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Short communication

Electrogenerated chemiluminescence of blue emitting ZnSe quantum dots and its biosensing for hydrogen peroxide

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ABSTRACT

A novel method for the detection of hydrogen peroxide (H_2O_2) has been developed, based on electrogenerated chemiluminescence (ECL) of blue emitting ZnSe quantum dots (QDs) in aqueous solution. The weak ECL emission of ZnSe QDs was generated when the electrode potential was scanned from 0.0 to −1.4 V (versus Ag/AgCl), and the ECL intensity was greatly enhanced in the presence of H_2O_2 or dissolved oxygen. Under the optimal conditions, the dynamic range is from 6.10×10^{-7} to 3.10×10^{-4} M H_2O_2, and the detection limit (at $S/N = 3$) is 2.00×10^{-7} M. The relative standard deviation (at 5.00×10^{-5} M of H_2O_2) is 2.70% ($n = 11$). A possible reaction mechanism of ECL of ZnSe QDs was also investigated. As a novel biosensor, it was used to detect H_2O_2 in the pig kidney (pk-15) cell, vero cell and mineral water, respectively. Moreover, the proposed biosensor showed a good reproducibility and potential application in real samples.

1. Introduction

Hydrogen peroxide (H_2O_2) is a very important intermediate in biological reactions. The level of H_2O_2 in living cells is associated with the normal physiologic functions. Therefore, the analysis of H_2O_2 is of great importance in cell biology field. At present, several methods were used to detect H_2O_2, such as spectrophotometric method [Matos et al., 2006], colorimetric assay [Wu et al., 2007a,b], electrochemical sensor [Tong et al., 2007] and chemiluminescence (CL) biosensor [Chen et al., 2009], and so on.

Compared with CL biosensor, electrogenerated chemiluminescence (ECL) sensor has both benefits of electrochemistry and CL. Some ECL reagents, including organic compounds, metal complexes and quantum dots (QDs), can be used to produce ECL emission [Fang et al., 2008]. Among them, ECL sensor of QDs has size-dependent and surface structure-dependent ECL properties [Hua et al., 2009; Jie et al., 2007]. In 2004, Ju and co-workers developed a H_2O_2 sensor for the first time based on the ECL of CdSe QDs [Zou and Ju, 2004]. After that, the ECL behavior of CdX (X = S, Se, Te) QDs has been extensively investigated [Chen et al., 2007; Jie et al., 2008; Han et al., 2007a]. However, CdX QDs are often liable to release Cd^{2+}, which is high cytotoxicity and often lead to significant cell death [Derfus et al., 2004]. Therefore, a research on the ECL of non-cadmium QDs is of interest in biological area.

ZnSe QDs, wide band gap II–VI semiconductor nanomaterials, have attracted significant amount of interests [Qian et al., 2006; Xu et al., 2008; Geng et al., 2007]. Due to their low cytotoxicity and intense blue luminescence properties, ZnSe QDs also show great potential applications in low-voltage electroluminescent devices, blue diode lasers and biolabeling materials [Andrade et al., 2009]. In this paper, we reported the ECL of low cytotoxicity and blue emitting ZnSe QDs in aqueous system. The ECL reaction mechanism of ZnSe QDs was investigated. As a novel ECL biosensor, ECL of ZnSe QDs was used to detect H_2O_2 in pk-15 cell, vero cell and mineral water samples, respectively, with satisfactory results.

2. Materials and methods

2.1. Chemicals

$Zn(NO_3)_2 \cdot 6H_2O$ (AR), $NaBH_4$ (96%) were purchased from Sinopharm Chemical Reagent Co. Ltd, China. Selenium Power (99.95%) was purchased from Shanghai Meixing Chemical Co. Ltd, China. Glutathion (GSH) was obtained from Biosharp Co., America. H_2O_2 was purchased from Shanghai Yuanda Feroxide Co. Ltd., China. The mineral water was obtained from Nongfu spring water, China. All other reagents were of analytical grade, and ultra pure water was used throughout. The pig kidney (pk-15) cell and vero cell were both obtained from State Key Laboratory of Agricultural Microbiology (China). Cells were scraped, sonicated by VC130PB ultrasonic processor (Sonics and Materials Inc., America) in ice-bath for 10 min at 20 kHz. The homogenate was centrifuged at 12000 g

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for 10 min. The pellet was discarded and the supernatant was collected and dispersed in PBS buffer (pH 7.4) for further experiments.

2.2. Instruments

The size and morphology of the ZnSe QDs were characterized by a JEM-2010FEF high-resolution transmission electron microscopy (HR-TEM) (JEOL Ltd., Japan). ECL of ZnSe QDs was performed by Model MPI-B electrochemiluminescence analytical systems (Xi'An Remax Electronic Science & Technology Co. Ltd., China). A conventional three-electrode system (a glassy carbon electrode as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl electrode as the reference electrode) was used. The voltage of photomultiplier tube (PMT) was set at −900 V during the whole process of detection. The ultraviolet and visible (UV–vis) absorption spectrum was obtained with 1.0 cm × 1.0 cm-quartz cuvette on a Model evolution 300 (Thermo Nicolet Corporation, America). Fluorescence (FL) spectra were recorded by a LS-55 FL spectrometer (PerkinElmer Corporation, America) equipped with a 20 kW xenon discharge lamp as a light source. An X-ray diffraction (XRD) pattern was derived by using a Rigaku D/MAX-rA diffractometer (Rigaku Corporation, Japan) with Cu Kα radiation (λ = 1.5418 Å).

2.3. Preparation of GSH-capped ZnSe QDs

GSH-capped ZnSe QDs were synthesized according to the method reported by Zheng et al. [2007]. Briefly, 23 mg of sodium borohydride, 15.8 mg selenium powder and 5 mL of ultrapure water were transferred to a small flask in ice-bath. After reacting for 1 h, the black selenium powder disappeared and NaHSe precursor was produced. Meanwhile, the pH value of the zinc nitrate solution in the presence of GSH at N_2 atmosphere was adjusted to 11.0 with 0.1 M NaOH. Then, the NaHSe precursor was added to the above mixture, and the molar ratio of Zn^{2+}: Se^{2-}: GSH was fixed at 5: 2: 6. Keep heating at 95 °C for 30 min, GSH-capped ZnSe QDs could be obtained.

3. Result and discussion

3.1. HR-TEM images of blue emitting ZnSe QDs

Fig. 1 shows the HR-TEM image and size distribution histogram of as-prepared GSH-capped ZnSe QDs. The results indicate that ZnSe QDs are monodisperse and uniform, and the average particle size is 2.2 nm.

3.2. ECL behavior of blue emitting ZnSe QDs

ECL of blue emitting ZnSe QDs was investigated as shown in Fig. 2A. Under the optimum conditions (ZnSe QDs concentration: 2 mM; potential window: 0 to −1.4 V; scan rate: 300 mV/s; 50 mM PBS, pH 7.4), a significant ECL peak of ZnSe QDs was observed around −1.3 V (curve c, Fig. 2A). It indicated that ZnSe QDs had a high ECL ability in aqueous solution. In contrast, ECL emission was not found in the blank solution without ZnSe QDs (curve a, Fig. 2A), suggesting that the presence of ZnSe QDs was necessary to observe the ECL emission. Moreover, the dissolved oxygen, H_2O_2 and NaOH could enhance the ECL intensity of ZnSe QDs (Fig. 2A).

3.3. The possible ECL mechanism of blue emitting ZnSe QDs

Based on the above results and some references [Zheng et al., 2007; Bae et al., 2004; Han et al., 2007b; Myung et al., 2002], a possible reaction mechanism for the ECL of ZnSe QDs is suggested as follows:

$$QDs + e \rightarrow QDs^- \quad (1)$$

$$O_2 + H_2O + 2e \rightarrow OOH^- + OH^- \quad (2)$$

$$2QDs^- + OOH^- + H_2O \rightarrow 3OH^- + 2QDs* \quad (3)$$

$$QDs* \rightarrow QDs + h\nu \quad (4)$$

Some ECL emission was often generated through a so-called preannihilation mechanism, which was produced from the reaction between the reduced state or oxidized state and coreactions [Myung et al., 2002]. In our experiments, only reduced species were produced (Eq. (1)) when the electrode potential was scanned from 0.0 to −1.4 V. During the process, the dissolved oxygen could be reduced and its reduced product OOH^- was one of the coreactants which participated in the ECL reaction (Eqs. (2)–(4)).

When H_2O_2 was added to the ECL system of ZnSe QDs, it could make OOH^- under base condition (Eqs. (5)). The more OOH^- reacted with QDs^-, the more QDs* were produced, leading to the higher ECL intensity.

$$H_2O_2 + OH^- \rightarrow OOH^- + H_2O \quad (5)$$

During the ECL emission from ZnSe QDs, the surface state plays an important role. According to Bard's work [Bae et al., 2004], the passivation of surface traps is very important to obtain a highly efficient luminescence. In this work, the ECL enhancement of ZnSe

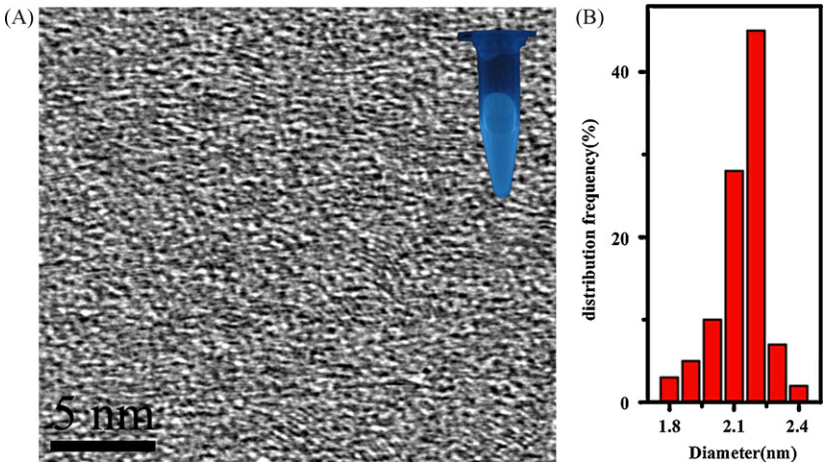

Fig. 1. (A) HR-TEM image and (B) size distribution histogram of the ZnSe QDs.

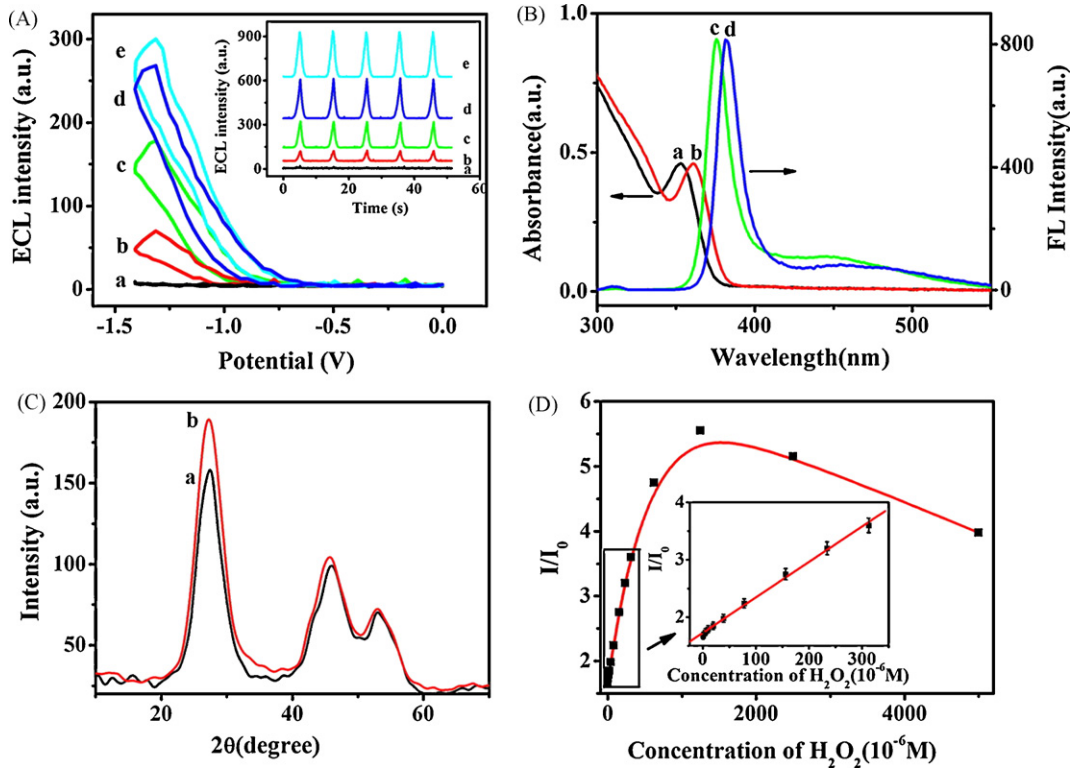

Fig. 2. (A) The ECL potential curve of (a) blank, (c) ZnSe QDs, (d) ZnSe QDs after add NaOH, ZnSe QDs after (e) add and (b) remove dissolved oxygen. Inset: The ECL time curve of (a) blank, (c) ZnSe QDs, (d) ZnSe QDs after add NaOH, ZnSe QDs after (e) add and (b) remove dissolved oxygen. (B) UV–vis absorption spectra of ZnSe QDs (a) before and (b) after adding NaOH; FL spectra of ZnSe QDs (c) before and (d) after adding NaOH. (C) XRD patterns of ZnSe QDs (a) before and (b) after adding NaOH. (D) Effect of H_2O_2 concentration on ECL of ZnSe QDs. Inset: Calibration curve for H_2O_2 with a correlation coefficient of 0.9985 ($n = 10$).

QDs after the addition of NaOH was possibly caused by the surface passivation. UV–vis, FL and XRD spectra were employed to study passivation mechanism. It can be seen that the absorption peak of ZnSe QDs in the absence (curve a, Fig. 2B) and presence of NaOH (curve b, Fig. 2B) occurred at 353 nm and 361 nm, respectively. Otherwise, the maximum FL peak of ZnSe QDs in the absence of NaOH (curve c, Fig. 2B) and presence of NaOH (curve d, Fig. 2B) was located at 376 nm and 382 nm, respectively. The red shift of the UV–vis and FL peaks suggested that the size of ZnSe QDs must be changed, which indicated a formation of ZnSe/$Zn(OH)_2$ core/shell structure. Fig. 2D shows XRD patterns of the as-prepared ZnSe QDs with (curve a, Fig. 2C) and without (curve b, Fig. 2C) NaOH. It can be seen that the three main diffraction peaks appeared at 27.2°, 45.9°, and 53.0°. Compared with the XRD peaks of the ZnSe QDs, the three main diffraction peaks of ZnSe/$Zn(OH)_2$ QDs were broadened. The slight broadening of the XRD line of ZnSe/$Zn(OH)_2$ QDs was probably caused by the covering of $Zn(OH)_2$. Based on the above results, after adding NaOH, the surface passivation of $Zn(OH)_2$ was the one that caused the ECL enhancement of ZnSe QDs.

3.4. Analytical application

H_2O_2 is the analyte of great importance in connection with many electrochemical biosensors [Lin et al., 2005; Wu et al., 2007a,b]. Based on the enhanced effect of H_2O_2 on the ECL of ZnSe QDs, a novel method for the detection of H_2O_2 was developed (Fig. 2D). The results showed that the ECL intensity was linearly increased along with the concentration of H_2O_2. The linear regression equation obtained from the calibration curve (Fig. 2D inset)

Table 1
Analytical results, recovery yields, and the 95% CIs of H_2O_2 in mineral water, pk-15 cell and vero cell, respectively. Each value is the average of ten measurements.

Samples	Add (10^{-6} M)	H_2O_2 found (95% CI) (10^{-6} M)	RSD (%)	*p*-value	Recovery (%)
Mineral Water	0	0	1.2	/	/
	25	25.09 (8.37, 41.82)	1.8	<0.01	100.37
	50	51.34 (34.82, 67.85)	2.4	<0.01	102.67
	100	104.08 (87.70, 120.47)	2.9	<0.01	104.08
	200	202.40 (185.22, 219.58)	1.8	<0.01	101.20
pk-15 Cell	0	0	1.5	/	/
	25	24.93 (8.20, 41.66)	2.3	<0.01	99.72
	50	49.72 (33.19, 66.25)	1.8	<0.01	99.44
	100	102.34 (85.95, 118.72)	2.3	<0.01	102.34
	200	203.81 (186.60, 221.01)	2.4	<0.01	101.90
Vero Cell	0	0	2.0	/	/
	25	25.58 (8.86, 42.30)	1.5	<0.01	102.30
	50	51.77 (35.26, 68.28)	2.5	<0.01	103.54
	100	103.94 (87.55, 120.32)	1.4	<0.01	103.94
	200	204.58 (187.36, 221.80)	2.6	<0.01	102.29

is $y = 0.0062x + 1.7284$ ($r = 0.9985$, $n = 10$), and the linear range is 6.10×10^{-7}–3.12×10^{-4} M. The limit of detection was 2.0×10^{-7} M at a signal-to-noise ratio of 3. Moreover, the reproducibility was estimated by replicate detection ($n = 10$) of a standard solution containing 5.0×10^{-5} M H_2O_2. The relative standard deviation (RSD) was estimated to be 2.7%. Compared with previously reported methods (Spectrophotometric method, Colorimetric assay, Electrochemical sensor and UV–vis Absorption Spectrometry) [Matos et al., 2006; Wu et al., 2007a,b; Tong et al., 2007; Xu et al., 2009], the proposed method had low limit of detection and wide linear range for the determination of H_2O_2.

The proposed ECL sensor was used to detect H_2O_2 in the pk-15 cell (1.0×10^5 cells), vero cell (1.0×10^5 cells) and mineral water samples, respectively, by the standard addition method. As shown in Table 1, the relative standard deviations (RSD) were all below 3.0% for ten successive measurements, which indicated that the proposed biosensor possesses a good reproducibility. The 95% confidence intervals (CI) did not overlap zero and the p-values were all below 0.01, thus, the effect had statistically significant. The recovery was preferable, indicating that the proposed method could be used for the determination of H_2O_2 in real samples.

4. Conclusion

In summary, we synthesized non-cadmium and low-toxicity ZnSe QDs, and developed a novel method for the determination of H_2O_2 based on the ECL of the as-prepared ZnSe QDs in aqueous solution. The proposed sensor had a high sensitivity, and was applied to detect H_2O_2 in pk-15 cell, vero cell and mineral water samples, respectively, with satisfactory results. This preliminary work would promote the ECL applications of biocompatibility QDs in bioassays.

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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.2009.12.021.

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