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Detection of thrombin using electrogenerated chemiluminescence based on $Ru(bpy)_3^{2+}$-doped silica nanoparticle aptasensor *via* target protein-induced strand displacement

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Abstract

A sensitive and selective aptasensor using tri(2,2′-bipyridyl)ruthenium(II)-doped silica nanoparticles ($Ru(bpy)_3^{2+}$-doped SNPs) as DNA tags for detection of thrombin is developed based on the target protein-induced strand displacement of the DNA probe. For the proposed aptasensor, the aptamer was assembled on the surface of the Au electrode through Au-S binding. The hybridization event between the DNA probe labeled by the $Ru(bpy)_3^{2+}$-doped SNPs and the aptamer was evaluated by electrogenerated chemiluminescence (ECL) measurements. Then, the DNA probe was displaced by thrombin and the binding event between the thrombin and the aptamer was monitored by ECL measurements again. The difference of ECL intensity (ΔI_{ECL}) of the two events could be used to quantify the thrombin. Other proteins, such as bovine serum albumin and bovine hemoglobin, had almost negligible ΔI_{ECL}. Under the optimal conditions, the ΔI_{ECL} was linearly related to the concentration of the thrombin in the range of 10 fM to 10 pM and the detection limit was down to 1.0 fM since SNPs containing a large number of $Ru(bpy)_3^{2+}$ molecules were labeled on the DNA probe.

Keywords: Thrombin; Electrogenerated chemiluminescence; $Ru(bpy)_3^{2+}$-doped silica nanoparticles; Aptasensor; Target protein-induced strand displacement

1. Introduction

Thrombin, an important physiological protease existed in the blood, is composed of two polypeptide strands through cross-linking interaction of disulfide bonds. It plays an essential role in some physiological and pathological processes, such as blood solidification, wound cicatrization and inflammation. Therefore, specific recognition and quantitative detection of thrombin is extremely crucial in fundamental research as well as in clinical practice [1,2]. Aptamers [3–7], artificial nucleic acids selected by the SELEX technique, have recently attracted considerable attention for their ability to bind target proteins with high affinity and specificity [8,9]. The tight-binding property, long-term stability, target versatility and convenient regeneration make aptamers ideal candidates as molecular recognition elements in sensors when applied together with different analysis methods, such as fluorescence, radiolabeling, electrochemical quartz crystal microbalance, electrochemistry, electrogenerated chemiluminescence (ECL), and so on [10–13]. Therefore, aptasensors can be used in wide range of bioassays and the development of protein arrays.

The rapidly evolving field of nanotechnology has opened up a new and promising era, in which nanoparticles of various shapes, sizes, and compositions have been successfully used in sensing, labeling and integrating of the biomacromolecules due to their unique properties [14–16]. Recently, tri(2,2′-bipyridyl)ruthenium(II)-doped silica nanoparticles ($Ru(bpy)_3^{2+}$-doped SNPs) have received considerable attention [17–19]. They, with core-shell structure, incorporate $Ru(bpy)_3^{2+}$ molecules in the silica matrix. So, the $Ru(bpy)_3^{2+}$ molecules can be prevented from the surrounding environment and the ECL intensity can also be enhanced due to the increase of the number of $Ru(bpy)_3^{2+}$ doped per nanoparticle. Furthermore, some functional groups, such as amines, thiols and carboxyls can be easily introduce to the surface of $Ru(bpy)_3^{2+}$-doped

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silica nanoparticles. So, $Ru(bpy)_3^{2+}$-doped silica nanoparticles are ideal tags in bioanalysis applications and they have been applied to detect DNA hybridization [20]. However, electrochemiluminescent detection of protein based on $Ru(bpy)_3^{2+}$-doped SNPs tags has not been reported.

Herein, we attempt to combine $Ru(bpy)_3^{2+}$-doped SNPs as DNA tags with special capturing mode between aptamer and protein as well as a sensitive electrochemiluminescent method to detect thrombin. Since the interaction between aptamer and thrombin is stronger than that between aptamer and its complementary DNA chain, replacement of $Ru(bpy)_3^{2+}$-doped SNPs-labeled DNA probe by thrombin is easily, which leads to remarkable decrease of ECL signal. ΔI_{ECL}, the difference of ECL intensity before and after the replacement, was correlated to the concentration of the thrombin, in this way, the thrombin was detected. Furthermore, the ECL signal was amplified through boosting up the amount of $Ru(bpy)_3^{2+}$ labeled on DNA probe. So, the aptasensor displayed high specificity, sensitivity and wide linear range.

2. Experimental

2.1. Apparatus and reagents

ECL was measured with MPI-E electrogenerated chemiluminescence analyzer (Xi'an Remax Electronic Science Tech. Co. Ltd.), cyclic voltammogram (CV) was recorded with CHI 660 electrochemical analyzer (CHI Instruments Inc., USA).

Oligonucleotides were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China). The sequence of aptamer: 5′-HS-$(CH_2)_6$-ATATA*GGTTGGTGTGGTTGG*-3′. Complementary sequence of aptamer: 5′-NH_2-$(CH_2)_6$-TGAGT*CCAACCACACCA*-3′. Purified thrombin (TB, the activity of enzyme was 10 U mg^{-1}, freeze-dry powder), bovine serum albumin (BSA), bovine hemoglobin (BHb) and fish DNA were purchased from Dingguo Biological Technology Corporation of Beijing. $Ru(bpy)_3^{2+}$ (99.95%) and 6-mercapto-1-hexanol (SH-$(CH_2)_6$-OH, >97.0%) were purchased from Sigma (USA). Tetraethylorthosilicate (TEOS) and trimethoxysilylpropydiethylenetriamine (DETA) were purchased from United Chemical Technologies (Bristol, PA). The following buffers were used: 10 mM phosphate buffer solution (PBS, pH = 7.3), 10 mM PBS (0.3 M NaCl, pH = 7.3), 10 mM PBS (2 mM Mg^{2+}, 10 mM K^+, pH = 7.0). 1.0 mM tri-*n*-propylamine (TPrA) and 5.0 mM $LiClO_4$ in 20 mM PBS (pH = 8.7) was used as detecting buffer solution. Plasma supplied by the Central Hospital of Putuo District of Shanghai. Other reagents were of analytical reagent grade. All of the solutions were prepared with ultrapure water from a Millipore Milli-Q system.

2.2. Preparation of $Ru(bpy)_3^{2+}$-doped SNPs and $Ru(bpy)_3^{2+}$-doped SNPs-labeled DNA probe

Silica nanoparticles were prepared with water-in-oil (W/O) microemulsion method according to the literatures [21,22]. 1.77 mL of TritonX-100 was mixed with 7.5 mL of cyclohexane, 1.8 mL of *n*-hexanol and 340 μL of $Ru(bpy)_3^{2+}$ (40 mM). Then, a polymerization reaction was initiated by adding 60 μL of $NH_3 \cdot H_2O$ in the presence of 100 μL of TEOS. After 24 h, the nanoparticles, $Ru(bpy)_3^{2+}$-doped SNPs, were obtained. They were deposited with acetone, washed with ethanol and water for several times to remove the residual surfactant molecules and physically adsorbed $Ru(bpy)_3^{2+}$ on the surface of the particles.

Surface modification of $Ru(bpy)_3^{2+}$-doped SNPs and covalent conjugation of oligonucleotides onto the nanoparticle surface were prepared according to the references [23,24]. 1.0 mL of $Ru(bpy)_3^{2+}$-doped SNPs was immersed in 5.0 mL freshly prepared solution containing DETA (1%) and acetic acid (1.0 mM) for 30 min at room temperature. After rinsed with deionized water, the above amine-functionalized $Ru(bpy)_3^{2+}$-doped SNPs were reacted with 5.0 mL of glutaraldehyde solution (5%) for 2 h with shaking in a water bath of 37 °C. Then, 1.0 OD of 5′-amine-capped oligonucleotide in 10 mM PBS (pH = 7.3) was added into the solution (final oligonucleotide concentration was 10 μM), the reaction was continued for 2 h with stirring in a water bath of 37 °C. The $Ru(bpy)_3^{2+}$-doped SNPs-labeled DNA probe was obtained. It was washed, centrifuged, resuspended in 10 mM PBS (pH = 7.3) and stored at 4 °C for later use.

2.3. The procedure of detecting the thrombin via target protein-induced strand displacement

Fig. 1 represents the procedure of preparing electrochemiluminescent aptasensor for detecting the thrombin *via* target protein-induced strand displacement. The self-assembled monolayer of aptamer on Au electrode was prepared first (step a). In order to avoid the unspecific adsorption of $Ru(bpy)_3^{2+}$-doped SNPs-labeled DNA probe on the aptamer assembled Au electrode and increase the hybridization efficiency, SH-$(CH_2)_6$-OH was dropped to the aptamer assembled Au electrode to hold the unassembled surface and adjust the distributing degree of the aptamer on the electrode (step b). At step c, the aptamer immobilized on Au electrode was hybridized with $Ru(bpy)_3^{2+}$-doped SNPs-labeled DNA probe to introduce the luminescent substance on the electrode surface, and the first ECL signal was detected. Lastly, aptamer-thrombin complex (ATC) was formed *via* target protein-induced strand displacement of the $Ru(bpy)_3^{2+}$-doped SNPs-labeled DNA probe on Au electrode (step d), then the ECL signal was detected again. A remarkable decrease of ECL signal should be presented if the $Ru(bpy)_3^{2+}$-doped SNPs-labeled DNA probe was replaced by thrombin. In this way, the ΔI_{ECL} of the two events was obtained, and it can be used to quantify the thrombin. The following is the detailed preparation processes.

2.3.1. Formation of self-assembled monolayer of aptamer on Au electrode (steps a and b)

The self-assembled monolayer of aptamer on Au electrode was prepared according to the reported procedures [25,26]. The surface of the Au electrode was polished with alumina slurry, rinsed with water and ethanol in an ultrasonic bath briefly. The Au electrode was further treated electrochemically in H_2SO_4