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A novel aptasensor for lysozyme based on electrogenerated chemiluminescence resonance energy transfer between luminol and silicon quantum dots

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ABSTRACT

In the present work, electrogenerated chemiluminescence (ECL) of luminol was investigated in neutral condition at a gold electrode in the presence of silicon quantum dots (SiQDs). The results revealed that SiQDs can not only greatly enhance luminol ECL, but also act as energy acceptor to construct a novel ECL resonance energy transfer (ECL-RET) system with luminol. As a result, strong anodic ECL signal was obtained in neutral condition at the bare gold electrode, which is suitable for biosensing application. Lysozyme exhibited apparent inhibiting effect on the ECL-RET system, based on which an ECL aptasensor was fabricated for the sensitive detection of lysozyme. The proposed method showed high sensitivity, good selectivity, and wide linearity for the detection of lysozyme in the range of 5.0×10^{-14}–5.0×10^{-9} g mL^{-1} with a detection limit of 5.8×10^{-15} g mL^{-1} (3σ). The results suggested that as-proposed luminol/SiQDs ECL biosensor will be promising in the detection enzyme.

1. Introduction

Luminol is one of the most famous luminescent reagents and electrogenerated chemiluminescence (ECL) behaviors of luminol have been intensively reported (Richter et al., 2004; Miao et al., 2008). In the past few decades, luminol has been widely used as ECL labels in biochemical analysis, such as enzyme biosensors (Borisov et al., 2008). Generally, luminol can generate strong ECL signal in alkaline media but its signal in neutral media is very weak at bare electrodes (Fahnrich et al., 2001). Unfortunately, strong alkaline condition is not suitable for the fabrication of biosensor. To overcome this problem, the catalytic effects of metal nanoparticles (NPs), such as gold, silver, platinum, and palladium NPs on ECL reactions have been demonstrated (Cui et al., 2004; Li et al., 2010; Haghighi et al., 2011; Zhang et al., 2013, 2014). For example, luminol ECL can be enhanced 2–3 orders of magnitude at the gold NPs modified gold electrode in neutral condition, which exhibited great potential applications in biosensing fields (Cui et al., 2004). Inspired by the successful applications of metal NPs in luminol ECL, the effects of semiconductor quantum dots (QDs) on traditional ECL system were investigated (Chen et al., 2014; Sun et al., 2012; Dong et al., 2014; Taokaenchan et al., 2015). These results revealed that semiconductor QDs could catalyze the traditional ECL reactions, and bring new ECL behaviors due to the resonance energy transfer (RET). For example, CdSe@ZnS QDs exhibited excellent catalytical effect on luminol ECL reactions. Subsequently, ECL resonance energy transfer (ECL-RET) occurred between luminol and QDs, which generated stronger ECL signal, and could be applied in the sensitive detection of thrombin (Dong et al., 2014). Although the semiconductor QDs have been widely used in ECL investigation, heavy metals as the essential elements in these QDs have raised serious health and environmental concerns (Derfus et al., 2004). Therefore, searching for alternative eco-friendly nanomaterials with good ECL activities has become an urgent challenge. The ECL behavior of silicon quantum dots (SiQDs) has already been reported in nonaqueous condition in 2002, however, SiQDs ECL has been rarely reported in aqueous condition for their poor aqueous dispersibility (Ding et al., 2002; Baker et al., 2010). In recent years, intensive researches have been done in producing hydrophilic SiQDs to make them suitable for biological applications (Lu et al., 2012; Liu et al., 2012; Michalet et al., 2005; Song et al., 2010; He et al., 2009, 2010, 2011; Zheng et al., 2009; Zhong et al., 2013; Erogbogbo et al., 2008). The successful synthesis of water dispersible SiQDs makes it possible to explore SiQDs ECL in aqueous condition and its biosensing application. It was found in our lab that water-soluble SiQDs can generate strong cathodic ECL in the presence of $K_2S_2O_8$, and

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can be used in the sensitive detection of DNA. However, the effect of SiQDs on traditional ECL systems, such as luminol, has not been explored.

Lysozyme (Lyz) is a ubiquitous protein serving as "body's own antibiotic" by cleaving acetyl groups in the polysaccharide walls of many bacteria. Therefore, the Lyz level in blood is regarded as the clinical index for many diseases such as leukemia and meningitis (Vocadlo et al., 2001). Owing to the physiological importance of Lyz, developing rapid and effective biosensor for its detection has been given considerable attention. To date, a variety of strategies for the Lyz detection have been reported including fluorescence resonance energy transfer, photoelectrochemical biosensor, aptamer-based biosensor, and electrochemical biosensor (Wang et al., 2009; Zhang et al., 2011; Cheng et al., 2007; Tang et al., 2011; Zhang et al., 2015). However, as a sensitive analytical technique, ECL has been seldom reported in Lyz detection (Huang et al., 2009). Herein, ECL-RET system involving luminol and SiQDs was established. Subsequently, a novel aptasensor based on ECL-RET was fabricated and used in the sensitive detection of Lyz.

2. Experimental section

2.1. Materials

Luminol, lysozyme (from egg white), 3-aminopropyl trimethoxysilane, 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC), tris(2-carboxyethyl)phosphine (TCEP), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA), 6-mercapto-1-hexanol (MCH), thrombin, and horseradish peroxidase, were obtained from Sigma. All other chemicals were of analytical reagent grade, and double distilled water was used throughout. 0.1 mol L^{-1} pH 7.4 phosphate buffer solution (PBS) was used to prepare the working solution.

The DNA used in this work was synthesized and purified by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. The sequences of two oligomers employed in the text are given as follows (The italicized part is the complementary strand of oligomers):

The anti-lysozyme aptamer (probe): 3′-SH-(CH2)6-CCC ***ATC AGG GCT AAA GAG TGC AGA*** GTT ACT TGA-5′.

The complementary DNA: 5′-COOH-TAG TCC CGA TTT CTC ACG TCT-3′.

2.2. Synthesis of SiQDs

3 mL of 3-aminopropyl trimethoxysilane was added into 12 mL N_2-saturated aqueous solution containing 0.632 g trisodium citrate to obtain precursor solution (Zhong et al., 2013). After 15 min of stirring, the precursor solution was transferred into a microwave reactor under 160 °C for 15 min. After microwave irradiation, the SiQDs sample was removed when the temperature was naturally decreased to room temperature. The residual impurities were removed by dialysis (1 kDa). The obtained SiQDs were characterized by HRTEM, FL, and UV–vis absorption spectroscopy and shown in Fig. S1–S3. The results revealed that spherical SiQDs have an average size of 6.72 nm, with wide absorbance band at 350 nm and symmetrical FL peak at 453 nm.

2.3. Preparation of luminol/DNA solution

200 μL of 2.5 μM complementary DNA was activated with 10 mg mL^{-1} EDC and 5 mg mL^{-1} NHS for 30 min at room temperature. Then, 150 μL of 10 mM luminol was added and reacted at 37 °C for 1 h to form luminol/DNA solution.

2.4. Fabrication of ECL sensor

A gold electrode (GE) was mechanically polished to a mirror with alumina pastes of 0.05 μm, and cleaned thoroughly in an ultrasonic cleaner with alcohol and water sequentially. Then, the electrode was cycled between 0 and 1.50 V (vs. SCE) in 0.50 mol L^{-1} sulfuric acid at a scan rate of 100 mV s^{-1}. This potential cycling was continued until a reproducible voltammogram for gold oxide formation/reduction was obtained, which meant a clean surface of gold electrode was obtained. The electrode was again rinsed with redistilled water and cleaned in an ultrasonic bath and was dried with blowing N_2. 165 μL of 1.818 μM probe was activated with 1.85 μL of 10 mM TCEP for 1 h to open disulphide bonds. 10 μL of activated probe was spread on the cleaned gold electrode surface for 2 h at 37 °C. Next, 10 μL of 10 mM MCH was spread on the electrode for 30 min at room temperature to remove the nonspecific probe adsorption. After the electrode was washed with PBS, 10 μL of luminol/DNA solution was dropped onto the electrode surface to incubate at 37 °C for 2 h. The electrode was washed with 10 mM PBS to remove the nonspecific binding luminol/DNA on the surface. Finally, the resulted electrode was incubated with different concentration of lysozyme for 1 h at 37 °C. After washing with PBS, the modified electrode was used in the ECL measurements.

3. Results and discussion

3.1. ECL and electrochemistry of SiQDs/luminol system

Generally, luminol ECL is extremely weak at the bare gold electrode in neutral condition. Intense luminol ECL emission can be obtained at metal NPs modified electrodes due to their electrocatalytical effects (Cui et al., 2004). Previous work revealed that CdSe@ZnS QDs could enhance luminol ECL and generate new ECL emission, suggesting that semiconductor QDs have great potential in ECL investigation (Dong et al., 2014). However, the ECL behavior of low-toxicity SiQDs has been seldom reported although SiQDs exhibited excellent FL characters and were widely used in biosensor (Lu et al., 2012; Liu et al., 2012; Michalet et al., 2005; Song et al., 2010; He et al., 2010). Herein, water soluble SiQDs were synthesized and ECL behaviors of SiQDs, luminol, and SiQDs/luminol mixing solutions were comparatively studied at a bare gold electrode as shown in Fig. 1A.

No light emission was obtained in PBS, and extremely weak luminol ECL was obtained at 0.50 V as shown in the inset of Fig. 1A, which is in accordance with the previous result (Cui et al., 2004). One broad and weak anodic ECL peak was obtained at ~1.30 V in SiQDs solution, which should be result from the reaction between QDs and the dissolved oxygen (Liu et al., 2007). When luminol was mixed with SiQDs, one strong ECL emission located at 1.30 V with a shoulder peak at 0.75 V was obtained. The ECL intensity increased nearly 50-times compared with pure luminol and SiQDs solutions. The shoulder peak can be assigned to luminol ECL. The maximum peak emission should be result from the interaction between luminol and SiQDs, because this peak can be obtained in SiQDs solution and was greatly enhanced in the presence of luminol.

Electrochemical behaviors of SiQDs and luminol were investigated as shown in Fig. 1B. The oxidation peak at 0.96 V and the reduction peak at 0.36 V obtained at the bare GE in PBS can be assigned to the oxidation/reduction of gold. In SiQDs solution, the redox peaks of gold were negatively shifted to 0.73 V and 0.16 V, respectively. Bard's work revealed that SiQDs can be oxidized to cation radicals through hole injection at Pt electrode (Ding et al., 2002). Although the corresponding oxidation peak of SiQDs can't be observed in the present work, the variation of cyclic voltammogram of gold electrode indirectly revealed that SiQDs radicals were generated. In luminol solution, one strong oxidation peak at 0.50 V can be assigned to the oxidation of luminol because the peak current increased with the increase of luminol concentration. The oxidation current of luminol was greatly decreased in the presence SiQDs, revealing that SiQDs can react with luminol, which could compete with the electrochemical oxidation of luminol and decrease its oxidation current.

It is well-known that luminol ECL is often influenced by the