

A new copper mediated on-off assay for alkaline phosphatase detection based on MoO_x quantum dots

Li Zhang^{a,b}, Ming Fang Hong^a, Zhao Jun Chu^c, Han Xu^c, Shan Ping Wang^c, Xiao Jing Zhao^d, Sai Jin Xiao^{c,e,*}

^a College of Chemistry, Nanchang University, Nanchang 330031, PR China

^b State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, PR China

^c School of Chemistry, Biology and Material Science, East China University of Technology (ECUT), Nanchang 330013, PR China

^d Jiangxi Entry-Exit Inspection and Quarantine Bureau, Nanchang 330038, PR China

^e Jiangxi Key Laboratory of Mass Spectrometry and Instrumentation, ECUT, Nanchang 330013, PR China



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ABSTRACT

We present a new on-off assay for alkaline phosphatase (ALP) detection using molybdenum oxide quantum dots (MoO_x QDs) based on three favorable properties: the quenching ability of Cu²⁺ on MoO_x QDs, the strong binding affinity between Cu²⁺ and PPI, and the catalysis ability of ALP to hydrolyze PPI to orthophosphate (Pi). Benefiting from the strong affinity of Cu²⁺ and PPI, the effective quenching of MoO_x QDs by Cu²⁺ was hampered when PPI was added into the system containing MoO_x QDs and Cu²⁺, leading to a significant photoluminescence enhancement (“ON”). Further addition of ALP, PPI was catalytically hydrolyzed into Pi, which would disable the formation of Cu²⁺-PPI complex and release free Cu²⁺, resulting in photoluminescence quenching (“OFF”). The on-off assay allows the analysis of PPI and ALP by measuring the photoluminescence intensity, and quantitative detection of ALP in a linear range from 0.1 to 5.0 U/L with the detection limit of 0.02 U/L (3σ/K) is realized. Moreover, the present assay was successfully applied in ALP inhibitor screening and ALP detection in diluted human serum samples, and satisfactory recoveries between 99.6% and 103.0% were obtained. The on-off photoluminescence assay shows many merits, including convenient operation, cost-saving, high sensitivity and superior selectivity, which allow the assay for ALP detection in real human serum samples.

1. Introduction

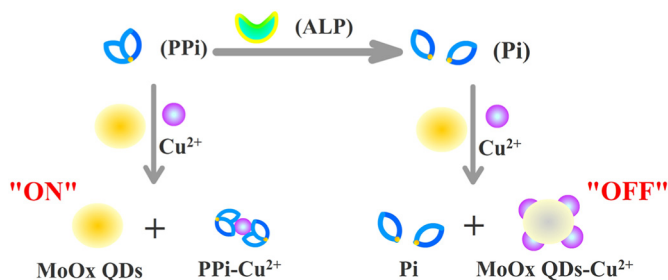
Alkaline phosphatase (ALP) is responsible for the dephosphorylation process of proteins, nucleic acids, and small molecules (such as pyrophosphate ion, PPI) [1]. As a widely distributed enzyme, ALP is participated in signal transduction and the regulation of cell growth and apoptosis, and the abnormal level of ALP is associated with several diseases, such as diabetes, prostate cancer, bone disease, liver diseases, and so on [2–5]. Moreover, ALP is an important indicator and is one of the most used biomarkers in clinic diagnosis and diseases therapy. Therefore, convenient and reliable assays for ALP detection are profound important for the clinic diagnosis and therapy of some diseases. Until now, a variety of assays have been applied for ALP detection in different samples, which include the assays using electrochemistry [6,7], colorimetry [8–10], chemi-luminescence [11–13], surface-enhanced Raman scattering [14], and chromatography [15] as readout signals. However, the methods mentioned above usually have the limitations of requiring sample processing and/or complex

instrumentation, and thus the fluorescent assay is extremely attractive since it is a nondestructive method possessing the advantages of simplicity, cost-saving, high sensitivity and real-time detection.

Recently, various fluorescence methods have been developed for ALP detection on the basis of organic dye [16,17], fluorescent polymers [18,19], semiconductor quantum dots [20,21], and other fluorescent nanomaterials [3,22–24]. Nevertheless, organic dyes and semiconductor quantum dots often have the disadvantages of poor water solubility, high toxicity and/or poor biocompatibility while fluorescent polymers have the drawbacks of the complex synthesis and purification processes, and other fluorescent nanomaterials still have certain limitations, such as sophisticated probe preparation processes, multistep operations, and time-consuming. Therefore, the development of a label-free, nontoxic, sensitive and simple method for ALP detection based on new fluorescent nanomaterials is of profound important.

Molybdenum oxide quantum dots (MoO_x QDs) are a new type of photoluminescence probe which is synthesized by a one pot method using commercial molybdenum disulfide (MoS₂) powder and hydrogen

* Corresponding author at: School of Chemistry, Biology and Material Science, East China University of Technology (ECUT), Nanchang 330013, PR China.
E-mail address: xiaosj@ecit.cn (S.J. Xiao).



Scheme 1. Schematic representation of the label-free assay for PPI and ALP detection using a copper-mediated On – Off switch based on photoluminescent MoO_x QDs.

peroxide (H₂O₂) as precursor and oxidant according to our previous reports [25,26], and possesses the advantages of facile synthesis, large Stokes shifts, low toxicity, superior photostability and biocompatibility, and excellent chemical stability. Owing to these interesting features, MoO_x QDs show a great promising in various fields, such as metal ion sensing, small biomolecule detection, and biological labeling and imaging. Herein, a new label-free assay for simple and sensitive ALP detection was developed using MoO_x QDs as the fluorescent probe, as shown in Scheme 1. Inspired by the facts that Cu²⁺ effectively quenches the photoluminescence of MoO_x QDs [27,28] and PPI has a strong affinity for Cu²⁺ [29,30], we speculated the chelation between PPI and Cu²⁺ would result in the dissociation of Cu²⁺ from MoO_x QDs surface, leading to the photoluminescence recovery of MoO_x QDs (ON state). When ALP coexists with PPI and Cu²⁺, the photoluminescence of MoO_x QDs is quenched again since ALP catalyzed the hydrolysis of PPI into Pi with the result of releasing Cu²⁺ (OFF state). To summarized, the present assay can be utilized to both PPI and ALP detection since the photoluminescence enhancement and quenching of MoO_x QDs is directly related to the addition and consumption of PPI. What's more, PPI is a natural substrate in physiological condition, implying that the ALP functions can be evaluated more accurately by the present assay in biological systems.

2. Experimental section

2.1. Chemicals and reagents

MoS₂ powder, alkaline phosphatase (ALP), acetylcholinesterase (AChE), thrombin (THR), exonuclease III (Exo III), telomerase (TELM), bovine serum albumin (BSA) and glucose oxidase (GOx, from *Aspergillus niger*) were purchased from Sigma-Aldrich (<http://www.sigmaaldrich.com>). Sodium orthovanadate (Na₃VO₄), PPI, Pi and other chemicals were purchased from Shanghai Maikun Chemical Reagents Co., Ltd. All chemicals and solvents were analytical grade without further purification. Deionized water was used throughout.

2.2. Preparation of MoO_x QDs

According to our previous work, MoO_x QDs were synthesized as follow: [25,26] 10 mL 10% H₂O₂ were added into 10.0 mg MoS₂ powders under intensive stir for an hour at room temperature. Then, 425 g L⁻¹ sodium hydroxide solution (NaOH) was added into the solution with the purpose of reacting with excess H₂O₂ and further exfoliating MoS₂ [31], which will induce the color from black to brown. Finally, MoO_x QDs solution was obtained by centrifuging at 8000g for 10 min.

2.3. Photoluminescence quenching of MoO_x QDs by Cu²⁺

For the quenching of MoO_x QDs by Cu²⁺, MoO_x QDs solutions (20 μL 0.5 mg mL⁻¹) was incubated with certain concentration of Cu²⁺

in the presence of 20 μL Tris-HCl buffer (20 mM pH 7.0) at room temperature for 10 min, and the fluorescence spectra were recorded by the USB-4000FL spectrophotometer under excitation at 405 nm.

2.4. Detection of PPI by the “ON” photoluminescence assay

Certain concentrations of PPI were added into the mixture of 20 μL MoO_x QDs (0.5 mg mL⁻¹) and 20 μL Cu²⁺ (300 μM) in the presence of 20 mM Tris-HCl buffer (pH 7.0). After mixed thoroughly, the mixture was stand at room temperature for 10 min and the fluorescence spectra were recorded on the USB-4000FL spectrophotometer under excitation at 405 nm.

2.5. ALP detection with the “OFF” photoluminescence assay

In a typical experiment for ALP detection, 50 μL ALP with an activity ranging from 0 to 300 U/L was added to Tris-HCl buffer solution (20 mM, pH 7.0) containing 150 μM PPI. The mixture was incubated at 37 °C for 90 min and then the enzyme was deactivated at 90 °C for 10 min. Then, 20 μL MoO_x QDs (0.5 mg mL⁻¹) and 20 μL Cu²⁺ (300 μM) were added, and the fluorescence intensity was recorded at an excitation wavelength of 405 nm after 10 min.

2.6. ALP detection in human serum sample

The human blood samples were kindly provided by the hospital of Nanchang University from healthy adult volunteers, and were pre-treated to eliminate any protein interference according reference [32]. Briefly, 1.5 mL 15% (w/v) trichloroacetic acid was added into 0.5 mL serum to destroy the activity of proteins, and then shake vigorously for 15 min. After that, the mixture was centrifuged at 10000 rpm for 10 min, and the supernatant was obtained and adjusted to pH 7.0 using NaOH. The treated serum samples were diluted using deionized water. Different concentrations of ALP were spiked in 1% diluted human serum sample, and the detection procedure is the same as that showed above except 60 μM Cu²⁺.

3. Results and discussions

Although numerous fluorescent nanomaterials have been applied in ALP activity monitoring, certain drawbacks, including sophisticated probe preparation processes, multistep operations, low sensitivity and time-consuming, still exist and trigger the need of developing ALP assays based on new fluorescent nanomaterials. There force, MoO_x QDs, a new type of photoluminescent nanomaterials synthesized by a one pot, rapid and environmentally friendly method according to our previous work [25,26], were utilized as a new photoluminescent probe for ALP detection. Since Cu²⁺ is employed frequently as a quencher of numerous fluorescent nanomaterials [33–37], we firstly assessed the influence of metal ions including K⁺, Mg²⁺, Ca²⁺, Hg²⁺, Zn²⁺, Fe³⁺, Ni²⁺, Co²⁺ and Cu²⁺ on photoluminescence of MoO_x QDs (seen in Fig. S1). The presence of K⁺, Mg²⁺, Ca²⁺, and Zn²⁺ shows little influence on the photoluminescence intensity of MoO_x QDs whereas the addition of Fe³⁺, Hg²⁺, Ni²⁺, Co²⁺ and Cu²⁺ decreases the photoluminescence intensity with distinct quenching effect. Among these metal ions, Cu²⁺ has the strongest quenching ability, and thus was chosen as the quencher to MoO_x QDs. As seen in Fig. 1, with the addition of increasing concentration of Cu²⁺, the photoluminescence of MoO_x QDs gradually decreased and nearly 80% of photoluminescence was quenched observed when Cu²⁺ was 30 μM. The quenching mechanism of MoO_x QDs by Cu²⁺ can be mainly account for the formation of non-fluorescent MoO_x QDs-Cu²⁺ complex since Cu²⁺ are able to coordinate with oxygen atoms on the surface of MoO_x QDs. [27] As the photoluminescence quenching of MoO_x QDs by Cu²⁺ is reversible and can be completely recovered by ethylenediaminetetra acetic acid [27], we suspect that the complex of MoO_x QDs-Cu²⁺ might also be dissociated

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