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A convenient and label-free fluorescence "turn off–on" nanosensor with high sensitivity and selectivity for acid phosphatase

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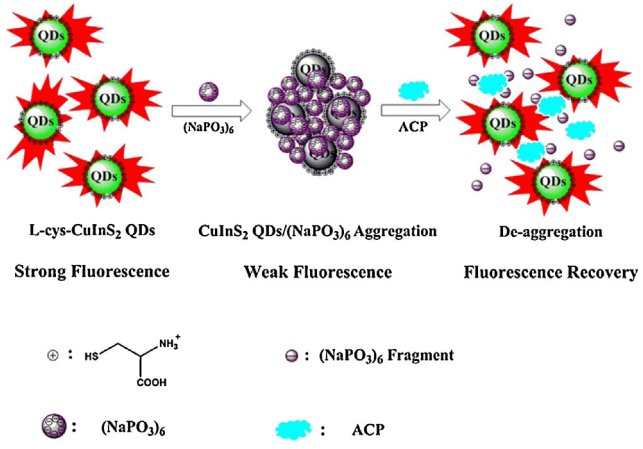
HIGHLIGHTS

- A novel convenient and label-free fluorescence "turn off–on" nanosensor for rapid detection of acid phosphatase was established.
- The simple and straightforward method was based on the aggregation-caused quenching and enzymolysis approach.
- Highly sensitivity and selectivity were obtained.
- Acid phosphatase in human serum sample was detected with satisfactory results.

GRAPHICAL ABSTRACT

A convenient and label-free near-infrared fluorescence "turn off–on" nanosensor was developed for rapid detection of acid phosphatase based on aggregation-caused quenching and enzymolysis approach.



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ABSTRACT

In this study, we reported a convenient label-free fluorescence nanosensor for rapid detection of acid phosphatase on the basis of aggregation-caused quenching (ACQ) and enzymolysis approach. The selectivity nanosensor was based on the fluorescence "turn off–on" mode, which possessed high sensitivity features. The original strong fluorescence intensity of $CuInS_2$ QDs was quenched by sodium hexametaphosphate $(NaPO_3)_6$. The high efficiency of the quenching was caused by the non-covalent binding of positively charged $CuInS_2$ QDs to the negatively charged $(NaPO_3)_6$ through electrostatic interactions, aggregating to form a $CuInS_2$ QDs/$(NaPO_3)_6$ complex. Adding acid phosphatase caused intense fluorescence of $CuInS_2$ QDs/$(NaPO_3)_6$ to be recovered, and this was because of enzymolysis. $(NaPO_3)_6$ was hydrolyzed into small fragments and the high negative charge density decreased, which would weaken the strong electrostatic interactions. As a result, the quenched fluorescence "turned on". Under the optimum conditions, there was a good linear relationship between I/I_0 (I and I_0 were the fluorescence intensity of $CuInS_2$ QDs/$(NaPO_3)_6$ system in the presence and absence of acid phosphatase, respectively) and acid phosphatase concentration in the range of 75–1500 nU mL^{-1} with the detection limit of 9.02 nU mL^{-1}. The proposed nanosensor had been utilized to detect and accurately quantify acid phosphatase in human serum samples with satisfactory results.

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1. Introduction

Acid phosphatase (ACP) is a kind of hydrolase that can catalyze the hydrolysis of phosphate monoester to release phosphate groups during digestion under acidic condition. It is widespread in nature [1] and can be found in mammalian tissues including prostate, liver, kidney and hematological system [2,3]. ACP plays an essential role in regulating a variety of cellular functions although its concentrations are usually found low in mammalian cells. An abnormal level of serum ACP has proven to be associated with several diseases, such as prostate cancer, hepatitis, Gaucher disease, Paget's disease, thrombophlebitis, hyperparathyroidism, kidney disease, multiple myeloma [1,4–6]. Therefore, the precisely monitoring of ACP level in physiological media is quite helpful in the pathologic diagnosis of these relative diseases. Clinically, the determination of ACP has been used for the auxiliary diagnosis of human prostatic diseases and prostate cancer for many years [4–6].

To date, a few common methods for ACP assay have been proposed, including spectrophotometry [7], fluorimetry [8], immunoassay [9–11], high performance liquid chromatography, surface acoustic wave (SAW) methods and electrochemical methods [12]. Although these methods offer reasonable sensitivities, accurately quantify the amount of acid phosphate in the solution has seldom been reported. Furthermore, some ACP assays are time-consuming or have poor reproducibilities, some require expensive equipments and complicated instrumental setups, alkaline pH condition and many in situ or in vivo applications are limited by the necessity of requiring labeled or providing electrical connections to the detector [13,14]. Therefore, developing a simple-operation, rapid, low-cost, reliable method for ACP detection is especially expected.

Fluorescence methods are the most promising alternatives for sensing because they offer advantages over other techniques, including their ease and speed of use and their sensitivities. Furthermore, fluorescence turn-on sensors have more advantages than fluorescence quenching sensors, giving fewer chances of false positive signals, better sensitivities and selectivities, which have been demonstrated in numerous studies [15]. Recently, a fascinating spectrophotometry for the ACP detection has been reported, which is based on the squaraine dyes. It is a dual-channel including colorimetric and fluorometric sensor and exhibits turn-on response in near infrared (NIR) region. But the response time is long and the organic fluorescent dyes usually have lots of shortcomings, such as narrow excitation spectra and broad emission bands. Further developing an environmentally friendly turn-on probe for detecting ACP is, therefore, still a challenge [16].

Compared with organic dyes, QDs possess some unique optical properties including high quantum yields, long fluorescence lifetimes, tunable size-dependent emission, highly stable against photobleaching and broad absorption spectra coupled with narrow and symmetrical emission spectra [17–21]. In recent years, the newly emerging $CuInS_2$ QDs have attracted particular research interests in vivo fluorescence sensing and biomedical imaging applications [22]. This is mainly due to its low cell toxicity and environmental friendliness in comparison with traditional semiconductor usually containing toxic elements (Cd, Hg, Pb, As, Te and Se).

In this paper, a novel simple fluorescence "turn off–on" nanosensor for rapid sensing of ACP was reported for the first time. As illustrated in Scheme 1A, the present method was based on the aggregation-caused quenching (ACQ) and enzymolysis. In aqueous solution, the NIR $CuInS_2$ QDs with L-cysteine as the stabilizer were positively charged at appropriate pH. Upon addition of sodium hexametaphosphate $(NaPO_3)_6$ with six negative charges in the six-membered ring, the $CuInS_2$ QDs favored to form aggregate assembly with opposite charged $(NaPO_3)_6$ via electrostatic interactions. Accordingly, the initial strong fluorescence of the dispersive $CuInS_2$ QDs had a dramatically decrease due to the aggregation-caused quenching (ACQ) property. In the presence of ACP, $(NaPO_3)_6$ could specifically digest $(NaPO_3)_6$ into phosphate fragments. Therefore, the aggregation equilibrium between $CuInS_2$ QDs and $(NaPO_3)_6$ was disturbed and the assembly were disaggregated. As a result, the quenched fluorescence was restored. Thus, a convenient and label-free fluorescent "turn off–on" nanosensor for ACP detection was fabricated.

2. Experiment

2.1. Materials and instruments

All chemicals and reagents were analytical grade and used directly without further purification. Copper(II) chloride dehydrate ($CuCl_2{\cdot}2H_2O$), sulfourea ($CS(NH_2)_2$), sodium hydroxide (NaOH), sodium dehydrogenized phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Beijing Chemical Works. Indium(III) chloride tetrahydrate ($InCl_3{\cdot}4H_2O$) and K_2MoO_4 were purchased from Sigma–Aldrich Corporation. Acid phosphatase (ACP), adenosine-5′-triphosphate (ATP), trypsin (Try) and carboxypeptidase Y (CPY) were purchased from Sigma–Aldrich Corporation. Heparinase (Hep) was purchased from Si Qing Yuan Biotechnology (Beijing). L-Cysteine, sodium hexametaphosphate [$(NaPO_3)_6$] and other materials were obtained from Beijing Dingguo Biotechnology Co., Ltd. (Beijing). The water used in all experiments had a resistivity greater than 18 $M\Omega\,cm^{-1}$. The human serum was obtained as a gift from the University Hospital.

Fluorescence measurements were performed on a Shimadzu RF-5301 PC spectrofluorophotometer (Shimadzu Co., Kyoto, Japan), and a 1 cm path-length quartz cuvette was used in experiments. UV–vis absorption spectra were obtained using a Varian GBC Cintra 10e UV–vis spectrometer. All pH measurements were taken with a PHS-3C pH meter (Tuopu Co., Hangzhou, China). All temperature measurements were accomplished using a water bath. Transmission electron microscopy (TEM) experiments were performed on a Philips Tecnai F20 TEM operating at 200 kV.

2.2. Synthesis of $CuInS_2$ QDs

The water-soluble NIR $CuInS_2$ ternary QDs with L-cysteine as the stabilizer used in our work were synthesized via a hydrothermal synthesis method as described in our previous papers [23]. In a typical experiment, L-cysteine (3.60 mmol) was dissolved in 7.5 mL distilled water. Then $CuCl_2{\cdot}2H_2O$ (0.15 mmol) and $InCl_3{\cdot}4H_2O$ (0.15 mmol) were injected into the solution and the solution turned light green. The pH value of the mixture solution was adjusted to 11.3 by adding 4 mol L^{-1} NaOH solution with stirring. During this process, the solution changed from light green to clear brown. After stirring for 10 min, $CS(NH_2)_2$ (0.30 mmol) was dissolved in the solution. The Cu-to-In-to-S and Cu-to-L-cysteine precursor ratios were 1:1:2 and 1:12, respectively. All the above mentioned experimental procedures were performed at room temperature, and then the solution was transferred into a Teflon-lined stainless steel autoclave with a volume of 15 mL. The autoclave was maintained at 150 °C for 23 h and then cooled down to room temperature by a hydro-cooling process. The final concentration of $CuInS_2$ QDs, as measured by the Cu^{2+} concentration, was 1.36×10^{-4} mol L^{-1}.