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Sensitive optical detection of alkaline phosphatase activity with quantum dots

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ABSTRACT

A simple method has been developed to detect the activity of alkaline phosphatase (ALP) by the changing of fluorescence intensities of the quantum dots (QDs). In this system, the fluorescence intensities of the QDs were quenched by p-nitrophenol (pNP) which was produced in the process of ALP catalytic reaction. A series of linear calibration curves of the activity of ALP were obtained in different pH buffer solutions. The wide linear range was 3–1000 U L^{-1} and the detection limit was 3 U L^{-1} (S/N=3). Furthermore, the experimental conditions of biosensor were optimized, and anti-interference ability was presented. The activity of ALP was also detected in serum and the recovery of ALP in serum samples was more than 95%. The excellent performance of this biosensor indicates that it can be used in practice detection of ALP.

1. Introduction

The use of semiconductor quantum dots (QDs) for sensing and biosensing has attracted growing research efforts [1–6]. The unique photophysical properties of QDs, such as narrow emission bands, high fluorescence quantum yields, high Stokes shifts, and stability against photobleaching, make them a superior sensing material. Various QD-based biosensors have been developed, including sensors that follow the activities of enzymes or their substrates [7–9], immunosensors [10], and sensors that based on DNA hybridizations [11,12]. Most of these studies operate by the mechanism of fluorescence resonance energy transfer (FRET) [13–16]. However, the assembly of FRET-based QD sensors is usually a complicated chemical process involving the modification of the QD surface, the immobilization of biological receptors, and the adsorption of a chosen dye quencher. Therefore, the QDs-based biosensors followed by other processes, such as electron transfer (ET) [17–19], are also highly desired.

Alkaline phosphatase (ALP) is a nonspecific esterase that catalyzes hydrolysis of many monoesters of phosphoric acid. It is an enzyme which can be found in human serum and assayed in routine clinical analysis, because ALP in blood is mainly derived from liver and bone, and its activity increases if there are problems in these organs [20,21]. In addition to diagnosing ailments, monitoring ALP level is useful in treatment for many liver diseases. Therefore, needs exist to detect ALP sensitively and selectively in many diagnostic and clinical assays. Current monitoring techniques for ALP commonly use an enzymatic substrate of ALP, and upon reactions with ALP, products of the substrate or its derivatives produce strong electrochemical [22,23], colorimetric [24], chemiluminescent [25], chromatographic [26], or fluorometric [27] signals. However, the above detection methods still have some problems, such as complex process, long detection time and expensive instruments. These would block the developments of the rapid diagnosis and home medical equipment for ALP detection. So the demands still exist to develop a sensitive, selective, simple convenient and cost-effective detection way for ALP determination.

Herein, we investigated a fluorescent biosensor for quantitative analysis of the activity of ALP based on the water-soluble and stable CdTe/CdS QDs, The obtained optical biosensor showed low detection limit, wide linear detection range and good selectivity. The practical detection in serum has been studied and the results indicated this detection system can be used for practical application. This system is very convenient, simple and rapid (only about 15 min from assembly to detection), because it avoids the complex process of the QDs' modification or immobilization which were involved in FRET-based biosensor. This present approach develops a sensitive, selective and cost-effective QD-based fluorescence sensor for the activity of ALP.

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2. Experimental

2.1. Material and methods

Alkaline phosphatase (ALP, 28.09 U mg^{-1}), uric acid (UA) and glucose were products of Sigma Company. p-nitrophenyl phosphate (pNPP) was purchased from Beijing Solarbio Science and Technology Company. L-lactic acid (LL) was from Alfa Aesar Company. Phosphate buffers containing 0.1 M KCl consisted of KH_2PO_4 and Na_2HPO_4 (0.2 M, pH=7.0). Borate buffers consisted of borax and boric acid (0.2 M, pH=8.7). Carbonate buffers consisted of Na_2CO_3, $NaHCO_3$ and $MgSO_4$ (0.2 M, pH=10). All reagents were used without further purification and adjusted with redistilled water.

The photoluminescence spectra were recorded by a Cary Eclipse fluorescence spectrophotometer (Varian, Inc.). If not specially stated, the samples were excited at 450 nm, and the exciting slit and the emission slit were 10 and 10 nm, respectively. The optical properties of solution were measured with quartz cuvettes of 10 mm path length.

2.2. Synthesis of the QDs

The CdTe/CdS QDs used in this study were obtained by the chemical aerosol flow method from our research group [28,29]. 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, [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 575 nm were synthesized with temperature of 250 °C and a flow rate of 3 L min^{-1}. The average size of the CdTe/CdS QDs was 3.2 nm.

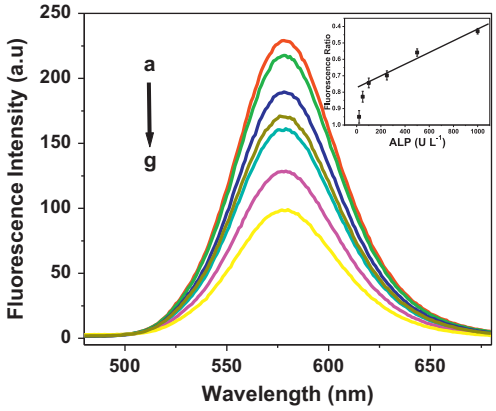

Fig. 1. Fluorescence changes upon the interaction of CdTe/CdS QDs with different amounts of ALP (from a to g the corresponding amount of the ALP is 0, 20, 50, 100, 250, 500, 1000 U L^{-1}, respectively) in phosphate buffer (pH=7). The inner shows the linear curves that reveal detectable concentration range of 100–1000 U L^{-1} and the correlation coefficient is 0.986.

2.3. QDs system for ALP detection

The ALP detection procedure was described as follows: 300 μL of QDs was mixed with pNPP and different concentrations of ALP solution (concentration from 3 to 1000 U L^{-1} in buffer solution). The mixture was diluted into 2 mL by buffer solution and reacted for 10 min. Then the fluorescence intensities of the solution were recorded.

3. Results and discussion

3.1. Detection of the activity of ALP

Fig. 1 showed typical fluorescence intensities curves obtained for different activity units (U L^{-1}) of ALP in phosphate buffer (pH=7) at optimal condition. The QDs interacted with different amounts of ALP (from 20 to 1000 U L^{-1}, see curve b–g) in the present of pNPP for a fixed interval time of 10 min. It could be seen that the fluorescence was decreased by the increasing amounts of ALP. The figure in the inset showed the calibration curve that corresponds to the fluorescence intensities of QDs (wavelength 575 nm) upon the different amounts of ALP. The linear curve revealed detectable concentration range of 100–1000 U L^{-1} and the correlation coefficient was 0.986.

3.2. Effect of pH value on the ALP detection

ALP is a non-specific phosphomonoesterase, which exhibits optimum activity at alkaline pH. So we investigated the effect of pH value on the performance of the biosensor. Fig. 2A showed typical fluorescence intensities curves obtained for different activity units of ALP at pH 8.7. The QDs interacted with different amounts of ALP (from 20 to 900 U L^{-1}, see curve b–g) in the present of pNPP for a fixed interval time of 10 min. As the

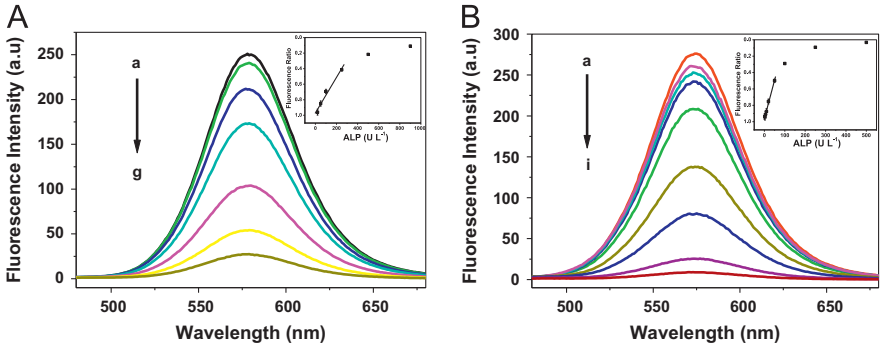

Fig. 2. (A) Fluorescence changes upon the interaction of CdTe/CdS QDs with different amounts of ALP (from a to g the corresponding amount of the ALP is 0, 20, 50, 100, 250, 500, 900 U L^{-1}, respectively) in borate buffer (pH=8.7). The inner shows the linear curve that reveals detectable concentration range of 20–250 U L^{-1} and the correlation coefficient is 0.988. (B) Fluorescence changes upon the interaction of CdTe/CdS QDs with different amounts of ALP (from a to i the corresponding amount of the ALP is 0, 3, 5, 10, 20, 50, 100, 250, 500 U L^{-1}, respectively) in carbonate buffer (pH=10). The inner shows the linear curve that reveals detectable concentration range of 3–50 U L^{-1} and the correlation coefficient is 0.998.