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Turn-on near-infrared electrochemiluminescence sensing of thrombin based on resonance energy transfer between CdTe/CdS $core_{small}$/$shell_{thick}$ quantum dots and gold nanorods

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ABSTRACT

Here we designed a near-infrared electrochemiluminescence (NECL) aptasensor for turn-on ultrasensitive determination of thrombin. It was based on the ECL resonance energy transfer (ECL-RET) of CdTe/CdS $core_{small}$/$shell_{thick}$ quantum dots (QDs) to gold nanorods (AuNRs). AuNRs which functioned as ECL acceptors were assembled onto CdTe/CdS film by DNA hybridization between aptamers and their complementary oligonucleotides. In the absence of thrombin, the NECL of QDs was quenched as a result of the ECL-RET of QDs to AuNRs. In the presence of thrombin, the NECL of the system was "turned on" because thrombin can replace the AuNRs onto the QDs film, owing to the specific aptamer-protein affinity interactions. In this way, the increment of ECL intensity and the concentration of thrombin showed a logarithmic linear correlation in the range of 100 aM to 10 fM with a detection limit of 31 aM (S/N=3). Importantly, the developed aptasensor was successfully applied to thrombin sensing in real serum samples.

1. Introduction

Near-infrared (NIR) fluorescence detection is widely recognized as an effective method for high sensitivity bioapplication benefiting from its attractive merits such as improved tissue penetration, lower background interference, and reduced photochemical damage (Amiot et al., 2008; Ma and Su, 2010). Similarly to NIR fluorescence, NIR electrochemiluminescence (NECL) analysis has emerged as an alternative to traditional visible-range ECL bioassays, especially in some complex biological systems (Wang and Han, 2013). This is because the incorporation of NIR spectral region with ECL technique could minimize the background signal and provide the possibility to fulfill the requirement of interference-free sensing. There have been tremendous efforts to develop high-efficiency ECL emitters for NECL analysis. Semiconductor quantum dots (QDs) hold great promise as a new generation of ECL emitters owing to their size or surface-dependent luminescence and photochemical stability (Ding et al., 2002). While QDs as ECL probes in visible-range have been well developed (Bertoncello and Forster, 2009; Hu and Xu, 2010), NECL from QDs emitters have been much less explored because of their relatively weak and unstable emission signal, as well as low sensitivity captured by the detectors of ECL instruments (Cui et al., 2012; Sun et al., 2008). Furthermore, such rational sensing strategies of traditional visible ECL may be not suitable for NECL detecting in some cases. Only recently, the dual-stabilizer-capped CdTe QDs were synthesized through a convenient one-pot approach (Liang et al., 2011b) and have been used as NECL probes for small molecule and target antigen detection with high sensitivity (Liang et al., 2012; Liang et al., 2011a; Zou et al., 2011). Our group also reported the NECL behaviors from core/shell CdTe/CdS QDs (Wang et al., 2011a) and several NECL biosensors have been constructed in both cathodic and anodic regions (Wang et al., 2012; Wang and Jiang, 2015). Despite these advances, much work is still needed to obtain robust NECL emitters for practical applications.

Luminescence resonance energy transfer (LRET), often occurring between a suitably matched acceptor and donor pair, is an attractive technique for sensitive detection of biomolecules (Sapsford et al., 2006). Various types of LRET including fluorescence resonance energy transfer, chemiluminescence resonance energy transfer and bioluminescence resonance energy transfer have established a sensing platform in the bioapplication (Frigerio et al., 2012). Nowadays, with the progress of ECL technical analysis, ECL resonance energy transfer (ECL-RET) received increasing

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attention in sensing applications of ion, small molecule, protein, and DNA (Wu et al., 2014). Although the current ECL-RET systems were mainly located in the visible range (Lei et al., 2015; Liang et al., 2016; Qi et al., 2013; Shan et al., 2009, 2010; Wang et al., 2016), their sensing strategy could be applicable for NECL biosensors provided that the energy-overlapping donor-acceptor pair is found. Notably, gold nanorods (AuNRs) with distinctive shape-dependent surface plasmon resonance are highly absorbent of light in the NIR region and can act as an acceptor by absorbing the NIR emission (Xu et al., 2011). Inspired by this, a NECL protocol involving ECL-RET from double shelled CdSeTe/CdS/ZnS QDs to AuNRs was reported (Li et al., 2013).

Herein, a turn-on NECL aptasensor for thrombin was developed using an ECL-RET system consisting of CdTe/CdS $core_{small}/shell_{thick}$ QDs and AuNRs. Thrombin is an important physiological protease that plays significant role in many life processes, such as blood solidification, wound cicatrization, and inflammation (Hwang et al. 2001). Therefore, quantitative detection of thrombin at low concentrations is extremely important in clinical diagnosis. The CdTe/CdS QDs film at the glassy carbon electrode (GCE) surface showed a strong and stable NECL emission of 707 nm at *ca.* − 1.45 V. After immobilization with capturing DNA (cDNA, the aptamer of thrombin) and sequential hybridization with probing DNA-modified AuNRs (pDNA-AuNRs), the NECL signal was tremendously quenched through ECL-RET. The spectrum- and distance- dependent quenching efficiency of ECL-RET was studied. After adding thrombin in the system, thrombin G-quadruplex conformation could be formed, and this will dehybridize the double-stranded DNA to release the pDNA-AuNRs from electrode surface. Thus the cathodic NECL of the QDs was recovered. On the basis of concentration-dependent ECL recovery, aptamer-mediated "turn-on" mode combination with the low-interference NIR optical window, this approach exhibited good performance in the detection of thrombin at ultralow concentration. Furthermore, the applicability of the proposed NECL aptasensor in human serum was explored.

2. Material and methods

2.1. Apparatus

The ECL emissions and spectra were performed using a Model MPI-EII ECL Analyzer Systems, and the details on manipulation could be found in previous report (Wang et al. 2012). Data of absorption spectra, photoluminescence (PL) spectra, transmission electron microscopy (TEM), hydrodynamic diameters and zeta potentials were acquired according to the reported method (see details in Supplementary Data, SD).

2.2. Reagents

Thrombin (freeze-dry powder), mercaptopropionic acid (MPA), bovine serum albumin (BSA), tris(2-carboxyethyl)phosphine hydrochloride solution (TCEP), cetyltrimethylammonium bromide (CTAB), and sodium dodecyl sulfate (SDS) were obtained from Sigma-Aldrich Chemical Co. Tellurium powder, cadmium chloride ($CdCl_2 \cdot 2.5H_2O$), sodium borohydride ($NaBH_4$), chloroauric acid ($HAuCl_4 \cdot 3H_2O$), chitosan (> 85% deacetylation), glutaraldehyde (25% aqueous solution), ascorbic acid (AA) and silver nitrate ($AgNO_3$) were purchased from Sinopharm Chemistry Reagent Co., Ltd (Shanghai, China). All other common solvents and salts were of analytical grade and all solutions were prepared with ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ-cm, Milli-Q, Millipore).

All the oligonucleotides were purchased from Shenggong Bioengineering Ltd Company. The sequences of these oligonucleotides employed were given in SD.

2.3. Preparation

The CdTe/CdS $core_{small}/shell_{thick}$ QDs, the CTAB-stabilized AuNRs, and pDNA-AuNRs were synthesized according to previously published procedure with small modifications (see details in SD).

2.4. Fabrication of the NECL aptasensor

First, the bare GCE was polished in sequential order with 1.0, 0.3, and 0.05 μm alumina slurry to obtain a mirror-like surface, followed by sonication in the ultrapure water and ethanol in turn, and finally, dried in air. Next, 6 μL of the prepared QDs solution (1 mg/mL) was cast onto the bare GCE surface and evaporated in air to form a homogeneous film. Because of the abundant active sites (such as –OH, –C=O, and –COOH) on the fresh surface of pretreated GCE, the CdTe/CdS QDs could adsorb on it firmly. Subsequently, to increase the reproducibility of the modified electrode, 3 μL of 0.05% chitosan (Ch) solution was dropped on the above modified electrode (GCE/QD) to produce a smoother and more uniform surface microenvironment for DNA immobilization. After rinsing thoroughly with ultrapure water, the modified electrode (GCE/QD/Ch) was incubated with 5 μL of 2.5% glutaraldehyde solution for 2 h at room temperature to activate the amino groups. After rinsing with 0.1 M Tris–HCl buffer (pH 7.4), the resulting electrode was soaked in 50 μL of 1 μM cDNA solution at 4 °C for at least 12 h. Afterward, 50 μL of 2 wt% BSA was dropped on the electrode (GCE/QD/Ch/cDNA) at 4 °C for 2 h to block the non-specific binding sites. The resultant modified electrode (GCE/QD/Ch/cDNA,BSA) was incubated with 50 μL of pDNA-AuNRs dispersion at 37 °C for 2 h. The obtained modified electrode was thoroughly cleaned with ultrapure water and Tris–HCl buffer to remove the physically absorbed species. The finished aptasensor (GCE/QD/Ch/cDNA,BSA, pDNA-AuNRs) was stored in the refrigerator at 4 °C for further use.

2.5. ECL detection of thrombin

The ECL responses of the proposed aptasensors were investigated in 3 mL of 0.1 M phosphate buffered saline (PBS, pH=7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl, which are incubated with different concentrations of thrombin solution for 1 h at 37 °C. The working potential was from 0 to − 1.5 V (vs Ag/AgCl) at a scan rate of 200 mV/s, and the voltage of the photomultiplier tube (PMT, R8630) was at 900 V. ECL signals related to the thrombin concentrations could be measured. The schematic graph of the fabrication process of aptamer based assay for thrombin was illustrated in Scheme 1.

3. Results and discussion

3.1. Preparation and Characterization of CdTe/CdS $core_{small}/shell_{thick}$ QDs and AuNRs

MPA-capped CdTe/CdS $core_{small}/shell_{thick}$ QDs with a NIR emission peak at 702 nm (Fig. 1A, curve a) were synthesized on the basis of a previous method (Deng et al., 2010). As a characteristic of type-II QDs (produced by lattice-mismatch strain tuning), the distinctive peak of absorption spectra was unconspicuous after the thick shell coating (Fig. 1A, curve b) (Smith et al., 2008). Representative TEM image (Fig. 1B) shows that the obtained CdTe/CdS $core_{small}/shell_{thick}$ QDs were nearly monodispersed with an average size of 4.6 ± 0.5 nm. The AuNRs, synthesized in the