J Fluoresc (2011) 21:125–131
DOI 10.1007/s10895-010-0696-z

ORIGINAL PAPER

Determination of L-tyrosine Based on Luminescence Quenching of Mn-Doped ZnSe Quantum Dots in Enzyme Catalysis System

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Received: 29 March 2010 / Accepted: 22 June 2010 / Published online: 8 July 2010

Abstract In this paper, we attempted to develop a novel application of Mn-doped ZnSe quantum dots (Mn: ZnSe d-dots) as probes to detect L-tyrosine (L-Tyr). The bioconjugates of horseradish peroxidase (HRP)-conjugated Mn: ZnSe d-dots were used in the enzyme catalyzed reaction of L-Tyr with H_2O_2. Compared with traditional CdTe QDs, Mn: ZnSe d-dots have better biocompatibility and less negative impact on enzyme catalyzed system. In HRP-conjugated Mn: ZnSe-L-Tyr-H_2O_2 system, electron transfer occurred between Mn: ZnSe d-dots and HRP. It resulted in the luminescence quenching of the Mn: ZnSe d-dots., which can be used to detect L-Tyr. The coupling of efficient quenching of Mn: ZnSe d-dots photoluminescence (PL) and the effective enzyme-catalysis can afford a simple and sensitive method for L-Tyr detection. The Mn: ZnSe d-dots-enzyme catalyzed system displays great potential in the development of enzyme-based biosensing systems for various analytes.

Keywords Mn: ZnSe d-dots · Luminescence quenching · Horseradish peroxidase · L-tyrosine

Introduction

Over the past several years, the fundamental studies on nanoparticals have experienced huge development and a great widening of the fields of applications (e.g. catalysis [1], electronics [2], electrochemistry [3, 4], biology [5–7]). In particular, the assembly of nanoparticals and biomolecular complexes (e.g. nanostructure-conjugated DNA, proteins and enzymes) as nanosensors for biological analysis and applications has become a hot research field [8–10]. Semiconductor nanoparticles (or quantum dots, QDs), with their unique properties such as good optical characteristics, large surface-to-volume ratio, high electron-transfer efficiency, biocompatibility, dimensional similarities with biological macromolecules and high surface reaction activity have been favorably adopted as potential materials in enzyme-based biosensing systems [11–13]. CdTe quantum dots (q-dots) have been widely explored as biomedical labeling reagents [14, 15]. But some results indicate that any leakage of cadmium from the CdTe semiconductor nanoparticles would be toxic and fatal to a biological system [16]. Moreover, cadmium-containing products are eventually environmentally problematic. Mn-doped ZnSe quantum dots (Mn: ZnSe d-dots) have low toxicity by replacing cadmium in CdTe quantum dots with zinc and d-dot emitters can also overcome a couple of intrinsic disadvantages of q-dot emitters, that is, strong self-quenching caused by their small ensemble Stokes shift and sensitivity to thermal, chemical, and photochemical disturbances [17–21].

Owing to rapidity and high selectivity, enzymatic assays have been widely used in biochemistry fields and practical analytical applications, such as diagnostic kits and enzyme immunoassays. Horseradish peroxidase (HRP) is one of the most important enzymes obtained from a plant source [22] and has been introduced into various fields such as biotechnological applications [23], biosensors [24, 25] and synthesis [26]. The horseradish peroxidase (HRP)-catalyzed reaction is one of the most widely used enzymatic reactions in bioanalytical chemistry [27–30]. Now it is systematically studied with H_2O_2 as an oxidizing agent and various substances as substrates [27]. L-tyrosine is one of the hydrolysates of protein and mostly obtained by extraction

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from protein hydrolysates [31]. It is used as a dietary supplement and can represent an important raw material for the synthesis of the anti-Parkinson's disease 3, 4-dihydroxyphenyl-L-alanine, L-dopa [32]. Moreover, L-tyrosine is a precursor of p-hydroxycinnamic acid, a valuable monomer for the production of liquid crystal polymers, which can be used for electronic applications [33].

In this paper, we attempted to investigate the effects of different kinds of QDs in enzyme catalyzed system and constructed a simple and sensitive detection method for L-tyrosine with the new non-cadmium Mn: ZnSe d-dots. Mn: ZnSe d-dots and CdTe QDs were prepared in aqueous solution and introduced directly into the bio-reaction system after their conjugation with HRP via electrostatic force, respectively. Based on electrons transfer from QDs to enzyme (HRP) in redox process, L-Tyr can be detected via the luminescence quenching of the QDs. The influences of the amounts of HRP on the sensitivity of Mn: ZnSe d-dots-HRP biosensing system was also investigated in this work. Furthermore, the spectral characteristics of bio-conjugates of HRP-conjugated QDs and the optimum reaction conditions were also investigated.

Experimental

Reagents

All chemicals used were analytical reagent grade or the higher grade without further purification. The water used in all experiments had a resistivity higher than 18 MΩ cm^{-1}. Mercaptosuccinic acid (MSA) (99%) was purchased from J&K Chemical Co.. Tellurium powder (~200 mesh, 99.8%), $CdCl_2 \cdot 2.5H_2O$ (99%), $NaBH_4$ (99%), mercaptopropionic acid (MPA) (99%), selenium powder (~200 mesh, 99.9%), $Zn(NO_3)_2 \cdot 6H_2O$ (99.9%) and $MnCl_2 \cdot 4H_2O$ (99.9%) were purchased from Sigma-Aldrich Chemical Co.. Avidin labeled HRP (store: −20 °C) and L-tyrosine (purity >99%) were purchased from Beijing Dingguo Biotechnology Co. Ltd., China. H_2O_2 (30%, A.R.) was purchased from Beijing Chemical Works. Stock solutions of HRP and L-tyrosine were prepared with 2 mmol/L phosphate buffered saline solution (PBS, pH 7.4), having final concentrations of 10% (*v/v*) and 1 mg/mL, respectively. The above solutions were all stored at 0–4 °C and diluted with PBS to the concentrations used only immediately prior to use. H_2O_2 solution (3.0×10^{-4}% (*v/v*)) was prepared just before using in the experiments.

Apparatus

The photoluminescence (PL) spectrum and intensity were recorded using a Shimadzu RF-5301 PC spectrofluorometer equipped with a xenon lamp using right-angle geometry and a 1.0 cm quartz cell. All optical measurements were carried out at room temperature under ambient conditions. A bath ultrasonic cleaner (Autoscience AS 3120, Tianjin, China) was used to fully mix the solution. All pH measurements were made with a Delta 320 pH meter (Hangzhou, China).

Synthesis of water-compatible CdTe QDs and Mn: ZnSe d-dots

Water-compatible CdTe QDs used in this study were synthesized by refluxing routes with mercaptosuccinic acid (MSA) as stabilizer [34]. Briefly, sodium hydrogen telluride (NaHTe) was produced in an aqueous solution by the reaction of sodium borohydride ($NaBH_4$) with tellurium powder at a molar ratio of 2:1 at first. Later, fresh NaHTe solution was added to 1.25 mM N_2-saturated $CdCl_2$ solution at pH 11.2 in the presence of mercaptosuccinic acids (MSA) and the molar ratio of Cd^{2+}/MSA/HTe^- was fixed at 1:1.5:0.2. The CdTe precursor solution was then subjected to a reflux at 100 °C under open-air conditions with condenser attached. Stable water-compatible MSA-capped CdTe QDs with PL emission wavelength at 590 nm were obtained and used in the present experiments.

Mn: ZnSe d-dots were prepared in aqueous solution by nucleation doping method [35]. Generally, 0.2 mL $MnCl_2$ solution (1.25×10^{-2} M) and 90 mL H_2O were loaded into a 250 mL three neck flask and degassed for 30 min by bubbling with nitrogen. Fresh NaHSe solution was added to N_2-saturated $MnCl_2$ solution at pH 11.2 in the presence of MPA as stabilizing agent. The reaction was then switched from nitrogen bubbling to nitrogen flow and subjected to a reflux at 100 °C for 40 min. 10 mL of the stock solution of $Zn(NO_3)_2$ (1.25×10^{-2} M) was injected and the reaction was refluxed for 5 h. Stable water-compatible MPA-capped Mn: ZnSe d-dots with PL emission wavelength at 565 nm were obtained and used in the present experiments.

Bio-conjugation of QDs with HRP and enzyme catalyzed reaction

The first step of the experiment is to fabricate the QDs (CdTe QDs or Mn: ZnSe d-dots)-HRP bio-conjugates. In a 5 mL centrifuge tube, 50 μL HRP (1% (*v/v*)) and 200 μL Mn: ZnSe d-dots (1.25 mM) or 25 μL CdTe QDs (10 mM) were added and mixed separately at room temperature with gentle agitation for 30 min. Avidin labeled HRP provides positively charged outer surface to facilitate the adsorption of the negatively charged QDs (CdTe QDs or Mn: ZnSe d-dots) capped with MSA or MPA, utilizing the electrostatic interaction between the negatively charged carboxy ($-COO^-$) groups on the surface of the QDs and positively charged tryptophan residues of avidin. After

conjugation, 50 μL L-Tyr (10.0 μg/mL) and 50 μL H_2O_2 (3.0×10^{-4}%, *v/v*) were transferred sequentially into the centrifuge tube and diluted to 2 mL with PBS (pH 7.4). Then, the mixture was incubated at 35 °C for 35 min. Finally, the photoluminescence (PL) emission spectra of the reaction system were measured.

Results and discussion

Spectra characteristics of QDs and QDs-HRP bio-conjugates

The PL emission spectra of CdTe QDs-HRP bio-conjugates are excited at 400 nm, and that of Mn: ZnSe d-dots-HRP bio-conjugates at 330 nm (Fig. 1). It can be seen that the maximum PL emission peak of the CdTe QDs slightly shifts red from 590 nm to 592 nm after its conjugation with HRP and for Mn: ZnSe d-dots, the maximum PL emission peak (565 nm) also slightly shifts red, which are attributed to the increase of the final sizes of the QDs (CdTe QDs or Mn: ZnSe d-dots)-HRP bio-conjugates after surface modification of HRP. The PL emission intensity of the QDs-HRP solutions increases gradually (11% for CdTe, and 20% for Mn: ZnSe) with the increasing concentrations of HRP, as shown in the Fig. 1. Flat slopes of PL emission intensity appear after the concentrations of HRP reached 0.025% (*v/v*), which is the saturated concentration for two kinds of QDs with the same concentration.

Effects of different kinds of QDs on the HRP-L-Tyr-H_2O_2 system

Two kinds of QDs (CdTe QDs or Mn: ZnSe d-dots) were conjugated with HRP respectively, and then the QDs-HRP bio-conjugates were introduced directly into the L-tyrosine-H_2O_2 reaction system. With the present of L-tyrosine and H_2O_2, electrons transfer from QDs to HRP contributed to the luminescence quenching of QDs. The electrons can further participate in the catalyzed reaction of H_2O_2 occurred on the surface of HRP [36]. Therefore, the novel assembled Mn: ZnSe d-dots-HRP biosensing system is based on the luminescence quenching process, caused by electrons-transfer from Mn: ZnSe d-dots to peroxidase (HRP), which catalyzed the oxidation/reduction reactions of L-tyrosine and H_2O_2.

Figure 2 shows the PL emission intensity changes of L-Tyr in different catalyzed system (Fig. 2 a–d) and the quenching effects of two kinds of QDs (Fig. 2 e–h). In the absence of QDs, the PL emission intensity of L-Tyr (ex/em= 275 nm/305 nm) decreases only 19.63% (Fig. 2 a and b), whereas the PL emission intensity of L-Tyr decreases

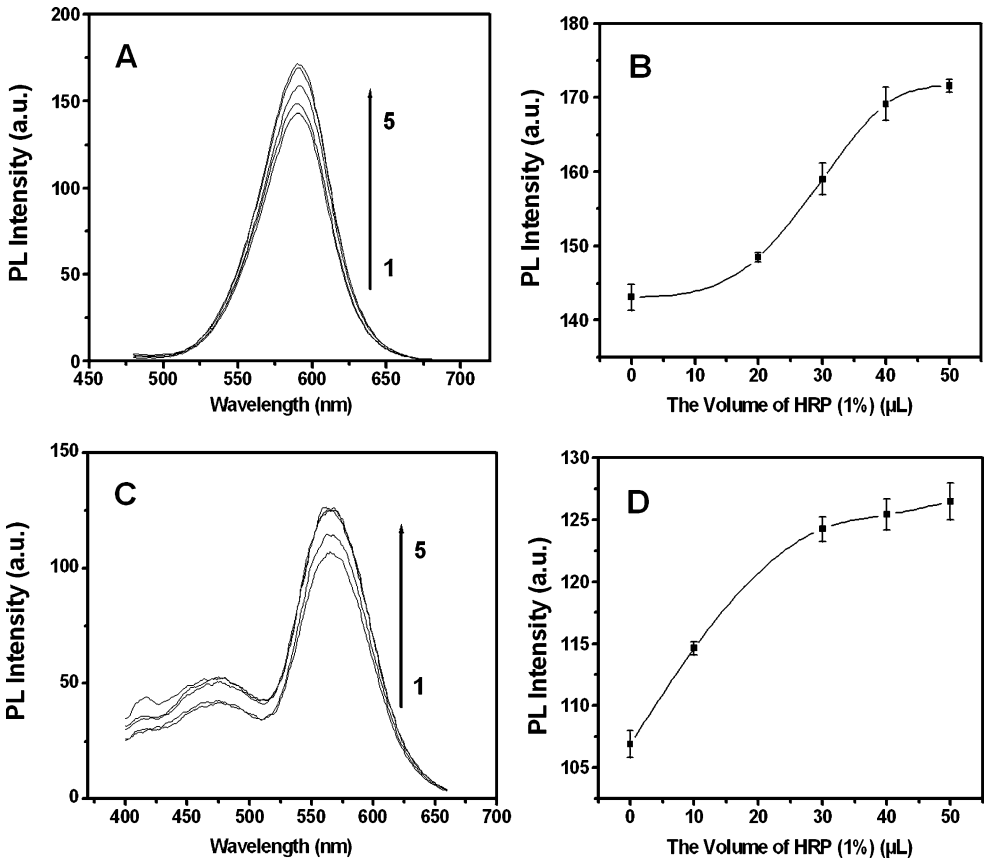

Fig. 1 **a** PL emission spectra of CdTe QDs-HRP catalyzed system. The volumes of HRP solution (1% (*v/v*)) added are 0, 20, 30, 40, and 50 μL (curve1–5). **b** The relationship between the PL intensity of CdTe QDs-HRP catalyzed system and the volume of HRP solution added. **c** PL emission spectra of Mn: ZnSe -HRP catalyzed system. The volumes of HRP solution (1% (*v/v*)) added are 0, 10, 30, 40, and 50 μL (curve1–5). **d** The relationship between the PL intensity of Mn: ZnSe -HRP catalyzed system and the volume of HRP solution added

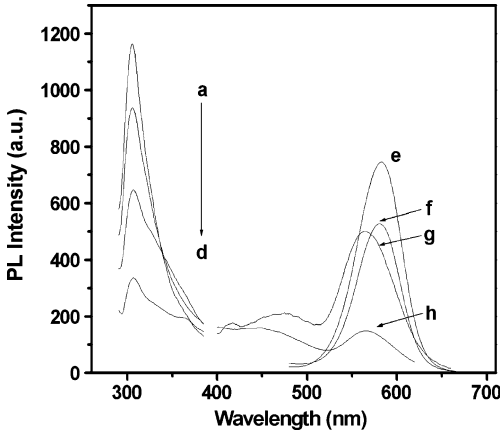

Fig. 2 PL emission spectra of L-Tyr (*a*), in the HRP catalyzed system (*b*), and in the QDs-HRP catalyzed system, CdTe QDs (*c*), Mn: ZnSe d-dots (*d*). PL emission spectra of CdTe-HRP (*e*) and Mn: ZnSe-HRP (*g*) in the absence of L-Tyr. PL emission spectra of CdTe-HRP (*f*) and Mn: ZnSe-HRP (*h*) in the presence of L-Tyr. The QDs-HRP catalyzed system includes 0.125 mM QDs, 50 μL HRP solution (1% (*v*/*v*)), 50 μL L-Tyr solution (10.0 μg/mL), and 50 μL H_2O_2 solution (3×10^{-4}% (*v*/*v*))

44.46% with CdTe QDs labeled HRP (Fig. 2 c) and decreases 71.15% with Mn: ZnSe d-dots labeled HRP (Fig. 2 d), which is attributed to the increased surface area of QDs, which can provide a better matrix for the immobilization of more HRP molecules. The combination of HRP to the surface of nanoparticles can improve the HRP-substrate interaction effectively by avoiding the potential aggregation between free HRP molecules, which would lead to the enhancement of enzymatic activity [37, 38]. Furthermore, the HRP attached on nanomaterial surface can reduce protein molecule unfolding and turbulence to ensure stability of enzyme [39]. It is also clearly shown in Fig. 2 that Mn: ZnSe d-dots are more efficient than CdTe QDs from the point of view of the catalysis of QDs labeled-HRP. The PL emission intensity of CdTe QDs decreases 29.20% in the HRP catalyzed system (Fig. 2 e and f), while the PL emission intensity of Mn: ZnSe d-dots decreases 70.27% (Fig. 2 g and h). This is due to the different components of QDs. Because of no cadmium-containing, Mn: ZnSe d-dots are better biocompatible than CdTe QDs, and have little negative impact on enzyme catalyzed system. Therefore, non-cadmium doped Mn: ZnSe d-dots were employed in the further experiments.

There are some advantages for Mn: ZnSe d-dots used in this study. Firstly, many proteins have autofluorescence signals around the range of 300 nm, which will interfere with the determination of L-tyrosine [40]. Secondly, QDs with their interesting properties such as photo and chemical stability, high sensitivity, high catalytic efficiency, and high surface reaction activity have been favorably adopted as potential materials to play a catalytic role in enzyme-based biosensing systems [11–13]. Moreover, this study aims at constructing a method based on efficient PL quenching of QDs and the effective enzyme-catalysis. Therefore, the detection of PL intensity changes of Mn: ZnSe d-dots in the HRP catalyzed system is superior to direct determination of luminescence signals of L-tyrosine. In addition, the Mn: ZnSe d-dots-enzyme catalyzed system can be further applied to other analytes without luminescence signals.

Optimization of the general procedure

A series of experiments were carried out to optimize pH, ionic strength, incubation temperature, and reaction time. Enzymatic activity was monitored by the decrease of QDs photoluminescence signal. The effect of pH in the range 6.0–8.5 on the efficiency of luminescence quenching was studied (Fig. 3). When pH value is lower than 6.5, QDs will become unstable in the aqueous environment. So the PL emission intensity of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system at 565 nm increases gradually from pH 6.0 to 6.5. And then the PL emission intensity tends to decrease in the range of 6.5–8.0 in the catalyzed reactions. When the pH value is too high (>8.0), the HRP is prone to denaturalization and lose catalyzed activity. An appropriate pH value of 7.4 was used in the further experiments.

In the above mentioned studies, the experiments were carried out in a low concentration of PBS buffer solution (2 mmol/L). To examine the influence of higher ionic strength on the PL of Mn: ZnSe-HRP-L-Tyr-H_2O_2 system, the PL emission spectra of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system in different salt concentrations (NaCl) were recorded (Fig. 4). It can be seen that the PL emission intensity of Mn: ZnSe-HRP at 565 nm decreases sharply with the increase of ionic strength until the salt concentration reaches 0.003 mol/L. At high ionic strength condition (0.01–0.05 mol/L), the PL emission intensity could not be quenched effectively. It indicated that ionic strength has a

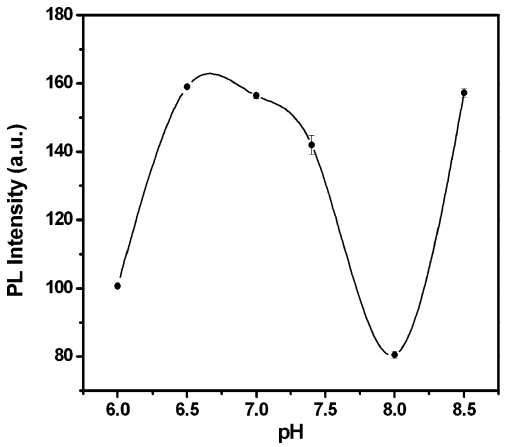

Fig. 3 The effect of pH on PL intensity of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system at 565 nm

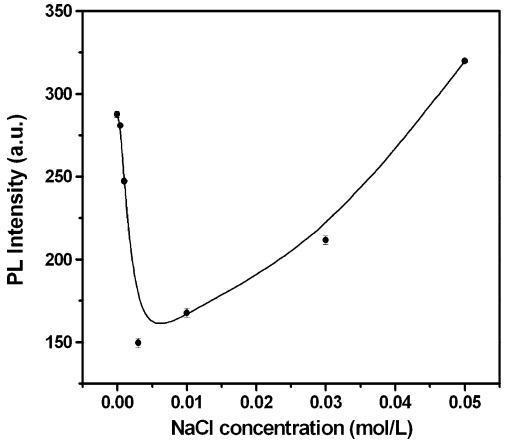

Fig. 4 The effect of ionic strength on PL intensity of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system at 565 nm

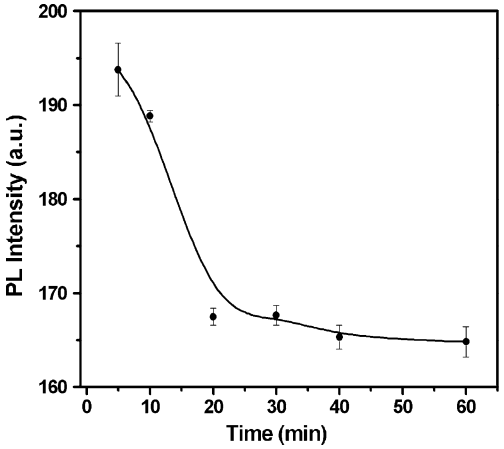

Fig. 6 The effect of reaction time on PL intensity of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system at 565 nm

great effect on the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system. This phenomenon is closely associated with the suppression of the electric double-layer of enzyme (HRP) in the presence of ionic strength. The appropriate ionic strength can decrease the bilateral electrostatic repulsion of enzyme and improve the interaction. Excessive salt makes an increase in the hydratability of enzyme and depresses the binding affinity. So PBS buffer solution with 3.0 mmol/L NaCl was chosen in this study.

The effect of incubation temperature on PL emission intensity of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system was also studied (Fig. 5). The PL emission intensity of the system at 565 nm decreases gradually with the temperature changed from 10 °C to 35 °C. However, the PL emission intensity begins to increase with the further increase of the temperature. The enzyme (HRP) provides bioactivity in a suitable temperature range, and will lose activity at too low or too high temperature conditions. The optimal incubation temperature in this study was chosen at 35 °C.

In order to study the dynamics of enzyme-catalyzed reaction, the effect of reaction time on PL emission intensity of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system was also investigated. And the results were shown in Fig. 6. The PL emission intensity of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system sharply decreases with the increase in reaction time until 30 min, while no major change is observed after that. Thus, 35 min was adopted as the suitable reaction time in this study in order to make reaction complete.

Calibration curves

On the basis of the luminescence quenching method, we performed the sensitive determination of L-tyrosine by using the assembled Mn: ZnSe d-dots-HRP biosensing system. The quenching process was based on the transfer of electrons from the QDs to enzyme (HRP), which catalyzes the oxidation/reduction reactions of L-tyrosine and H_2O_2. The more L-tyrosine was catalyzed, the more PL quenching

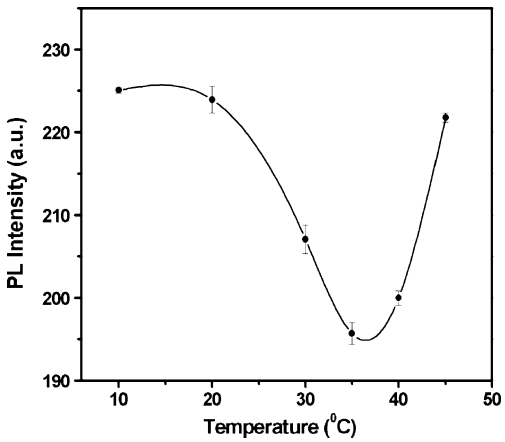

Fig. 5 The effect of incubation temperature on PL intensity of the Mn: ZnSe-HRP-L-Tyr-H_2O_2 system at 565 nm

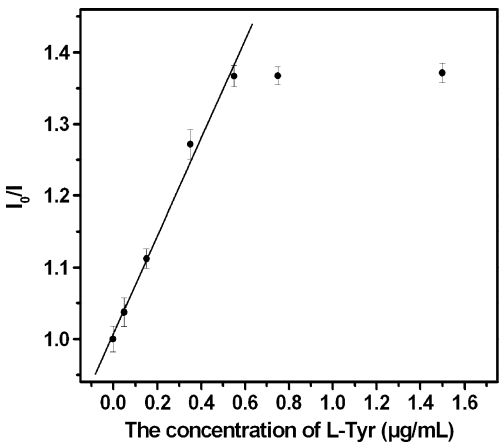

Fig. 7 Calibration curves and the detection limit

of the QDs was observed. In this study, the amount of H_2O_2 was excess to ensure the detection of total L-tyrosine. In the presence of 50 μL HRP solution (1% (*v/v*)) and 50 μL H_2O_2 solution (3×10^{-4}% (*v/v*)), the detection of L-tyrosine can be achieved within a certain range (Fig. 7). Under optimal experimental conditions, the relation between the concentration of L-tyrosine and the PL emission intensity was shown in Fig. 7. The results show that the PL emission intensity decreases almost linearly with the concentration of L-tyrosine. The linear regression equation is as follows:

$$I_0/I = 1.00738 + 0.683C_{L-Tyr}(\mu g/mL) \quad (1)$$

where I_0 and I are the PL emission intensity of Mn: ZnSe d-dots-HRP biosensing system in the absence and presence of L-tyrosine. The coefficient of correlation is 0.995. The linear dynamic range for L-tyrosine is 0.05–0.55 μg/mL, and the detection limit of L-tyrosine is as low as 0.01 μg/mL. The results validate the feasibility of luminescence quenching method based on Mn: ZnSe d-dots-HRP biosensing system. This new assembled Mn: ZnSe d-dots-HRP biosensing system can be applied to the detection of L-tyrosine with high sensitivity and simplicity.

Conclusion

In summary, we introduced two kinds of QDs (Mn: ZnSe d-dots and CdTe QDs) into the enzyme-catalyzed system. In contrast to CdTe QDs, Mn: ZnSe d-dots showed excellent characteristics such as excellent biocompatible, high sensitive and efficient enhancement of enzymatic activity on HRP catalyzed system [35]. We assembled a novel Mn: ZnSe d-dots-HRP biosensing system for L-tyrosine detection. Electron transfer occurred during the QDs-HRP-catalyzed oxidation of L-tyrosine, which can quench the photoluminescence of Mn: ZnSe d-dots. The proposed method provides an approach for the assembly of Mn: ZnSe d-dots with other redox enzymes to realize enhanced enzymatic activity, and to further the design of novel nanosensors applied in biological systems in the future.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (No. 20075009, No. 20875036) and the Development Program of the Ministry of Science and Technology of Jilin Province, China (No. 20080544).

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