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Short communication

Enzyme biosensor based on NAD-sensitive quantum dots

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ABSTRACT

A novel quantum dots (QDs) based biosensor has been developed to detect the activity of lactate dehydrogenase (LDH) by the change of fluorescence intensities of the QDs. In this system, the fluorescence intensities of the QDs are quenched by nicotinamide adenine dinucleotide (NAD, the coenzyme of LDH) first and then intensified with increasing amounts of the LDH because of the consumption of the NAD in the biocatalyzed reaction. A linear calibration plot of the activity of LDH is obtained in the wide amounts range from 150 to 1500 U/L and the detection limit is 75 U/L. Furthermore, the effect of several possible interfering substances, such as uric acid (UA), lactose, and glucose on the QDs-based biosensor is also investigated. This enzyme biosensor is of considerable interest due to its promise for simple procedure and the established method has great potential in detection of the physiological level of some biomolecules in clinical diagnostics of various diseases.

1. Introduction

Quantum dots (QDs) have gained increasing interest during the past decade due to their unique, novel properties and biomedical applications. As the optical properties of QDs strongly depend on the nature of the surface, the interaction between specific analytes and the surface of the QDs can result in dramatic changes in their optical properties (Gill et al., 2005; Deng et al., 2007). Recently, there are growing efforts have been directed to the application of QDs for the development of sensory systems based on the changes of fluorescence intensity due to the fluorescence resonance energy transfer (FRET), electron transfer (ET), or other interactions occurring at the QDs surface (Gill et al., 2008; Wang et al., 2009). Some articles have reported use of the fluorescence quenching or recovery of the QDs to assay glucose (Duong and Rhee, 2007; Huang et al., 2008; Yuan et al., 2008, 2009), nucleic acid hybridization (Algar and Krull, 2009; Feng et al., 2008), or some other biomolecules (Stoll et al., 2006; Freeman et al., 2009a,b). However, these analytic systems still have some deficiencies such as expensiveness, complicatedness, and require a long-term operation because most of QDs used in these systems need to be modified by different kinds of molecules as energy or electron donor. Moreover, few studies have concerned the selectivity of the biosensor based on the optical property of QDs.

In the biochemical analysis of human serum or urine, detection of the level of some important physiological species, such as glucose, cholesterol as well as some enzymes, is significant in clinical diagnostics of various diseases. For example, lactate dehydrogenase (LDH), as one of the nicotinamide adenine dinucleotide (NAD) dependent enzyme, plays an important role in diagnoses of diseases (Liang and Chan, 2007; Yin et al., 2009). Clinical research has revealed that the level of LDH in the environment of tissue cells is much higher than those of the normal ones if there are some physiological diseases (Sieber et al., 2004; Mok et al., 2008), such as congestive heart disease, hepatitis especially liver cancer. Thus, detecting of enzyme activity is more and more important in early diagnosis and postoperative monitoring of these diseases. A number of techniques have been developed for analysis of the activity of LDH, such as enzyme-linked immunosorbent assays (ELISA), colorimetric, fluorescence, or amperometric detection (Li et al., 2002; Hong et al., 2002; Zhao et al., 2009). Among these detected methods, optical analysis is the commercially available method for the evaluation of enzyme activities. However, most of those methods are expensive, time-consuming, and inadequate for in situ monitoring.

Herein, we investigated a fluorescent biosensor to quantitative analysis of the activity of LDH and its coenzyme NAD based on a water-soluble and stable CdTe/CdS QDs, which was synthesized by our simple chemical aerosol flow method (Li et al., 2009). Our work differed from the previous approach on the following points: (i) the CdTe/CdS QDs was synthesized in a rapid "one-step" process with large-scale production, and the QDs had high quantum yield and well stability. (ii) The analysis processing was very convenient and simple because QDs need not be modified by any special organic or biologic molecules. (iii) This biosensor showed low detection limit, wide linear detection range and good selectivity, so it had potential

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application in qualitative or quantitative analysis of LDH for early diagnosis of diseases. Moreover, NAD is well known as the important coenzymes for many enzymes during biocatalyzed reactions. So this optical analysis method could provide a generic way to analyze numerous NAD-dependent enzymes, as well as biocatalyzed reaction.

2. Experimental

2.1. Materials and methods

Lactate dehydrogenase (LDH, 30 U/mg) was purchased from Shanghai Kayon Biological Technology Company. Nicotinamide adenine dinucleotide (NAD) was from Roche Company. L-lactate (LL) was from Alfa Aesar Company. Uric acid (UA) and β-D Glucose were products of Sigma Company. D-Galactose was from Beijing Biodee Biotechnology Company. Phosphate buffers containing 0.1 M KCl consisted of KH_2PO_4 and Na_2HPO_4 (0.2 M, pH 7.0). All reagents were used without further purification and prepared with redistilled water.

The photoluminescence spectra were recorded with a Hitachi F-4500 fluorescence spectrophotometer. If not specially stated, the samples were excited at 450 nm, and the exciting slit and the emission slit were 5 and 5 nm, respectively. The optical properties of solution were measured using quartz cuvettes of 10 mm path length.

2.2. Synthesis of the QDs

The CdTe/CdS QDs used in this study were obtained via our research group developing the chemical aerosol flow method (Gao et al., 1998; Yu et al., 2003; Li et al., 2009). The emission peak of the QDs can be tuned by changing the heating temperature and gas flow rates. The precursor solution was prepared by adding freshly prepared NaHTe solution to a nitrogen-saturated $CdCl_2$ solution at pH 11.5 in the presence of thiolglycolic acid (TGA). The precursor concentrations were [Cd] = 10 mM, [TGA] = 24 mM and [Te] = 5 mM, respectively. This precursor solution was used in the chemical aerosol flow method to synthesize QDs. Water-soluble QDs with core/shell structure could be ultrafast synthesized in less than 10 s. In this work, the QDs with emission peak position at 535 nm were synthesized with temperature of 225 °C and a flow rate of 3 L min^{-1}. As shown in Fig. S(A) (see the Supporting Information), the obvious Ultraviolet–visible spectroscopy (UV–vis) absorption peaks (curve a) indicate that the CdTe/CdS QDs are closed to mono-dispersed. The photoluminescence emission maximum (curve b) appears at 535 nm and the photoluminescence full width at half-maximum is about 33 nm. The photoluminescence quantum yield is 30%. X-ray diffraction (XRD) patterns of the CdTe/CdS QDs are shown in Fig. S(B) (see the Supporting Information). The (1 1 1) peak is at 24.4°, which is slightly shifted to a high scattering angle compared with bulk CdTe pattern (JCPDS Card No. 75-2086). The XRD results indicate that the QDs have a core/shell structure, which is consisted with the reported results (Gao et al., 1998; Yu et al., 2003). As shown in Fig. S(C) (see the Supporting Information), transmission electron microscopy (TEM) images also suggest that the CdTe/CdS QDs have narrow size distribution. The average size of the CdTe/CdS QDs was 3.0 nm.

2.3. QDs system for LDH detection via NAD-dependent fluorescence of QDs

The detection procedure was described as follows: 400 μL of QDs was dissolved into 2 mL by PBS buffer (pH 7.0) and then reacted with different concentrations of NAD solution (concentration from 0 to 3 mM) for 10 min, then the fluorescence intensities of the solution were recorded. The detection procedure of the activity of LDH was the same as the previous description. After adding NAD (1 mM) into the QDs solution, then the equal molar amount of substrate L-lactate (LL) and the different amounts of enzyme LDH were also added, and the mixture was reacted for 10 min. The fluorescence changes of QDs enzyme biosensor were recorded by a F-4500 fluorescence instrument, the wavelength 450 nm of the laser source was used for the excitation of the QDs detection system. The fluorescence signal was recorded over a range from 470 to 620 nm.

3. Results and discussion

Fig. 1A shows typical fluorescence intensities curves obtained for different activity units (U/L) of LDH at optimal condition. Each sample is analyzed by reacting QDs with different amounts of LDH (from 75 to 4500 U/L, see curve a–h) in the present of coenzyme NAD and the substrate L-lactate (LL) for a fixed time interval of 10 min. It could be seen that the fluorescence is intensified by the increasing amounts of LDH. The inner shows the calibration curve that corresponds to the fluorescence intensities of QDs (wavelength 535 nm) upon the different amounts of LDH. The linear plot reveals detectable concentration range of 150–1500 U/L and the correlation

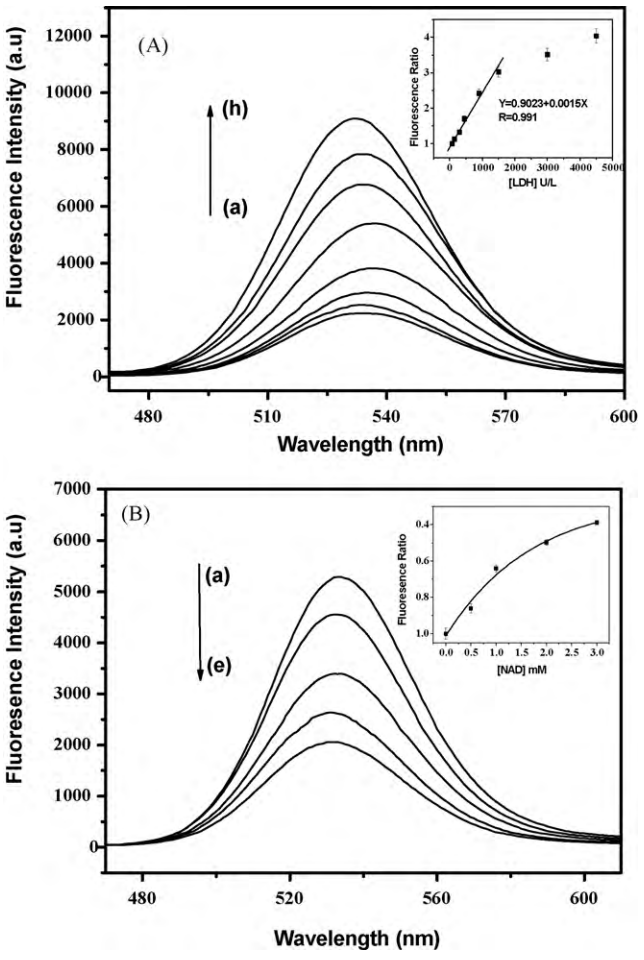

Fig. 1. (A) Fluorescence changes upon the interaction of CdTe/CdS QDs with different amounts of LDH (from a to h the amount of the LDH is 0.15, 0.3, 0.6, 0.9, 1.8, 3, 6 and 9 U in 2 mL solution, respectively). The inner shows the linear plot that reveals detectable concentration range of 150–1500 U/L and the correlation coefficient is 0.996. (B) Fluorescence changes upon the interaction of CdTe/CdS QDs with different concentration of NAD: (a) before addition of the NAD; (b)–(e) the concentration of the NAD is 0.5, 1, 2 and 3 mM, respectively. The inner is the calibration curve corresponding to the optical analysis of different concentration of NAD by the CdTe/CdS QDs.

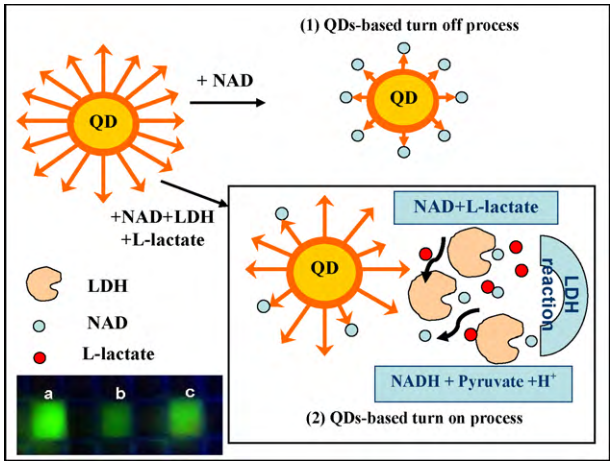

Fig. 2. Schematic principle for assay of LDH. The inner shows the fluorescence image of the QD in the absence (a) or presence (b) of NAD, and in the presence of NAD, LL and LDH (c).

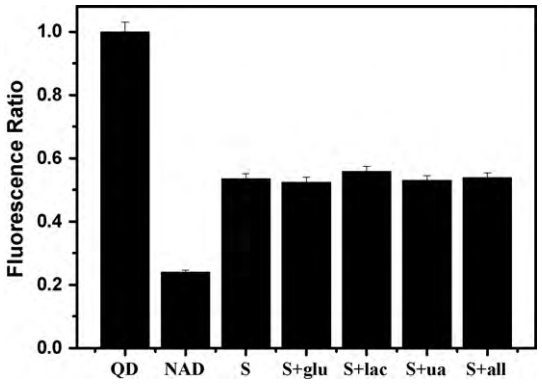

Fig. 3. Fluorescence changes of CdTe/CdS QDs in the detection system (S), the coexistence of the detection system with 2.2 mM glucose (S + glu), 0.5 mM lactose (S + lac) and 0.5 mM uric acid (S + ua), respectively and the coexistence of the detection system with all of these interferences (S + all).

coefficient is 0.996. The normal amount of the LDH in human blood serum is 100–300 U/L (come from spectrophotometric determination). When the amount of the LDH is >1000 U/L, there may be some differentiating disorders. So this new optical approach to detect the level of LDH could not only quantitatively detect the amounts of LDH in normal samples, but also have potential to qualitatively detect differentiating disorders in abnormal samples.

In the biochemical reaction, the LDH catalyzes the interconversion of L-lactate and pyruvate with the coenzyme NAD. The biochemical reaction of the LDH occurs as the following:

$$\text{L-lactate} + \text{NAD}^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + \text{H}^+ \quad (1)$$

In order to investigate the principle of optical analysis of LDH, the fluorescence intensities of the QDs affected by NAD were also measured. Fig. 1B depicts the fluorescence changes of the QDs mixing with different concentrations of NAD (curve a–e). Each fluorescence intensity is recorded when it levels off at a saturated value after 10 min. The inner shows the calibration curve that corresponds to the changes of fluorescence intensities of the QDs (wavelength 535 nm) upon treatment with different concentrations of NAD. From these figures we can see the fluorescence intensities of the QDs turn to decline with the increment of the concentration of NAD. NAD, and its reduced form NADH, the two nucleotides transfer hydrogen atoms and electrons from one metabolite to another in many cellular redox reactions (Xie et al., 2009). Recent papers have reported that the quenching of the fluorescence of the QDs by the NAD is presumable by an electron transfer (ET) process for modulating the surface chemistry of CdTe QDs (Han and Li, 2008; Freeman et al., 2009a,b; Freeman and Willner, 2009). So we suppose that NAD originally diffuses to the surface of CdTe/CdS QDs by random chemical adsorption or electrostatic force and affects the surface chemical bond of the QDs, consequently quenching the fluorescence of QDs. NAD is an ubiquitous coenzyme in the biological metabolism and is the substrate of over 300 species of cellular dehydrogenates (Li and Chen, 2002), so it is indicated that the QDs could not only be used as luminescent probe to analyze the important enzyme, such as LDH, also be applied for analyzing the activity of any NAD-dependent enzymes or biocatalyzed reaction in both analytical practice and biochemical synthesis.

We suppose the schematic illustration of LDH detection as shown in Fig. 2. In the QDs-based turn off process (1), NAD originally diffuses to the surface of CdTe/CdS QDs, and then makes electron transfer (ET) process with CdTe/CdS QDs. As the result, the fluorescence of the QDs is quenched by NAD. The proposed mechanism during the LDH detection is shown in process (2). As adding LDH and the substrates L-lactate (LL), biocatalyzed reaction (Eq. (1)) would consume NAD. The result is that only the residual NAD could interact with QDs and then the influence of NAD on the fluorescence intensities of QDs would be weakened. Therefore, the fluorescence of the QDs is increased with increasing amount of the LDH because the more LDH introduced may result more NAD consumed in the reaction.

We also got fluorescence image of the QDs (show in inner of Fig. 2) to show the changes of their fluorescence intensities. The left one (a) is the fluorescence image of the QDs without any analysis. The middle one (b) shows the green fluorescence declines as the result of being quenched by NAD. The fluorescence intensity of the right one (c) is intensified because the consumption of NAD in the biocatalyzed reaction by adding LDH and the substrate LL.

The interference study is one of the important evaluations of a biosensor. In this work, the effect of several possible interfering substances, such as uric acid (UA), lactose, and glucose on the QDs-based sensor was also investigated. Fig. 3 shows that significant fluorescence declined after the introduction of 1 mM NAD and then recovered by adding 1 mM NAD with 3 U LDH and 1 mM LL in the detection system (see "S" in Fig. 3). Then the additions of 0.5 mM UA (see "S + ua" in Fig. 3), 0.5 mM lactose (see "S + lac" in Fig. 3), and 2.2 mM glucose (see "S + glu" in Fig. 3) do not cause any observable changes of the fluorescence intensities of the QDs in the mixture, respectively. Moreover, even if all of these normal interfering substances are added into the detected system (see "S + all" in Fig. 3), the fluorescence intensities of the QDs are almost not affected. The results prove that the established method can provide selective detection of the activity level of enzyme in the complicated system.

4. Conclusions

In conclusion, an efficient NAD-dependent enzyme (LDH) analysis method by CdTe/CdS QDs is developed. The sensitive and selective detection of the LDH has been achieved based on the changes of fluorescence intensities of the CdTe/CdS QDs by the consumption of the NAD in the biocatalyzed reaction. A linear calibration plot is obtained in the wide amounts range from 150 to 1500 U/L and the detection limit is 75 U/L. This novel optical biosensor based on CdTe/CdS QDs provides a versatile tool to analyze the activities of NAD-dependent enzymes. Although the development of this LDH biosensor based on QDs is still in the preliminary stages, we believe this method has great potential in application of enzyme detection on the physiological level and accomplish early diagnosis of diseases.

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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.2010.05.014.

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