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Label-free fluorescent assay for high sensitivity and selectivity detection of acid phosphatase and inhibitor screening

Jing Wang[a,1], Yu Yan[a,1], Xu Yan[a,1], Tianyu Hu[a], Xiaojian Tang[b], Xingguang Su[a,*]

[a] Department of Analytical Chemistry, College of Chemistry, Jilin University, Changchun 130012, China
[b] Key Laboratory of Songliao Aquatic Environment, Ministry of Education, Jilin Jianzhu University, Changchun 130118, China

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ABSTRACT

In this work, we developed a convenient and label-free fluorescence sensing platform for sensitive detection of acid phosphatase (ACP) and its inhibitor. The selectivity fluorescent strategy was based on fluorescence enhancement mode of cysteamine-capped CdTe quantum dots (QDs). Upon addition of adenosine triphosphate (ATP), the amino groups on the surface of CdTe QDs can form both electrostatic and hydrogen bonding with ATP, leading to obvious fluorescence enhancement of QDs. ACP can easily catalyze the hydrolysis of ATP into adenosine and phosphate fragments under an acidic environment, causing dramatically decrease of the fluorescence intensity of QDs. Quantitative detection of ACP in a broad range from 1.0 to 50 $\mu U\,mL^{-1}$ with the detection limit of 0.45 $\mu U\,mL^{-1}$ can be achieved. The developed sensing platform has been successfully applied to the accurately analysis of ACP activity in human serum samples with good results. Furthermore, the proposed strategy also could be used for the detection of parathion-methyl which served as a model of ACP inhibitor. These results significantly demonstrated the established sensing platform can be used not only for ACP activity determination, but also for its inhibitor detection and screening.

1. Introduction

Acid phosphatase (ACP), which worked as digestive enzyme in mammalian body fluids and tissues, can efficiently catalyze the hydrolysis of phosphate esters under an acidic environment [1]. ACP in human serum is normally found in low concentrations but it plays a critical role in many mammalian physiological processes, especially the movement of humans [2]. An imbalance level of ACP may also cause a number of diseases, such as prostate cancer, multiple myeloma and Gaucher's disease [3,4]. Clinically, the measurement of ACP activity has been used for monitoring cell viability [5]. In fact, it has already been recognized as an important biomarker of metastatic prostate cancer [6–8]. Therefore, the precise detection ACP activity is great significance in pathologic diagnosis, postsurgical evaluation and drug screening.

Currently, a number of methods for ACP detection have been established, including high performance liquid chromatography [9], electrochemical methods [10,11] and immunoassay [12]. Although the above methods have good performance in sensitivity, the applications of these methods are limited due to tedious purification of samples, sophisticated instrumentation, costly bio-molecular reagents and time-costing immobilizing processes. Therefore, the development of a convenient, inexpensive and high sensitivity method for ACP detection is an important challenge to overcome. In order to circumvent these problems, fluorescence assay is regarded as a more desirable approach because of its convenience and high sensitivity. Up to date, few numbers of fluorometric assay using organic dyes [13,14] and fluorescent polymers [15] have been developed for ACP activity monitoring. Xu et al. established a fluorescent system for the determination of ACP based on the aggregation-caused quenching between cationic squaraine dyes and sodium hexametaphosphate [14]. Xie et al. established a sensitive fluorescent assay for ACP activity detection composed of a cationic conjugated polyelectrolyte and *p*-nitrophenyl phosphate [15]. As a result, most of established assays utilizing organic dyes are susceptible to poor stability, complex synthesis and tedious purification [16]. Compared with those organic dyes-based probes, quantum dots (QDs) offered several key merits, including better stability, easier preparation and size-tunable emission spectrum [17–19]. Recently, QDs have attracted increasing attention in chem/bio sensing, imaging and delivering drugs applications [20–22]. QDs-based fluorescent probe have already been regarded as a convenient system for sensitively analyzing for enzymes activ-

* Corresponding author.
E-mail address: suxg@jlu.edu.cn (X. Su).
[1] These authors contributed equally to this work.

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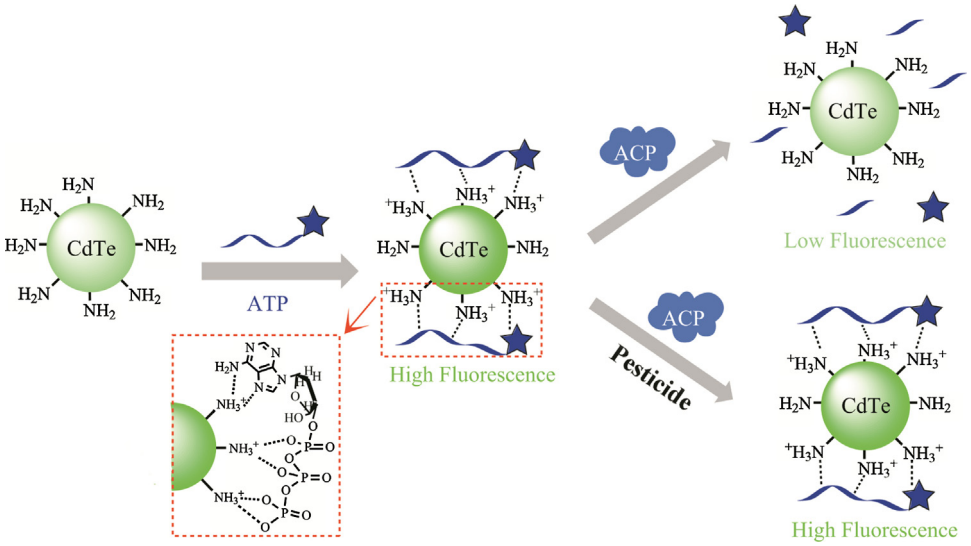

Scheme 1. The schematic illustration of the novel nanosensor for ACP and inhibitor detection.

ity. For instance, Yan et al. developed ratiometric fluorescence sensor by hybridizing two differently sized QDs for selective detection of tyrosinase [16]. Li et al. established a QD-based fluorescence biosensor for the detection of matrix metalloproteinase-2 in vitro and in vivo [23]. Therefore, the promising advantages of QDs make them serve as ideal sensor and imaging platforms.

With these insights, herein we designed a convenient assay with high sensitivity for ACP activity detection by using cysteamine-capped CdTe QDs as the fluorescent probe and adenosine triphosphate (ATP) as the substrate. As illustrated in Scheme 1, combining with ATP-triggered fluorescence enhancement and the ACP-caused catalytic hydrolysis, a novel sensing platform for ACP activity was proposed. The CdTe QDs capped with cysteamine possess strong green fluorescence at 539 nm. Upon addition of ATP into the system, the amino groups on the surface of QDs can interaction with the adenine base and phosphate group of ATP, resulting in the obvious fluorescence enhancement of the QDs. The presence of active ACP specifically catalyzes the hydrolysis of ATP into adenosine and phosphate fragments, and then the enhanced fluorescence of QDs can be dramatically quenched. On the basis of the fluorescence enhancement of QDs caused by ATP and following quenching with assistance of ACP, this label-free fluorescent assay possesses enough high sensitivity for ACP detection. Furthermore, the established fluorescence sensing system also could be used for the detection of the ACP inhibitor. In the presence of parathion-methyl (PM), the activity of ACP is inhibited [24], which prevents the hydrolyzation of ATP. This, in turn, will result in fluorescence recovery compared to that of QDs/ATP/ACP system. The present nanosensor based on ATP-triggered fluorescence enhancement of QDs for ACP activity detection has not been reported before.

2. Experiment

2.1. Reagents and instruments

$CdCl_2$ (99%), tellurium powder (99.8%), and $NaBH_4$ (99%), Cysteamine (95%), acid phosphatase (ACP), parathion-methyl (PM), and adenosine-5-triphosphate (ATP) were purchased from Sigma-Aldrich Corporation. Acetic acid and sodium acetate trihydrate were purchased from Beijing Chemical Corp. Other solvents and reagents were of at least analytical grade and used without further purification. The water which used throughout the experimental process had a resistivity greater than 18 MΩ cm^{-1}.

Fourier transform infrared spectra (FTIR) were collected on a Bruker IFS66 V spectrometer equipped with a DGTS detector. UV–vis absorption spectra were obtained on a Shimadzu UV-1700 spectrophotometer (Shimadzu Co., Kyoto, Japan). The fluorescence spectra were carried out with RF-5301 PC spectrofluorophotometer (Shimadzu, Japan), where a xenon lamp worked as the excitation source. All pH measurements were made with a PHS-3C pH meter (Tuopu Co., Hangzhou, China).

2.2. Synthesis of CdTe QDs

On the basis of our previous work [25], cysteamine capped CdTe QDs were synthesized by using a modified refluxing route. Briefly, sodium borohydride was firstly reacted with tellurium powder to produce sodium hydrogen telluride (NaHTe). NaHTe (0.25 mmol L^{-1}), $CdCl_2$ (1.25 mmol L^{-1}) and cysteamine (1.87 mmol L^{-1}) were mixed together at pH 5.7 in the presence of N_2 protection. Then, the solution was subjected to a reflux at 250 °C under condenser. Water-compatible cysteamine-capped CdTe QDs with fluorescence emission wavelength at 539 nm were obtained and used in the following experiments.

2.3. Fluorescence enhancement experiments induced by ATP

Cysteamine capped CdTe QDs (100 μL) and various concentrations of ATP (from 0 to 6.25 μmol L^{-1}) were introduced into 2.0 mL calibrated test tubes. And then, diluted to 2.0 mL with acetate buffer solution (10.0 mmol L^{-1}, pH = 5.0), followed by collecting the fluorescence spectrum with spectrofluorophotometer. The fluorescence emission spectra were measured with excitation wavelength at 360 nm.

2.4. Acid phosphatase (ACP) detection

The ACP standard solution were prepared by dissolved in acetate buffer solution (10.0 mmol L^{-1}, pH = 5.0). Various concentrations of ACP standard solution were mixed with 5 μmol L^{-1} ATP in a 2.0 mL calibrated test tube and kept in 37 °C for 40 min. After reaction, 100 μL CdTe QDs were introduced into the tube and diluted to 2.0 mL with acetate buffer solution (10.0 mmol L^{-1}, pH = 5.0). The