Enzyme–quantum dots architecture for highly sensitive electrochemiluminescence biosensing of oxidase substrates†

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A simple strategy for the fabrication of the first biosensor based on the intrinsic electrochemiluminescence of quantum dots coupled with an enzymatic reaction is proposed with glucose oxidase as a model, which could be applied in more bioanalytical systems for oxidase substrates.

Colloidal semiconductor nanocrystals, also called quantum dots (QDs), have attracted much attention due to their robust size-dependent optical and electronic properties, which play important roles in miscellaneous bioassays and the design of biomedical materials.[1] Owing to the advantages of electrochemiluminescence (ECL) for the detection of a wide range of analytes with much higher sensitivity than fluorescence methods,[2] the ECL of QDs has attracted great interest during the last 4 years.[3a] Since the first work on the ECL of silicon QDs was reported in 2002,[3b] great efforts have been focused on the ECL behavior and mechanism of QDs dispersed in organic solutions to examine the relationship between the ECL and the photoluminescence of QDs.[3] However, the instability of the generated radical species of QDs during the annihilation steps and the air-free and aqueous-free working conditions restrict their further applications in biosensing.[3c] Although the ECL phenomena of CdS spherical assemblies or nanotubes and Si QDs in aqueous solutions have been observed in later work,[4] the rigorous alkaline media (around pH 13) become a stubborn obstacle when QDs are incorporated with biomolecules.

Until recently a considerable cathodic ECL signal of QDs cast on a paraffin impregnated graphite electrode (PIGE) was obtained at physiological pHs in this laboratory,[5] which could be applied in H_2O_2 sensing. Subsequently, carbon nanotubes (CNT) have been used to enhance the ECL of CdS QDs film by reducing the injection barrier of electrons to the QDs.[6] However, to the best of our knowledge, no detection using the ECL of QDs for molecules other than peroxide has been developed, possibly due to the difficulty in preparing a biofunctionalized QDs film to obtain a sensitive and stable ECL signal.[7] This work thus develops a promising method by incorporating thioglycolic acid (TGA)-capped CdSe QDs with oxidases. The biocompatible shell structure improves greatly the stability of the coimmobilized biomolecules. With glucose oxidase (GOD) as a model the constructed enzyme–QDs architecture shows excellent analytical performance for ECL detection of glucose with low cost and sensitive, rapid and reproducible response.

TGA-capped CdSe QDs synthesized in an aqueous system[8] were selected as the ECL emitters. The sizes of the QDs could be tuned by simply varying the reflux time in a batch.[8] Both the size and concentration of the obtained QDs could be estimated from the UV–vis absorption spectrum by Peng's empirical equations.[9]† The QDs solution showed a photoluminescence peak at 574 nm, which was coincident with the luminescent peak-size relationships for reported CdSe QDs.[1] The presumed structure of QDs could be proposed as the excess TGA anions cover a cadmium-enriched CdSe core.[8]

The QDs films were prepared by casting equimolar QDs on PIGE with different sizes. The effects of QDs size on the ECL potentials and efficiency have been studied, and the QDs of 2.5 nm in size and 0.021 mmol L^{-1} in concentration showed the strongest ECL.† When the QDs film was immersed in the detection solution, *i.e.*, oxygen saturated pH 7.0 PBS containing 0.1 mol L^{-1} KNO_3, intense cathodic ECL could be observed at about −1.1 V accompanied by a shoulder peak near −1.5 V during the cyclic voltammetry scan (curve d in Fig. 1), while the bare electrode did not show an observable signal (curve c in Fig. 1). So these peaks were considered as the light emission by the lumophores generated from individual QD species and QD assemblies, respectively.[5,10] It was notable that an electrochemical peak appeared at about −0.9 V to produce reduced QDs (inset in Fig. 1), where ECL began to increase sharply due to the electron transfer reaction between the reduced QDs and dissolved oxygen to form excited

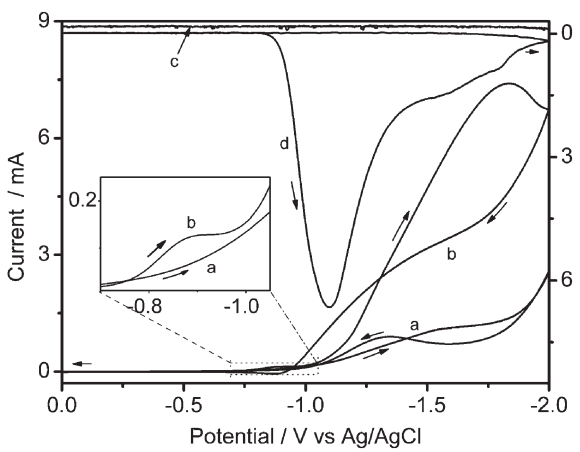

Fig. 1 Electrochemical (a, b) and ECL curves (c, d) at bare (a, c) and QDs film modified electrodes (b, d) in oxygen saturated pH 7.0 PBS. Inset: the amplification of the electrochemical peak at about −0.9 V.

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state species of QDs. The ECL peak potential at −1.1 V was more positive than those of bare CdSe[5] and CNT-CdS (both at about −1.2 V),[6] due to the low onset reduction potential of thiol capped CdSe QDs. This ECL peak was the indicator of detection in the following designs.

The preparation of enzyme–QDs architecture was performed by casting in succession GOD and QDs in appropriate amounts onto a PIGE. The GOD layer was first cast on a PIGE. After solvent evaporation the QDs solution was dropped on the formed film (Fig. 2), a portion of the immobilized GOD could be incorporated into the formed QDs layer due to its solubility in the coating solution, and some QDs could also be implanted in the GOD layer due to their great density. The formed GOD–QDs architecture measured by electrochemical impedance spectroscopy (EIS) showed a charge transfer resistance (R_{ct}) value of only 0.3 kΩ, which was much lower than that of the film made of the QDs–GOD bioconjugate (~7.4 kΩ).† The relative fine conductivity enabled fast electron transfer between the QDs and the electrode surface, which was a key point in obtaining efficient ECL.[7] As predicted, enough strong ECL emission could be obtained from the architecture, which provided the possibility of further applications. This architecture was rather stable. The semi-encapsulation of GOD by QDs also provided a convenient entrance for oxygen molecules.

The SEM image of bare PIGE displayed a predictably uneven surface (Fig. 2A). The coating of QDs solution on the PIGE produced a layer of QDs with an aggregate size less than 50 nm (Fig. 2B), which was smaller than those of 50–300 nm for unmodified CdSe QDs[5] due to the repulsion among these negatively charged (TGA^{2-}) capped CdSe QDs. The decline in aggregation was favorable for improving the ECL efficiency. The coating of GOD on PIGE showed obvious aggregation of enzyme molecules (Fig. 2C). After the casting of QDs on the GOD layer, the SEM image indicated both the QDs and the GOD molecules in the fabricated architectures could partly implant each other by precipitation and dissolution (Fig. 2D). The presence of GOD

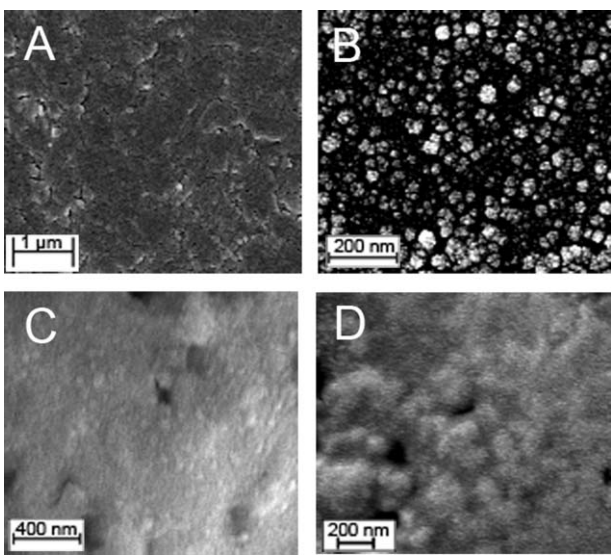

Fig. 2 SEM images of A) bare, B) QDs film, C) GOD film, and D) GOD–QDs architecture modified PIGE. The amount of deposition was 5 μg for GOD and 0.3 nmol for QDs.

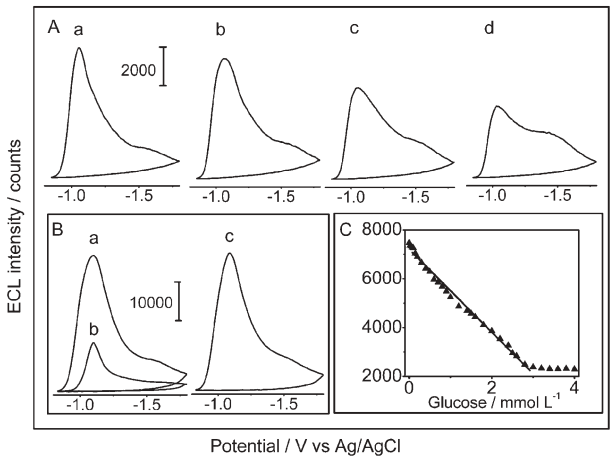

Fig. 3 Detection of glucose based on the decrease in ECL signal: (A) ECL of GOD–QDs architecture upon additions of 0 (a), 0.2 (b), 1.0 (c), and 1.8 (d) mmol L^{-1} glucose in the detection solution; (B) ECL of QDs film in the oxygen saturated detection solution (a), the deaerated detection solution containing 300 μmol L^{-1} H_2O_2 (b) and (a) + 5.0 mmol L^{-1} glucose (c), respectively; (C) Calibration plot for detection of glucose. The ECL counts were detected with the photomultiplier tube biased at −800 V.

molecules made QDs well dispersed. This resulted from the fact that the GOD molecules (pI ~ 4.2) were negatively charged at neutral pH, thus blocking the aggregation of the anion-capped QDs *via* electrostatic interaction.

Although both oxygen and hydrogen peroxide can act as the ECL coreactants,[5,10] the efficiencies of the two coreactants are different, since O_2 can capture more electrons from electrochemically reduced QDs than H_2O_2 and their reaction rates with the reduced QDs also differ. Curves a and b in Fig. 3B show the discrepancy of ECL signals enhanced by oxygen and peroxide by immersing the QDs film in the detection solution or deaerated detection solution containing 300 μmol L^{-1} H_2O_2, which was much larger than the saturated concentration of dissolved oxygen at room temperature (20 °C) under standard atmospheric pressure. As a consequence, the dissolved oxygen is a more efficient coreactant to enhance the ECL emission of QDs.

In a typical GOD catalytic cycle, the dissolved oxygen is consumed to produce equimolar hydrogen peroxide. According to the above description, the ECL response of GOD–QDs film should decrease upon addition of glucose, as observed from curves a–d in Fig. 3A. As a control, the addition of glucose did not change the ECL intensity of QDs film (curves a and c in Fig. 3B), indicating that the change in ECL response of GOD–QDs film resulted entirely from the GOD catalytic cycle. The concise ECL pathways in the presence of glucose can be expressed as follows (eqn. (1)–(6)):[5]

$$\text{CdSe/Cd(TGA) QDs} + ne \rightarrow nR^{\bullet -} \quad (1)$$

$$\text{Catalyzed by GOD: Glucose} + O_2 \rightarrow \text{Gluconate} + H_2O_2 \quad (2)$$

$$\text{For oxygen: } O_2 + 2R^{\bullet -} + 2H^+ \rightarrow 2R^* + H_2O_2 \quad (3)$$

$$H_2O_2 + 2R^{\bullet -} \rightarrow 2R^* + 2OH^- \quad (4)$$

$$\text{For peroxide: } H_2O_2 + 2R^{\bullet -} \rightarrow 2R^* + 2OH^- \quad (5)$$

$$\text{Then } R^* \rightarrow R + h\nu \quad (6)$$

The decrease in ECL response of GOD–QDs film upon addition of glucose provided the opportunity for glucose biosensing. The sensitivity of the glucose ECL biosensor depended on the amounts of both GOD and QDs contained in the architecture as well as the pH of the detection solution. The optimal amounts of GOD and QDs were 5 μg and 0.3 nmol, respectively. Considering a compromise between the linear range and the ECL sensitivity, pH 7.0 was used for the detection. The cyclic scan rate was also not a negligible parameter since the ECL efficiency was intensively dependent on the rate of generation/annihilation of the excited state R* during the electron transfer process. With the increasing scan rate the ECL intensity increased and tended to a plateau at the scan rate of 400 mV s^{-1}, indicating the emitted photons reached saturation. This rate was used in the following detection.†

Under optimal conditions the linear range for glucose was from 25.0 μmol L^{-1} to 3.0 mmol L^{-1} (Fig. 3C, R = 0.995) with a detection limit of 4.0 μmol L^{-1} at an S/N of 3. The limit of detection was much lower than those for most of the ECL glucose biosensors reported previously[11] and close to that of 3 μmol L^{-1} by an electrochemical method on a chitosan–carbon nanotubes electrode.[12] A series of six parallel measurements of 2.0 mmol L^{-1} glucose yielded a relative standard deviation (RSD) of 2.4%, and the ECL detected with five biosensors fabricated independently gave an RSD of 8%, indicating good reproducibility of this protocol. The scan time per detection was 15 s, thus it allowed rapid sample measurements. The proposed glucose ECL sensor showed good storage stability. 94% of the original ECL signal could be retained after 30-day storage in pH 7.0 PBS at 4 °C. Common inorganic and organic molecules hardly interfered with the detection. The successful serum sample examination showed that the protocol is promising for applications in clinical diagnostics.†

In summary, this work proposes a simple strategy for fabrication of the first architecture of QDs coimmobilized with enzyme. Using GOD as a model enzyme the biosensor for oxidase substrate is constructed by incorporating GOD in TGA-capped CdSe QDs film. The intrinsic cathodic ECL of the QDs can be applied as the indicator of enzymatic processes of oxidases for detection of their substrates. The architecture shows a sensitive ECL response to glucose in a wide linear range. The proposed ECL sensor shows satisfying reproducibility and acceptable stability. This strategy may be available in more exciting systems.

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