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Short communication

Electrochemiluminescence of CdSe quantum dots for immunosensing of human prealbumin

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ABSTRACT

We describe a non-labeled electrochemiluminescence (ECL) immunosensor based on CdSe quantum dots (QDs) for the detection of human prealbumin (PAB, antigen). The immunosensor was fabricated by layer by layer coupled with nanoparticle-amplification techniques. After two gold nanoparticle layers were self-assembled onto the gold electrode surface through cysteamine, anti-PAB (antibody) were conjugated with –COOH groups of both the CdSe QDs and cysteine, which were linked to the gold nanoparticle-modified electrode. The principle of ECL detection was that the immunocomplex inhibited the ECL reaction between CdSe QDs and $K_2S_2O_8$, which resulted in the decrease of ECL intensity. On the one hand, the immunocomplex increased the steric hindrance. On the other hand, the immunocomplex maybe inhibit the transfer of $K_2S_2O_8$ to the surface of the CdSe QD-electrode. The PAB concentration was determined in the range of 5.0×10^{-10} to 1.0×10^{-6} g mL^{-1}, and the detection limit was 1.0×10^{-11} g mL^{-1}. The developed CdSe QD-based ECL immunosensor provides a rapid, simple, and sensitive immunoassay protocol for protein detection, which could be applied in more bioanalytical systems.

1. Introduction

Highly luminescent semiconductor nanocrystals (NCs), often referred to as quantum dots (QDs), have received tremendous attention for their possible luminescent applications in aqueous solution (Bruchez et al., 1998; Chan and Nie, 1998). With size-tunable narrow emission spectra and broad excitation spectra, they have been widely used as multicolored photoluminescent probes, biological luminescent labels (Gill et al., 2006; Zhelev et al., 2006; Jin et al., 2005; Charbonniere et al., 2006) and bioimagings (Medintz et al., 2005; Larson et al., 2003). Since the breakthrough works on the use of QDs in 1998 (Bruchez et al., 1998; Chan and Nie, 1998), multifarious water-soluble QDs have been synthesized to show different optical properties, biocompatibilities, and cellular toxicities (Wang et al., 2006; Jin et al., 2006; Hoshino et al., 2004; Duan and Nie, 2007; Choi et al., 2006). Some bioinorganic conjugates made with CdSe/ZnS core-shell NCs and antibodies have shown their potential applications in fluoroimmunoassays (Goldman et al., 2002a,b, 2004; Mattoussi et al., 2000). Luminescent properties of semiconductor nanocrystals are usually investigated by photoluminescence (PL) (Qu and Peng, 2002), electrochemiluminescence (ECL) (Bae et al., 2004; Zou and Ju, 2004; Miao et al., 2005), cathodoluminescence, and chemiluminescence (CL) (Wang et al., 2005). Among them, ECL is a useful technique for both fundamental study and analytical applications (Bae et al., 2004; Ding et al., 2002; Myung et al., 2002, 2003). In recent years, Bard and co-workers (Ding et al., 2002; Myung et al., 2002, 2004; Bae et al., 2004) have reported ECL of semiconductor nanocrystals in organic solvent. Subsequently, ECL of CdS (Miao et al., 2005; Ding et al., 2006) and CdSe NCs in aqueous solution (Zou and Ju, 2004; Jiang and Ju, 2007a,b; Liu et al., 2007) have also been observed and applied to H_2O_2 or glucose sensing. However, so far no immunosensor using ECL of QDs has been reported. Therefore, the development of ECL immunosensor fabricated with CdSe QDs is of great significance for biological analysis.

Prealbumin (PAB), a stable circulating glycoprotein synthesized in the liver, is a reliable index of liver function. Blood serum PAB decreased in varieties of hepatitis, cirrhosis and hepatocarcinoma (Guo, 2006). So the detection of PAB was worthwhile for early diagnosing serious hepatitis and evaluating clinical results.

This work developed a promising CdSe QD-based ECL immunosensor for prealbumin detection via self-assembly and gold nanoparticle-amplification techniques. The CdSe QDs and anti-prealbumin were directly immobilized onto the gold nanoparticle-modified electrode. The specific immunoreaction of prealbumin with anti-prealbumin resulted in the decrease of ECL intensity which could be used to detect the prealbumin. This ECL immunosensor is rapid, specific, and ultrasensitive for bioassays. In

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particular, this approach would open new avenues to apply quantum dots ECL in immunoassays.

2. Experimental

2.1. Reagents

Prealbumin (PAB, antigen) with the iso-electric point of 4.7 and the molecular weight of 54,000 and anti-PAB (antibody) were obtained from Xiamen Yutaikang Imports and Exports Biological Co. The standard PAB stock solutions were prepared with 10 mM PBS (pH 7.4) and stored at 4 °C. *N*-Hydroxysuccinimide (NHS) and bovine serum albumin (BSA, 96–99%) were obtained from Sigma (St. Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Pierce (Rockford, IL). Gold nanoparticles with a diameter of ~12 nm were prepared by the sodium citrate reduction of $AuCl_4^-$ ions according to a well-known method (Frens, 1973). Mercaptoacetic acid-capped CdSe QDs were prepared according to the literature (Gaponik et al., 2002). 0.1 M PBS (pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl was used as the electrolyte in the measuring system. Doubly distilled water was used throughout.

2.2. Apparatus

The electrochemical measurement for ECL was carried out on a CHI 812 electrochemical working station (Shanghai CH Instruments Co., China) using a three-electrode system. The electrodes were a 4-mm-diameter Au disk working electrode, a saturated calomel reference electrode (SCE), and a Pt counter electrode. The ECL emission was detected with a Model MPI-A Electrochemiluminescence Analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) at room temperature. The spectral width of the photomultiplier tube (PMT) was 200–800 nm and the voltage of the PMT was at 800 V. Electrochemical impedance spectroscopy (EIS) was carried out with a CHI 660A electrochemical working station (Shanghai CH Instruments Co., China), using the same three-electrode system as that in the ECL detection. UV absorption spectra were acquired with a Ruili 1200 photospectrometer (Peking Analytical Instrument Co., Peking, China). Photoluminescence (PL) spectra were obtained on an RF-540 spectrophotometer (Shimadzu). The scanning electron micrographs were taken with a field-emission scanning electron microscopy (FESEM, JEOL JSM-6340 F).

2.3. Fabrication of the ECL immunosensor

A gold disk electrode was polished with 1.0-, 0.3- and 0.05-μm α-Al_2O_3 powder on abrasive paper and electrochemically pretreated in 0.5 M H_2SO_4. After cleaned, the electrode was immersed in 0.1 mol L^{-1} cysteamine aqueous solution for 10 h, and then in the colloidal gold at 4 °C for 10 h. The above two processes were repeated again. The gold nanoparticle-modified electrode was next treated with cysteine, and then soaked in 40 μL mercaptoacetic acid-capped CdSe QDs containing 20 mg mL^{-1} EDC at 4 °C for 10 h. After reacted with EDC, NHS, the CdSe QD-electrode was incubated in 40 μL of 500 μg mL^{-1} anti-PAB (antibody) at 4 °C for 12 h. Finally, the electrode was blocked with 20 μL of 2 wt% BSA at 37 °C for 1 h.

Scheme 1(A) outlines the fabricating steps of the ECL immunosensor.

2.4. ECL detection

For the immunoreaction, the immunosensor was incubated in different concentrations of PAB at 37 °C for 50 min. Then, the electrodes were scanned from 0 to −1.7 V in 0.1 M PBS (pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl, and ECL signals related to the PAB concentrations could be measured.

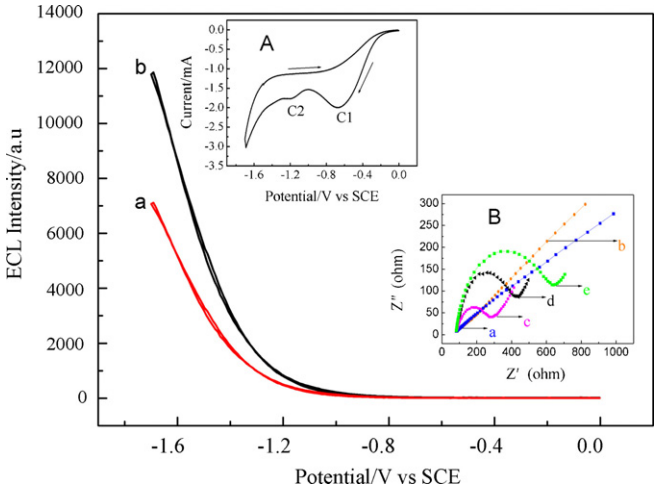


Fig. 1. Electrochemiluminescences intensity of CdSe QDs vs. potential. (a) On the bare Au electrode and (b) on the gold nanoparticle-modified electrode. Inset A: cyclic voltammogram of CdSe QDs on the gold nanoparticle-modified electrode. 0.1 M PBS (pH 7.4) containing 0.1 M KCl and 0.1 M $K_2S_2O_8$. Scan rate: 100 mV s^{-1}. Inset B: electrochemical impedance spectroscopy of the electrode at different stages. (a) Bare Au electrode, (b) after immobilization of gold nanoparticles, (c) after assembly of CdSe QDs, (d) after immobilization of antibody, and (e) after obturation with BSA. Supporting electrolyte: 10 mM PBS (2.5 mM $Fe(CN)_6^{4-/3-}$ + 0.1 M KCl, pH 7.4).

3. Results and discussion

3.1. Characterization of the CdSe QDs

The CdSe QDs showed a photoluminescence peak at 561 nm and absorption maximum at 451 nm (Fig. S1 in Supporting Information), indicating the consequence of quantum confinement (Murray et al., 1993). According to the literature (Yu et al., 2003), the particle size and band gap estimated from the UV–vis spectra, are 2.0 nm and 2.68 eV, respectively.

3.2. Electrochemical and ECL behaviors of the CdSe QDs on the Au electrode

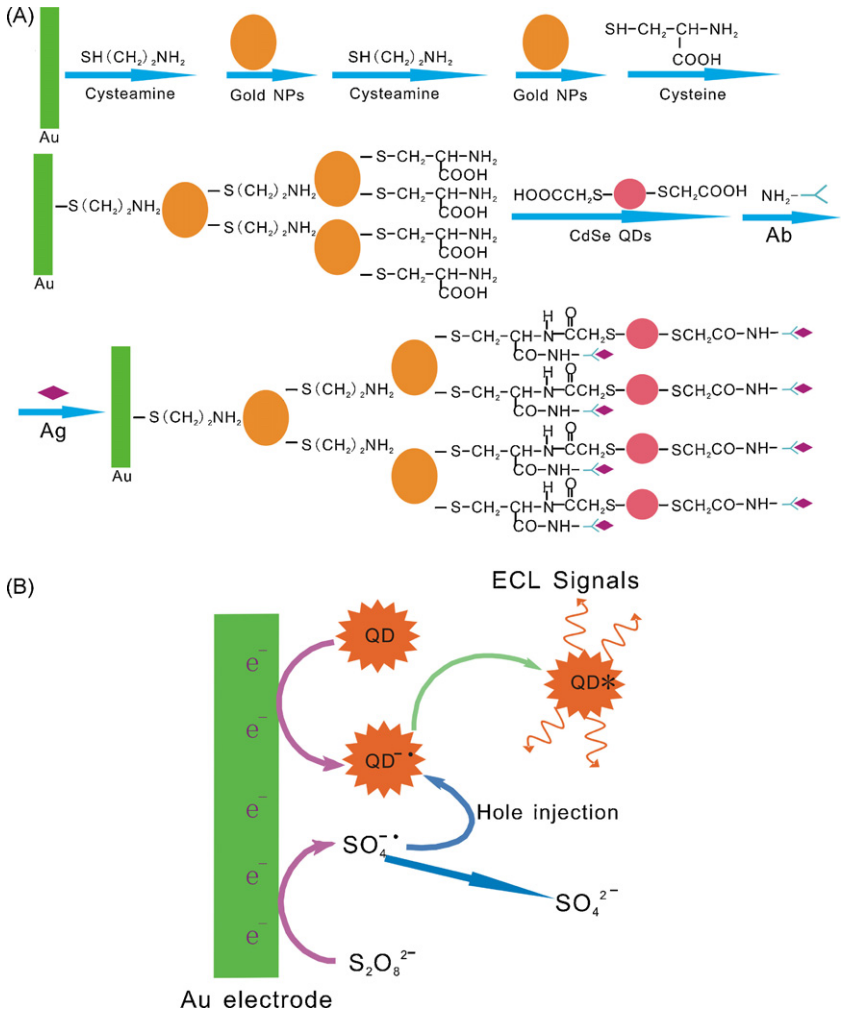
ECL and cyclic voltammogram (CV) (inset A) of the CdSe QDs on the Au electrode were shown in Fig. 1. In the CV, two cathodic peaks appeared at −0.67 V (C1) and −1.23 V (C2), corresponding to the reduction of $S_2O_8^{2-}$ and CdSe QDs, respectively. In the ECL curves (a and b), one ECL peak was observed at −1.68 V, resulting from the reaction between CdSe QDs and $S_2O_8^{2-}$. When the electrode was scanned between 0 and −1.7 V with an initial negative direction, the CdSe QDs were reduced to nanocrystal species ($CdSe^{\bullet -}$) (Myung et al., 2002). Reduction of $S_2O_8^{2-}$ produced a strong oxidant, $SO_4^{\bullet -}$, which could then react with the negatively charged QDs by injecting a hole into the HOMO, producing an excited state (QDs*) that then emitted light in the aqueous solution (Ding et al., 2002). The corresponding ECL mechanisms are as follows, which is shown in Scheme 1(B):

$$CdSe\ QDs + ne^- \rightarrow nCdSe^{\bullet -} \quad (1)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{\bullet -} \quad (2)$$

$$CdSe^{\bullet -} + SO_4^{\bullet -} \rightarrow CdSe^* + SO_4^{2-} \quad (3)$$

$$CdSe^* \rightarrow CdSe + h\nu \quad (4)$$



Scheme 1. Fabrication process (A) and ECL mechanisms (B) of the ECL immunosensor.

In addition, to investigate the gold nanoparticle-amplification for ECL analysis, we compared the ECL of CdSe QDs with and without gold nanoparticles. The ECL intensity of CdSe QDs was enhanced by ~1.7 orders of magnitude on the gold nanoparticle-modified electrode (curve b) than that on the bare Au electrode (curve a). This could be explained by the fact that the gold nanoparticles can provide more binding sites for assemble CdSe QDs, which could amplify the ECL signal, and thus enhanced the detection sensitivity.

3.3. Characterization of the ECL immunosensor

Electrochemical impedance spectroscopy (EIS) was an effective method to monitor the feature of modified electrode surface. The impedance spectra include a semicircle portion and a linear portion. The semicircle diameter corresponds to the electron-transfer resistance (R_{et}), and the linear part corresponds to the diffusion process. From the EIS of the electrode at different stages (Fig. 1 inset B), it was observed that the bare Au electrode exhibited an almost straight line (curve a), which was characteristic of a diffusional process. When the gold nanoparticles were assembled onto the electrode, the EIS was similar to that of the bare Au electrode (curve b). The reason may be that the gold nanoparticles play an important role similar to a conducting wire, which makes it easier for the electron transfer to take place. Subsequently, the CdSe QDs were linked to the electrode surface, and the EIS showed a low R_{et} (curve c) due to the conductivity of CdSe QDs. Additionally, EIS of the antibody-immobilized electrode showed a large increase in diameter (curve d), suggesting that the antibody formed an additional barrier and further prevented the redox probe to the electrode surface. Finally, BSA was used to block nonspecific binding sites of the electrode, which exhibited a greater increase in electron-transfer resistance (curve e).

The modified electrodes were also characterized by scanning electron microscope (SEM). As shown in the SEM images (Fig. S2 in Supporting Information), the bare Au electrode displayed a plane uniform structure (inset A). When the gold nanoparticles were assembled onto the electrode, many small bright particles could be observed (B). Then, the conjugation of CdSe QDs to the electrode produced a layer of QDs, and the corresponding microstructures obviously changed (C).

3.4. ECL detection of PAB with the immunosensor

ECL emission from the immunosensor upon continuous cyclic scan for 12 cycles was shown in Fig. 2 (inset A). The coincident signals indicated the reliability and stability of the immunosensor, which could be applied to the ECL detection.

Fig. 2 showed the ECL profiles of the immunosensor before (a) and after (b–g) incubating in different concentrations of PAB (antigen). The ECL signal after incubating in PAB (b) was higher than that before incubating in it (a), and decreased gradually with

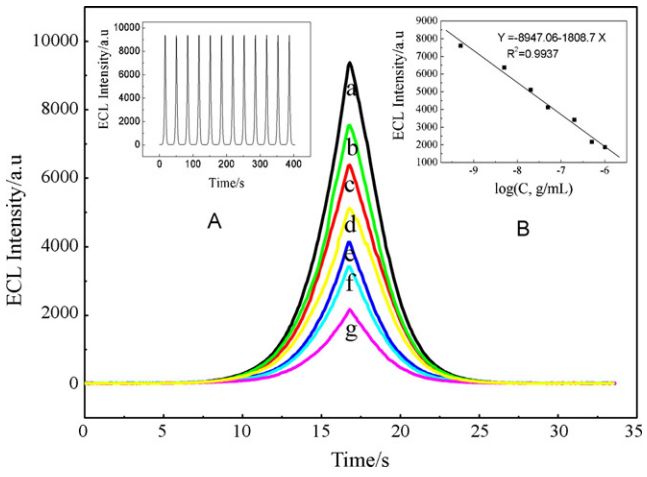


Fig. 2. ECL profiles of the immunosensor before (a) and after (b–g) incubating in different concentrations of PAB in pH 7.4 PBS containing 0.1 M KCl and 0.1 M $K_2S_2O_8$. PAB concentration (g/mL): (a) 0, (b) 5.0×10^{-10}, (c) 5.0×10^{-9}, (d) 2.0×10^{-8}, (e) 5.0×10^{-8}, (f) 2.0×10^{-7}, and (g) 5.0×10^{-7}. Inset A: ECL emission from the ECL immunosensor under continuous cyclic voltammetry for 12 cycles; inset B: linear plots of ECL intensity vs. PAB concentrations. Scan rate: 100 mV s^{-1}.

the increase concentrations of PAB (b–g). The reason was that the immunocomplex after immunoreaction inhibited the ECL reaction between CdSe QDs and $K_2S_2O_8$, and thus decreased the ECL intensity. On the one hand, the immunocomplex increased the steric hindrance, which slows down the electron-transfer speed in ECL reaction. On the other hand, the immunocomplex maybe inhibit the transfer of $K_2S_2O_8$ to the surface of the CdSe QD-electrode, which also decreased the ECL reaction. The results suggested the PAB concentration could be determined with the ECL immunosensor. The standard calibration curve for PAB detection was shown in Fig. 2 (inset B). The ECL intensity decreased linearly with the PAB concentrations in the range from 5.0×10^{-10} to 1.0×10^{-6} g mL^{-1}, and the detection limit was 1.0×10^{-11} g mL^{-1}. According to the linear equation, we could detect PAB concentration quantitatively.

3.5. Specificity, reproducibility and regeneration of the immunosensor

To investigate the specificity of the immunosensor, we detected the ECL response of the mixture containing 10 ng mL^{-1} PAB, 500 ng mL^{-1} human IgG, and 500 ng mL^{-1} low-density lipoprotein. Compared with the ECL response obtained from the pure PAB, no significant difference (R.S.D. = 8.8%) was found, indicating that the human IgG and low-density lipoprotein did not cause the observable interference, suggesting that this immunosensor is feasible for the determination of PAB in human plasma.

The reproducibility of the immunosensor for human prealbumin (PAB) was evaluated from the response to 10 ng mL^{-1} PAB at four different electrodes. A series of four measurements from the batch resulted in a relative standard deviation of 9.5%, indicating good electrode-to-electrode reproducibility of the fabrication protocol described above.

The regeneration of the immunosensor was tested. After detecting the PAB, the immunosensor was dipped into glycine–hydrochloric acid buffer solution for 8 min to remove PAB from anti-PAB. The consecutive measurements were repeated five times and a relative standard deviation (R.S.D.) of 7.4% was acquired.

4. Conclusions

In the present work, electrochemiluminescence of CdSe QDs was successfully used to develop a novel label-free ECL immunosensor for PAB determination. The CdSe QDs showed high ECL intensity and good biocompatibility. Besides, application of the self-assembled gold nanoparticle layers improved the absorption capacity of CdSe QDs and anti-PAB molecules, which enhanced the detection sensitivity of target PAB. Combining high sensitivity of ECL detection with specificity of immunoreaction, the immunosensor could become an alternative method to other immunoassays. This novel approach opens new avenues to apply QDs ECL in immunoassays, which has great potential to be applied in various analytical systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.02.028.

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