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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

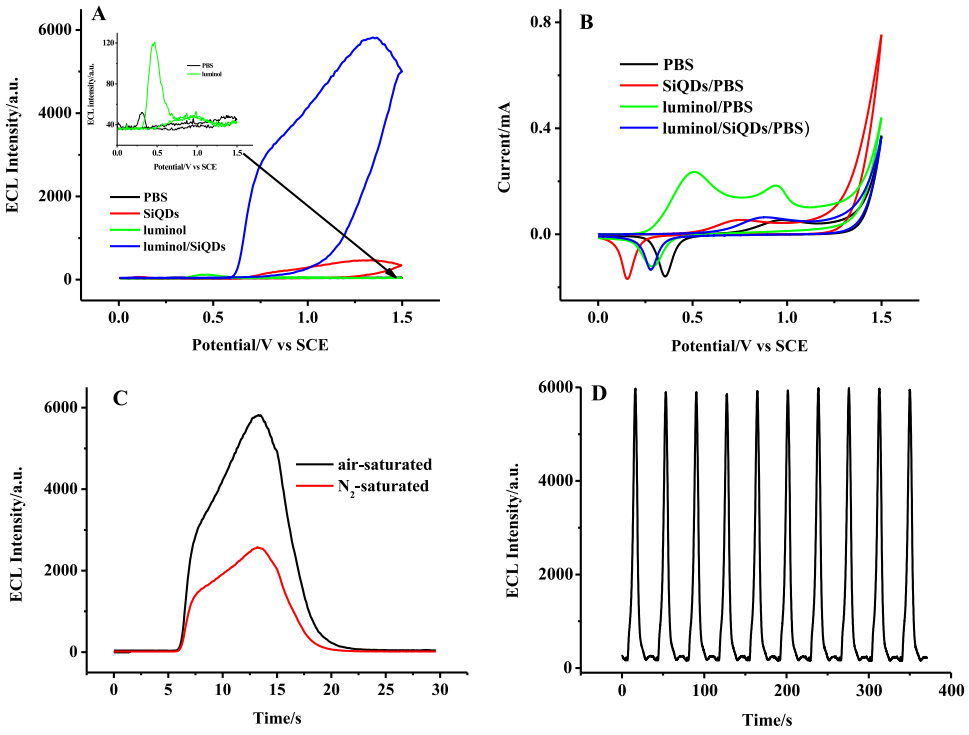

Fig. 1. ECL profiles (A) and Cyclic voltammograms (B) of SiQDs, luminol, SiQDs/luminol mixing solutions in PBS at the gold electrode. The inset is the enlarged ECL profiles in PBS and luminol. (C) ECL profiles of SiQDs/luminol mixing solution under different atmosphere. (D) Stability of ECL of SiQDS/luminol mixing solution under 10-cycles continuous potential scan.

dissolved oxygen. Therefore, luminol ECL was investigated under different atmospheres. It can be found from Fig. 1C that ECL intensity decreases greatly under N_2-saturated condition compared with that under air-saturated condition, suggesting that the dissolved oxygen participates in the ECL reactions. Fig. 1D displays the ECL intensities of SiQDs/luminol mixing solution under 10 repeated cyclic voltammetric scans. It can be found that the ECL intensity is quite stable with a relative standard deviation (RSD) of 1.3%. The strong and stable ECL emission in neutral aqueous condition reveals that the present ECL system has good application potential in bioassay field.

The effects of SiQDs and luminol concentrations on ECL signal were investigated and shown in Fig. S4. It can be found ECL intensities increase with the increase of luminol and SiQDs concentrations, revealing that luminol and SiQDs are responsible for ECL emission. Considering the consume of luminescent reagent, 0.01 mg mL^{-1} SiQDs and 1.0×10^{-5} mol L^{-1} luminol were selected in the following study, which can meet the requested sensitivity in the fabrication of biosensor.

3.2. ECL mechanisms

In order to elucidate ECL mechanism, the spectral behaviors of SiQDs, luminol, and SiQDs/luminol mixing solution were investigated as shown in Fig. 2. It can be found from Fig. 2A that luminol and SiQDs exhibited the maximum FL emission at 418 and 453 nm, respectively. When luminol was mixed with SiQDs, the maximum FL was observed at the same position of pure SiQDs, and the FL intensity of mixing solution is stronger than those of pure luminol and SiQDs solutions. The FL results revealed that resonance energy transfer process can occur between luminol and SiQDs. The UV–vis absorption spectra in Fig. 2B revealed that the absorption spectrum of SiQDs/luminol mixture is the simple overlap of luminol and SiQDs absorption spectra, suggesting that SiQDs can't react with luminol under the ground state. Fig. 2C revealed that the absorption spectrum of SiQDs can overlap with the ECL spectrum of luminol, which supported that ECL-RET is possible between luminol and SiQDs. It can be found from Fig. 2D that the light emitter of the shoulder ECL peak could be assigned to luminol ECL because the maximum ECL emission located at 410 nm which corresponds to luminol FL emission. However, when the ECL spectrum was recorded at 1.30 V, the maximum ECL peak is red-shifted to the position of the FL emission of SiQDs, revealing that the ECL emission should be the excited SiQDs.

The mechanisms of luminol ECL had been well documented previously (Richter et al., 2004; Miao et al., 2008). In general, luminol (LH^-) is electrochemically oxidized to produce diazo compound AP^{2-}. The compound is converted to its excited state AP^{2-*} in the presence of O_2. AP^{2-*} goes back to the ground state and emits light. In the present work, extremely weak luminol ECL can be observed in neutral condition at the bare GE according to the above mechanisms. In the presence of SiQDs, luminol ECL was greatly enhanced and one stronger ECL was observed at more positive potential (Fig. 1A). The UV–vis absorption spectra revealed that luminol can't react with SiQDs. However, the electrochemical results showed that the oxidation current of luminol decreased in the presence of SiQDs, suggesting that interaction between SiQDs and luminol occurred. Bard et al. revealed that SiQDs could be oxidized to cation radicals through hole injection (Ding et al., 2002). Therefore, it is reasonable to speculate that SiQDs can be oxidized to SiQDs radicals before the oxidation of luminol, which can then react with luminol. This process can compete with the electrochemical oxidization of luminol, resulting in the decreased oxidation current of luminol. ECL signal can't be completely inhibited under nitrogen saturated condition, revealing that there exists another pathway for the generation of ECL except the reaction of luminol with the dissolved oxygen. Combining the above results with the reference results (Cui et al., 2004), the mechanism for the shoulder peak can be described as follows:

$$LH^- - e \rightarrow L^{\bullet} \quad (1)$$

$$L^{\bullet} + O_2 \rightarrow O_2^{-\bullet} + L \quad (2)$$

$$L^{\bullet} + O_2^{-\bullet} \rightarrow \rightarrow AP^{2-*} \quad (3)$$

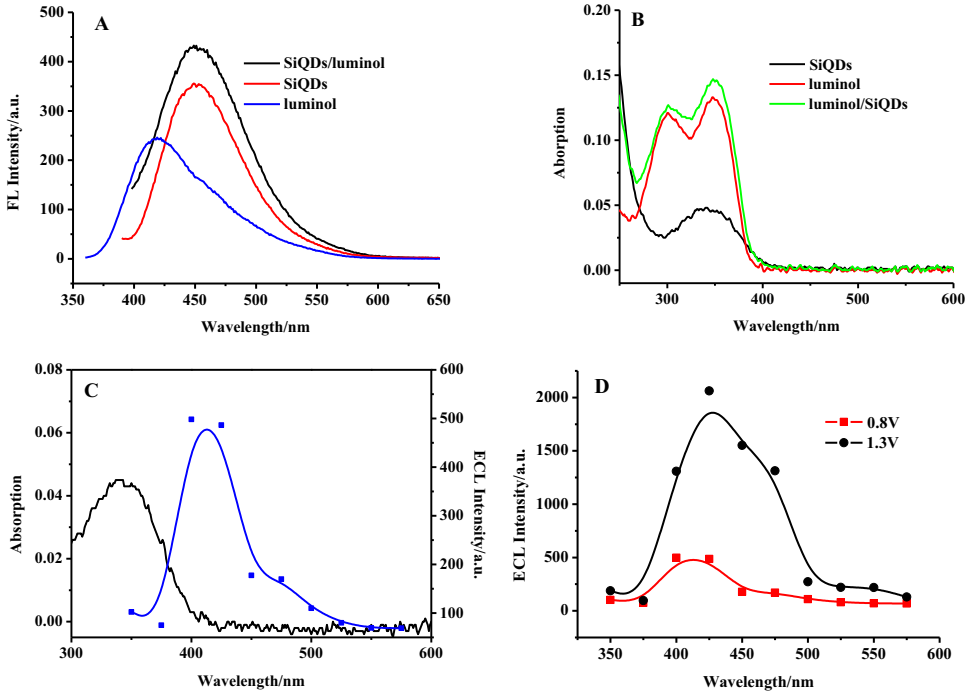

Fig. 2. FL (A) and UV–vis absorption (B) spectra of SiQDs, luminol, and SiQDs/luminol mixing solutions. (C) The overlap between ECL spectrum of luminol and UV–vis absorption spectrum of SiQDs. (D) ECL spectra of SiQDs/luminol system recorded at 0.80 and 1.30 V, respectively.

$$SiQDs - e \rightarrow SiQDs^{+\bullet} \quad (4)$$

$$SiQDs^{+\bullet} + LH^{-} \rightarrow AP^{2-*} \quad (5)$$

$$AP^{2-*} \rightarrow AP^{2-} + h\nu\ (410\ nm) \quad (6)$$

It was reported that the dissolved oxygen participated the ECL reactions of QDs in the species of $O_2^{-\bullet}$, which could inject electrons into the hole injected QDs and generate anodic ECL (Liu et al., 2007). Therefore, weak ECL observed in SiQDs solution should be generated as follows:

$$SiQDs - e \rightarrow SiQDs^{+\bullet} \quad (7)$$

$$SiQDs^{+\bullet} + O_2^{-\bullet} \rightarrow SiQDs^{*} + O_2 \quad (8)$$

$$SiQDs^{*} \rightarrow SiQDs + h\nu\ (450\ nm) \quad (9)$$

Because UV–vis absorption of SiQDs overlaps with the ECL spectrum of luminol, the excited SiQDs could be produced by the energy transfer from the excited state of luminol (Dong et al., 2014). In order to further confirm the energy transfer between luminol ECL and SiQDs, the ECL spectra of SiQDs/luminol system were recorded at 1.30 V by varying the amount of SiQDs. It can be found from Fig. S5 that the intensities of luminol ECL decreased while the intensities of SiQDs ECL increased with the increase of SiQDs amount, which support the energy transfer process between the excited state of AP^{2-*} and SiQDs. Therefore, the mechanism of ECL peak obtained at 1.30 V should be as follows:

$$SiQDs^{+\bullet} + AP^{2-*} \rightarrow SiQDs^{*} + AP^{2-} \quad (10)$$

SiQDs can not only enhance luminol ECL but also act as an ECL energy acceptor to generate anodic ECL which is even stronger than that of luminol and gives a new route for the bioapplication of anodic ECL in neutral condition.

3.3. Fabrication of biosensor

Nucleic acid aptamers are single-stranded DNA or RNA oligonucleotides with the high affinity and specificity toward their targets. Various aptamer-based biosensors have been developed using the specific identification of aptamer toward their target (Yin et al., 2012). Herein, the proposed ECL-RET system was used to fabricate ECL aptasensor for lysozyme detection. The protocol for the fabrication of ECL biosensor was shown in Fig. 3. Firstly, mercapto group terminated lysozyme aptamer can be connected onto the gold electrode by S-Au bond. Luminol/DNA can be connected with the aptamer through hybridization. The connected luminol can react with SiQDs to generate strong ECL signal. When the sensor was incubated in lysozyme solution, the specific interaction between lysozyme and aptamer will release luminol/DNA from the electrode surface, resulting in the decrease of ECL signal. Therefore, a simple and convenient sensing platform is proposed based on SiQDs, nucleic acid aptamer, and the intercalated probe, which can be used for the sensitive and selective detection of lysozyme.

Electrochemical impedance spectroscopy was employed to monitor the fabrication process of the biosensor. The equivalent circuit was shown in the inset of Fig. 4A, and was used as the model of the working electrode to fit the EIS data. The charge transfer resistance (R_{ct}) which equals the diameter of semicircle reflects the restricted diffusion of the redox probe through the electrode surface. R_s, Z_w, C_d are the electrolyte solution resistance, the Warburg impedance, and the double layer capacitance, respectively (Patolsky et al., 2001). It can be found from Fig. 4A that the bare GE exhibits nearly straight line in whole frequency range, revealing the facile electron transfer through the electrode surface. When the aptamer was modified on the GE, R_{ct} increased greatly due to the electrostatic repulsion between the negatively charged DNA aptamer backbone and the $Fe(CN)_6^{3-/4-}$ probe. When luminol/DNA was hybridized with the aptamer, the R_{ct} further increased because luminol/DNA solution was negatively charged. When luminol/DNA was replaced by lysozyme, the R_{ct} decreased because lysozyme is positively charged in pH 7.4 PBS (Rodriguez et al., 2005). The EIS results suggested the successful stepwise fabrication of the proposed ECL aptasensor.

ECL responses of different fabrication process were recorded as shown in Fig. 4B. Bare GE and aptamer modified GE exhibited

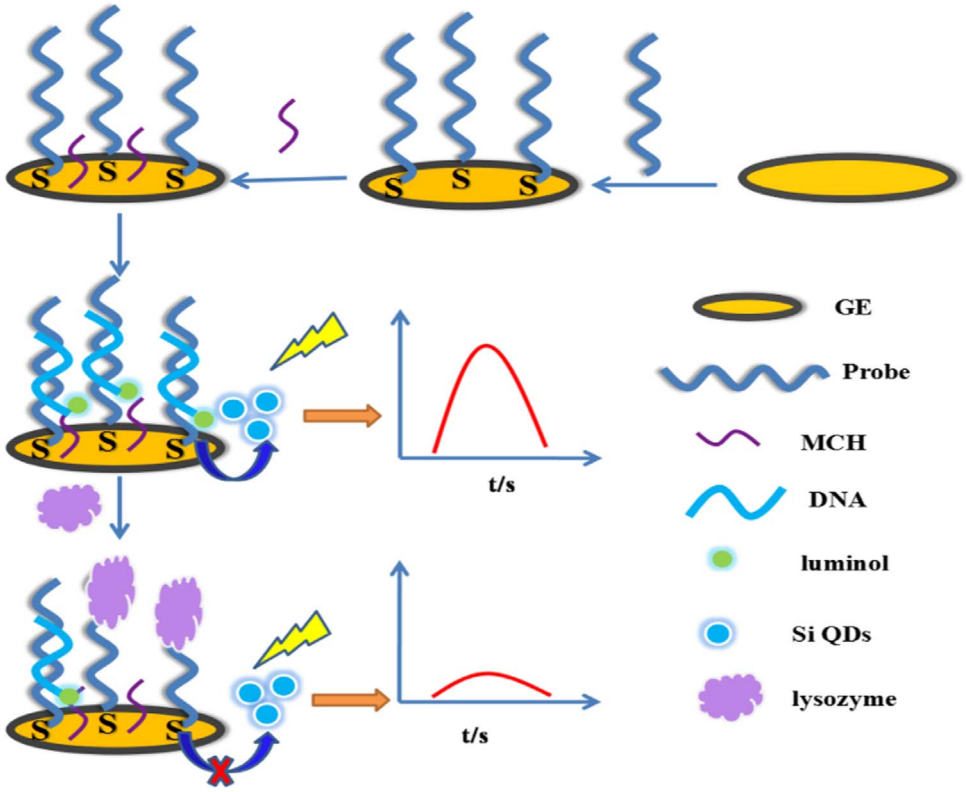

Fig. 3. Schematic representation of the modification of gold electrode and the detection of lysozyme.

extremely weak ECL in neutral SiQDs solution. When luminol/DNA was connected with aptamer through hybridization, the reaction between luminol and SiQDs resulted in strong light emission. When lysozyme was hybridized with its aptamer to release luminol/DNA from the electrode surface, the ECL-RET between luminol and SiQDs was hampered, resulting in the reduced ECL intensity instantly. The inhibiting effect of lysozyme on ECL signal could be used in the determination of lysozyme indirectly.

3.4. Analytical performance of the biosensor

The quantitative behavior of as-prepared ECL aptasensor for lysozyme detection was assessed by measuring the relationship between ECL intensity and lysozyme concentration under the optimized experimental condition. After the biosensor was incubated with different concentrations of lysozyme, the ECL intensities of the biosensor were tested as shown in Fig. 5A. It can be found that the ECL intensity decreased gradually with the increase of lysozyme concentration. The calibration curve showed a good linear relationship between ECL intensity and the logarithm of lysozyme concentration in the range from 5.0×10^{-14} to 5.0×10^{-9} g mL^{-1} with the correlation coefficient of 0.992 as shown in the inset of Fig. 5A. The detection limit of the biosensor was calculated at levels down to 5.8×10^{-15} g mL^{-1} (3σ, n=5). The reproducibility of the proposed biosensor for lysozyme was evaluated from the response to 5 pg mL^{-1} lysozyme at six different electrodes. The relative standard derivation (RSD) was 3.8%, demonstrating that the biosensor possessed acceptable reproducibility. When the biosensor was stored in pH 7.4 PBS at 4 °C for one week, the ECL signal retained 94.6% of the original ECL response, indicating a good stability of the biosensor.

In order to investigate the specific response of the biosensor to lysozyme, control experiments were performed by incubating the biosensor in several 5 pg mL^{-1} aqueous solutions of thrombin (Thr), bovine serum albumin (BSA), horseradish peroxidase (HRP), and the mixture of lysozyme with them. It could be observed from Fig. 5B that only the lysozyme sample and the mixture of lysozyme gave significant decreased ECL intensity. BSA, HRP, and thrombin had almost negligible interference for lysozyme detection. These results indicate good

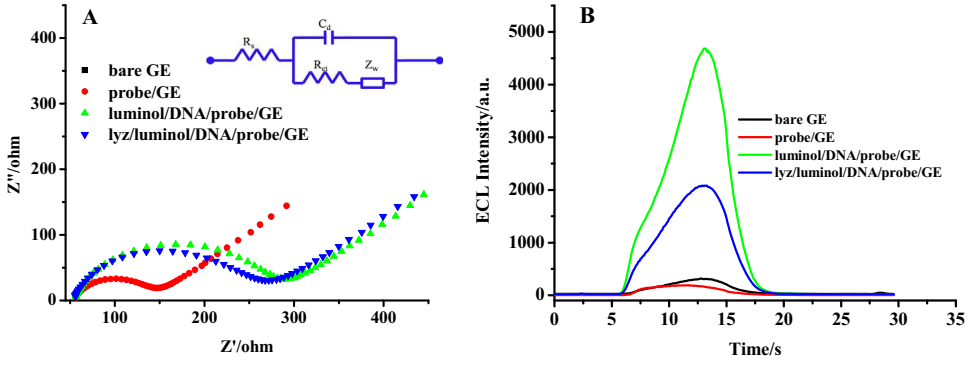

Fig. 4. (A) Nyquist diagram of electrochemical impedance spectra recorded from 0.01 to 10^5 Hz for $[Fe(CN)_6]^{3-/4-}$ (10 mM, 1:1) in 0.1 mol L^{-1} pH 7.4 PBS containing 0.1 mol L^{-1} KCl. (B) ECL profiles of different processes of the fabrication of biosensor. Lysozyme, 5 pg mL^{-1}; PBS, 0.1 mol L^{-1}; pH, 7.4; scan rate, 100 mV s^{-1}.

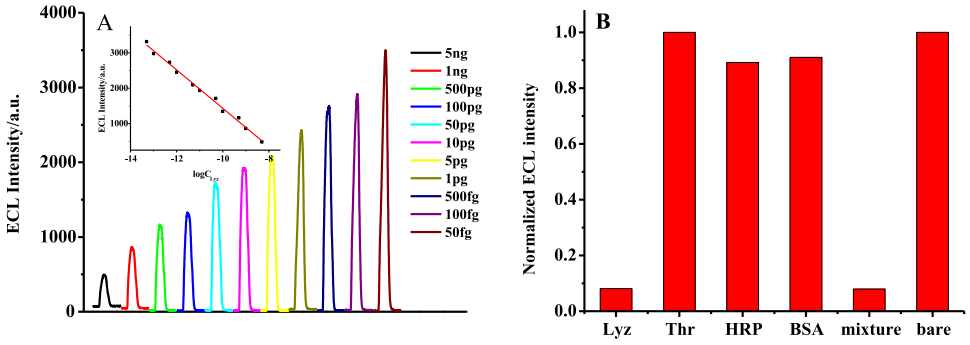

Fig. 5. (A) ECL signals of the biosensor incubated with different concentrations of lysozyme (from 50 fg mL^{-1} to 5 ng mL^{-1}). The inset is the corresponding logarithmic calibration curve. (B) Selectivity of the proposed biosensor to lysozyme by comparing it to the interfering proteins. BSA, HRP, Lyz, Thr at 5 pg mL^{-1}, respectively. Luminol, 1.0×10^{-4} mol L^{-1}; PBS, 0.1 mol L^{-1}; pH, 7.4; scan rate, 100 mV s^{-1}.

selectivity and high specificity of the proposed biosensor to lysozyme. The comparison of the various techniques for the detection of lysozyme was listed in Table S1. It can be found that the present ECL biosensor is superior to other reported biosensors.

3.5. Applications of the aptasensor

The potential applicability of the aptasensor in the detection of lysozyme in real sample was investigated by determining lysozyme in human blood serum samples (The sample was obtained from Nanjing Gulou Hospital). The serum 1:5 diluted with TE buffer (pH 7.4) was spiked with lysozyme at different concentrations. The results in Table S2 show the acceptable relative standard deviation (RSD) and good recoveries, implying that the present aptasensor has a promising analytical application in real biological samples.

4. Conclusions

Water soluble SiQDs can react with luminol to generate strong anodic ECL in neutral condition. ECL resonance energy transfer can occur with luminol as energy donor and SiQDs as energy acceptor, leading stronger ECL emission. An aptasensor for the detection of lysozyme was fabricated based on ECL-RET system. In the range of 5.0×10^{-14}–5.0×10^{-9} g mL^{-1}, lysozyme can be sensitively detected with a detection limit of 5.8×10^{-15} g mL^{-1} (3σ). The interaction between SiQDs and traditional ECL reagents reveals the new role of SiQDs in ECL techniques, and eco-friendly character of SiQDs will find more applications in ECL biosensing field compared with heavy metal involved semiconductor QDs. Most importantly, the proposed sensor can be widely used in the detection of other kinds of enzymes through varying the interaction method between DNA and aptamer. The successful construction of resonance energy transfer system between luminol and SiQDs will promote the research interesting in finding more suitable energy donor/acceptor pairs, which will enrich the investigation field of ECL-RET.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2017.03.044.

References

Baker, S.N., Baker, G.A., 2010. Angew. Chem. Int. Ed. 49, 6726–6744.
Borisov, S.M., Wolfbeis, O.S., 2008. Chem. Rev. 108, 423–461.
Chen, Y.Y., Tu, Y.F., 2014. Electrochim. Acta 135, 187–191.
Cheng, A.K.H., Ge, B.X., Yu, H.Z., 2007. Anal. Chem. 79, 5158–5164.
Cui, H., Xu, Y., Zhang, Z.F., 2004. Anal. Chem. 76, 4002–4010.
Derfus, A.M., Chan, W.C.W., Bhatia, S.N., 2004. Nano Lett. 4, 11–18.
Ding, Z.F., Quinn, B.M., Haram, S.K., Pell, L.E., Korgel, B.A., Bard, A.J., 2002. Science 296, 1293–1296.
Dong, Y.P., Gao, T.T., Zhou, Y., Zhu, J.J., 2014. Anal. Chem. 86, 11373–11379.
Erogbogbo, F., Yong, K.T., Roy, I., Xu, G.X., Prasad, P.N., Swihart, M.T., 2008. ACS Nano 2, 873–878.
Fahnrich, K.A., Pravda, M., Guilbault, G.G., 2001. Talanta 54, 531–559.
Haghighi, B., Bozorgzadeh, S., 2011. Anal. Chim. Acta 697, 90–97.
He, Y., Kang, Z.H., Li, Q.S., Tsang, C.H.A., Fan, C.H., Lee, S.T., 2009. Angew. Chem. 121, 134–138.
He, Y., Fan, C.H., Lee, S.T., 2010. Nano Today 5, 282–295.
He, Y., Zhong, Y.L., Peng, F., Wei, X.P., Su, Y.Y., Lu, Y.M., Su, S., Gu, W., Liao, L.S., Lee, S.T., 2011. J. Am. Chem. Soc. 133, 14192–14195.
Huang, H.P., Jie, G.F., Cui, R.J., Zhu, J.J., 2009. Electrochem. Commun. 11, 816–817.
Li, N., Gu, J., Cui, H., 2010. J. Photochem. Photobiol. A: Chem. 215, 185–190.
Liu, S., Tian, J.Q., Wang, L., Zhang, Y.W., Qin, X.Y., Luo, Y.L., Asiri, A.M., Al-Youbi, A.O., Sun, X.P., 2012. Adv. Mater. 24, 2037–2041.
Liu, X., Jiang, H., Lei, J.P., Ju, H.X., 2007. Anal. Chem. 79, 8055–8060.
Lu, W.B., Qin, X.Y., Liu, S., Chang, G.H., Zhang, Y.W., Luo, Y.L., Asiri, A.D., Al-Youbi, A.O., Sun, X.P., 2012. Anal. Chem. 84, 5351–5357.
Miao, W.J., 2008. Chem. Rev. 108, 2506–2553.
Michalet, X., Pinaud, F.F., Bentolila, L.A., Tsay, J.M., Doose, S., Li, J.J., Sundaresan, G., Wu, A.M., Gambhir, S.S., Weiss, S., 2005. Science 307, 538–544.
Patolsky, F., Lichtenstein, A., Willner, I., 2001. J. Am. Chem. Soc. 123, 5194–5205.
Richter, M.M., 2004. Chem. Rev. 104, 3003–3036.
Rodriguez, M.C., Kawde, A.N., Wang, J., 2005. Chem. Commun. 41, 4267–4269.
Song, S.P., Qin, Y., He, Y., Huang, Q., Fan, C.H., Chen, H.Y., 2010. Chem. Soc. Rev. 39, 4234–4243.
Sun, L.Y., Chu, H.H., Yan, J.L., Tu, Y.F., 2012. Electrochem. Commun. 17, 88–91.
Tang, D., Liao, D.L., Zhu, Q.K., Wang, F.Y., Jiao, H.P., Zhang, Y.J., Yu, C., 2011. Chem. Commun. 47, 5485–5487.
Taokaenchan, N., Tangkuaram, T., Pookmanee, P., Phaisansuthichol, S., Kuimalee, S., Satienperakul, S., 2015. Biosens. Bioelectron. 66, 231–237.
Vocadlo, D.J., Davies, G.J., Laine, R., Withers, S.G., 2001. Nature 412, 835–839.
Wang, J., Liu, B., 2009. Chem. Commun. 17, 2284–2286.
Yin, X.B., 2012. Trends Anal. Chem. 33, 81–94.
Zhang, H.R., Xu, J.J., Chen, H.Y., 2013. Anal. Chem. 85, 5321–5325.
Zhang, H.R., Wu, M.S., Xu, J.J., Chen, H.Y., 2014. Anal. Chem. 86, 3834–3840.
Zhang, X.R., Zhao, Y.Q., Zhou, H.R., Qu, B., 2011. Biosens. Bioelectron. 26, 2737–2741.
Zhang, Z.H., Zhang, S., He, L.H., Peng, D.L., Yan, F.F., Wang, M.H., Zhao, J.H., Zhang, H.Z., Fang, S.M., 2015. Biosens. Bioelectron. 74, 384–390.
Zheng, L.Y., Chi, Y.W., Dong, Y.Q., Lin, J.P., Wang, B.B., 2009. J. Am. Chem. Soc. 131, 4564–4565.
Zhong, Y.L., Peng, F., Bao, F., Wang, S.Y., Ji, X.Y., Yang, L., Su, Y.Y., Lee, S.T., He, Y., 2013. J. Am. Chem. Soc. 135, 8350–8356.