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RESEARCH PAPER

A Turn-on Fluorescent Probe Based on Quantum Dots for Detection of Trace Glutamate Dehydrogenase

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Abstract: We reported a simple and fast fluorescence system based on quantum dots (QDs) to detect glutamate dehydrogenase (GLDH), which inverted glutamate to *α*-ketoglutarate using nicotinamide adenine dinucleotide (NAD^+) as a cofactor. The fluorescence of CdTe QDs was quenched by NAD^+ through an electron transfer pathway, and the quencher could be consumed by adding NAD^+-dependent enzymes such as GLDH. In the NAD^+/GLDH system, the fluorescence of QDs was first quenched by NAD^+, and the restored fluorescence was in proportional to the amount of GLDH added. Based on this principle, we designed a fluorescence detection system to detect the concentration of GLDH ranging from 10 U L^{-1} to 1000 U L^{-1}, which is a critical concentration range in clinical diagnosis of different kinds of liver diseases.

Key Words: Fluorescence methods; Quantum dots; Glutamate dehydrogenase; NAD^+; Hepatotoxicity

1 Introduction

Quantum dots have been used in many research areas due to its unique electronic and optical properties[1,2]. As an inorganic fluorescent probe, quantum dots possess broad absorption profiles, narrow emission band, and high resistance to photobleaching. These advantages enable the QDs to be applied in in-vitro imaging[3–7], in-vivo targeting[8,9], bioanalytical assays[10] and biosensors[11,12]. Many QDs-based biosensors have been developed for detecting various analytes such as Ag^+, Cu^{2+} and Pb^{2+}, small reactive molecules such as NO and HClO, and functional biomolecules such as DNA, proteins and enzymes[13–19].

Herein, we reported a detection method by using CdTe QDs to detect glutamate dehydrogenase (GLDH), a NAD^+-dependent enzyme. Glutamate dehydrogenase (GLDH) is a mitochondrial enzyme present in many tissues, including heart, kidney, liver etc[20]. There are many serum biomarkers of hepatotoxicity, such as ALT, GLDH, AST, SDH, and ALP[21–24]. Among these biomarkers, GLDH is a sensitive enzymatic serum biomarker of liver toxicity. Compared to ALT, AST, SDH or ALP, GLDH is a more effective serum marker. GLDH level in serum is very sensitive to hepatocyte injury and increases after hepatocellular injury[25]. The existing methods for GLDH assay are colorimetry, spectrophotometry and enzyme-linked immunosorbent assay (ELISA). These methods sometimes are low sensitive and lead to false positive. Enhanced fluorescence methods based on Forster fluorescence resonance energy transfer and photoinduced electron transfer mechanism are generally more sensitive and anti-interference, hence it is very useful for the sensitive detection of peptides and enzymes[25].

2 Experiment

2.1 Materials and apparatus

All chemicals were of analytical grade and without further

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purification. 3-Mercaptopropionic acid (MPA) was purchased from Sigma, USA. Tellurium powder, sodium borohydride ($NaHB_4$), and cadmium chloride hydrate ($CdCl_2 \cdot 2.5H_2O$) were purchased from Shanghai Chemicals Ltd. Glutamate dehydrogenase (Type II, GLDH, 20 mg) was purchased from Sigma. Nicotinamide adenine dinucleotide (NAD) was purchased from Shanghai Sangon Biotechnology Co. Ltd. *L*-Glutamate was from Sinopharm Chemical Reagent Co. Ltd. All the solutions were prepared with water purified by a Millipore water purification system (18.2 MΩ cm). Fluorescence experiments were recorded by a PerkinElmer LS-55 luminescence spectrometer. The UV-Vis absorption spectra were obtained with a Shimadzu UV-2550 spectrometer. The pH value of solution was measured using PHS-3C.

2.2 Methods

The detection procedure of NAD^+ was described as follows: 8 μL CdTe QDs was added into 2 mL ultrapure water, different concentrations of NAD^+ (from 0 to 14.71 μM) were then added and the mixture was kept for 10 min. The fluorescence of the solutions was measured and the concentration of NAD^+ which could quench 65% of the fluorescence was obtained. The detection procedure of GLDH was described as follows: 8 μL QDs was diluted in 2 mL ultrapure water. The enzymatic catalytic reaction was carried out in a separate PCR centrifuge tube, which contains appropriate amount of the cofactor NAD^+, the equivalent amount of substrate *L*-glutamate, and different amounts of GLDH. The mixture was mixed at 25 ºC water bath for 10 min. The mixture was then added into a QDs solution and stirred for 5 min, followed by recording the fluorescence intensity I_j of the solution (j = 0, 10, 25, 50, 100, 250, 500, 750 and 1000 U L^{-1}; j is the amounts of GLDH added.). All fluorescence spectra were recorded using a 400-nm excitation wavelength from 450 nm to 650 nm with a 500 nm min^{-1} scan rate.

3 Results and discussion

3.1 Quenching effect of NAD+ on CdTe-MPA QDs

NAD^+ can effectively quench the fluorescence of QDs through an electron transfer pathway. Figure 1 shows the time-dependent fluorescence changes after adding 10 μM NAD^+. The spectra were recorded with a time interval of 2 min. The fluorescence intensity decreased with the addition of NAD^+ and reached nearly constant after 10 min. The dose-responsive quenching of the fluorescence to the amount of NAD^+ added was also performed and presented in Fig.2. As can be seen from Fig.2, The fluorescence intensities of CdTe QDs versus the concentrations of NAD^+ could be calibrated in a linear relationship (inset). The calibration curve shows the linearity dependence of the fluorescence intensity ratio I/I_0 (I

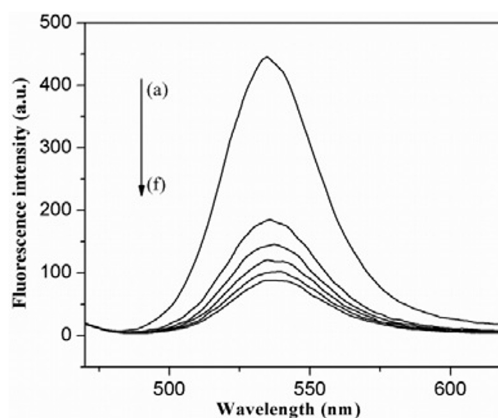

Fig.1 Time-dependent fluorescence changes of CdTe QDs after adding 10 μM NAD^+. The spectra were recorded at time intervals of 2 min. (a): before adding NAD^+, (b) to (f): after adding NAD^+ for 2, 4, 6, 8 and 10 min

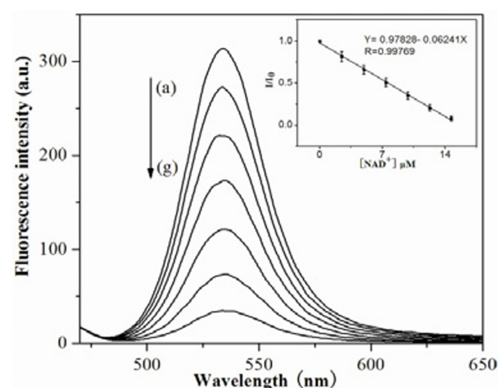

Fig.2 Fluorescence intensity changes of CdTe QDs after adding different concentrations of NAD^+. (a) to (g): 0, 2.48, 4.95, 7.41, 9.85, 12.29 and 14.71 μM. The inner plot is the calibration curve corresponds to the fluorescence intensity of the QDs versus the concentrations of NAD^+

and I_0 refer to the fluorescence intensities in the presence/absence of NAD^+) on the concentration of NAD^+.

3.2 Fluorescence detection of GLDH

NAD^+ was used as a cofactor in the catalytic reaction of glutamate to α-ketoglutarate. When adding GLDH and *L*-glutamate into the solution containing NAD^+, NAD^+ was consumed as the reaction went on. On the basis of this fact above mentioned, we have designed a fluorescence system for the determination of GLDH. Figure 3 illustrates the detection principle of GLDH using QDs and NAD^+. The reaction between GLDH and NAD^+ is favored in pH 7.2 phosphate buffer, however, the quenching efficiency of NAD^+ is very low in this condition, as shown in Fig.4. In order to understand this, we measured the UV-Vis absorption spectra of NAD^+ in ultrapure water and phosphate buffer, respectively. As shown in Fig.5, it can be seen that the absorption spectra are the same, suggesting the structure of NAD^+ are retained in

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