form double-stranded DNA (ds-DNA) oligonucleotides. At last, through the biotin-avidin system, avidin-QDs were bound to 5′-biotin-modified cDNA oligonucleotides. The ECL signal of the biosensor was responsive to the amount of QDs bonded to the cDNA oligonucleotides. However, these methods were relatively complex.

In order to simply the design process and improve the detection linear range and the sensitivity, here a novel ECL biosensor for the determination of thrombin was fabricated by using water soluble thioglycolic acid (TGA)-modified CdSe QDs. CdSe QDs can absorb more aptamer by the carboxyl groups and the amino groups to improve the electrochemiluminescence signal. Also, the construction method was very simple. To the best of our knowledge, there have not been the same reports as ours. The proposed method would have a potential value in clinical applications.

2. Experimental

2.1. Reagents and materials

The aptamer was purchased from Bioneer (Shanghai, China). The 5′-terminus of the aptamer was modified by the aliphatic amine and it contained 22 bases with its sequence as follows: 5′-NH_2-$(CH_2)_6$-TTC CAA CGG TTG GTG TGG TTG G-3′.

Thrombin (500 U), $C_8H_{17}N_3HCl$ (EDC), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA), lysozyme and IgG were purchased from Aldrich and used without further purification. Serum samples were provided by Jiangsu Institute of Cancer Prevention and Cure and they were obtained by centrifugation of blood for about 5 min with the rotation rate of 3000–4000 rpm. Other chemicals were all of analytical reagent grade. The ITO electrodes with a working area of 1 cm × 1 cm were purchased from Kangdake Photoelectric Technique Ltd. Co. (60 Ω).

2.2. Preparation of the water-soluble TGA-modified CdSe QDs

The preparation of the water soluble CdSe QDs was prepared according to the literature by Liu [25]. In brief, 50 mL of 2 mM $CdCl_2$ was mixed with 20 μL of TGA (pH = 10), then they were bubbled with highly pure N_2 for 30 min. 0.7 mL of 70 mM NaHSe (obtained from the reaction of Se powder and $NaBH_4$ in air-free double distilled water) was injected into this mixture to obtain a clear, lightly yellow solution of CdSe precursors. The final molar ratios of Cd^{2+}/Se^{2-}/TGA for the preparation of optimum QDs were 1:0.5:2.5. The obtained solution was then refluxed at 100 °C for 4 h to form TGA-modified CdSe QDs. After cooling, the QD solution was ultra-filtered at 6000 rpm for 5 min to remove unreacted reactants. Then, the water-soluble TGA-modified CdSe QDs were obtained which were stored in the refrigerator before used.

2.3. Modification process of the electrode

The modification process of the electrode was shown as Scheme 1. The CdSe QDs were dissolved by the deionized water

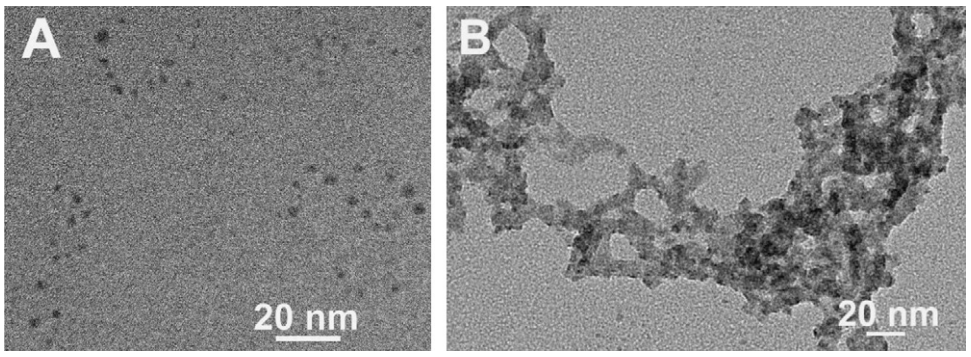

Fig. 1. High (A) and low (B) magnification TEM images of CdSe.