Sensors & Actuators: B. Chemical 279 (2019) 334–341

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Assembly-enhanced fluorescence from metal nanoclusters and quantum dots for highly sensitive biosensing

Yahua Liu, Ping Dong, Qunying Jiang, Fuan Wang, Dai-Wen Pang, Xiaoqing Liu*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, PR China

ARTICLE INFO

Keywords:
Biosensor
Gold nanocluster
Quantum dot
Self-assembly
Enzyme

ABSTRACT

Fluorescent nanoparticles exhibit unique optical properties for applications in imaging and sensing. Herein, the enhancement of luminescence emission from self-assembled nanoparticles including gold nanoclusters, cadmium sulphide and near-infrared silver selenide quantum dots are reported. Addition of zinc and pyrophosphate ions induces assembly and disassembly of the particle aggregates and the corresponding intensified and recovery of fluorescence intensity, respectively. The enhancement is due to assembly-enhanced emission of metal nanoclusters and passivation of the surface trap states of quantum dots. With the hydrolysis of pyrophosphate catalyzed by alkaline phosphatase, the released zinc ions from pyrophosphate-zinc ion complexes mediate nanoparticle assembly and lead to luminescence regeneration. The assembly-induced fluorescence enhancement can be applied for sensitive assay of alkaline phosphatase with a detection limit of 3.4 pM in a continuous and real-time way. This method is capable of screening enzyme inhibitor and analyzing biological samples.

1. Introduction

Alkaline phosphatase (ALP) is an important enzyme existing widely in intestine, liver, bone, kidney and placenta, which is responsible for the dephosphorylation process of proteins, nucleic acids, and small molecules in biological organisms [1,2]. In different tissues, ALP has different biological functions by dephosphorylation of different physiological substrates. The interest in ALP assay rests in its diverse possible applications such as acting as an important biomarker of clinical diagnosis and a common label for enzyme immunoassays [3,4]. For example, abnormal level of ALP can be used as an important anomaly index of bone disease, liver dysfunction, breast cancer, prostate cancer and diabetes. Additionally, ALP conjugated to antibodies and other proteins is widely used for ELISA, Western blotting, and histochemical detection [5,6]. So far, many methods have been developed for the detection of ALP activity such as colorimetry [7–9], fluorescence [10,11], surface enhanced Raman spectroscopy [12], nuclear magnetic resonance [13] and electrochemical methods [14]. Among these methods one appealing technique is fluorescent assay considering its simplicity and capability for real-time detection of ALP [15–17]. One kind of fluorescent assay involves nanomaterials-based hybrid system where fluorescent nanoparticles such as quantum dots [11], polymer nanoparticles [18] and supramolecular hydrogels [19] are covalently modified using phosphatase substrate. Another kind of fluorescent assay is based on fluorophore derivatives containing phosphate group for electrostatic interaction [20]. As a promising alternative, unmodified fluorescent materials including graphene quantum dots [21], silver clusters [22] and polymers [23] are also directly utilized for ALP activity assay by using copper ions as a quencher. The mechanism relies on the fact that copper ion has a higher affinity for pyrophosphate, a substrate for ALP, than its ALP-catalyzed product orthophosphate [8]. Despite these reported methods, simple but efficient approach for fluorescent detection of ALP is still in its infancy. Therefore, development of highly sensitive ALP activity assay is vitally important.

As a kind of metal nanoclusters, luminescent gold nanoclusters (AuNCs) typically contain several to tens of metal atoms [24,25]. They possess ultrasmall sizes, exhibit molecule-like properties including size-dependent fluorescence [26–28], and discrete size-dependent electronic state [29,30]. Owing to the ultra small size, colloid stability, low toxicity and good biocompatibility, AuNCs have been widely used for sensing and imaging [31–36]. More importantly, recent reports reveal that metal nanoclusters synthesized from small thiol-containing molecules possess unique property of aggregation-induced emission enhancement by addition of specific solvent or through polymer assembly. It is believed that high compactness can suppress intramolecular vibration and rotation of capping ligand and enhance the emission intensity of nanoclusters [37–39]. This optical property of AuNCs has been utilized for detection of chemical species including metal cations,

* Corresponding author.
E-mail address: xiaoqingliu@whu.edu.cn (X. Liu).

https://doi.org/10.1016/j.snb.2018.10.016
Received 22 June 2018; Received in revised form 1 October 2018; Accepted 4 October 2018
Available online 05 October 2018
0925-4005/

anions, and small molecules of interest [40–42]. However, to the best of our knowledge, only few fluorescent assays based on assembly-enhanced fluorescence of AuNCs have been proposed, and none of the fluorescent sensors have been designed to assay biomolecules such as enzyme to date.

Besides metal nanoclusters, fluorescence of cysteine-capped cadmium sulphide (CdS QDs) could be improved by formation of aggregates, however, no related study or applications have been reported [43]. Considering the unique optical properties of semiconductor QDs such as high quantum yield and extinction coefficient, size-tunable narrow emission, as well as photochemical stability [44,45], biosensors based on assembly-enhanced fluorescence of semiconductor QDs may find more biological applications. Moreover, further study on assembly-enhanced fluorescence using semiconductor QDs such as near infraed-emiting silver selenide (Ag_2Se) QDs, which are more environmentally-friendly and stable, will facilitate the development of fluorescence biosensors.

Herein, we developed a fluorescent method for biosensing of Zn^{2+}, pyrophosphate (PPi), and real-time detection of ALP activity based on assembly-enhanced fluorescence by using nanoparticles including AuNCs, CdS and Ag_2Se QDs. As shown in Scheme 1, all the studied fluorescence nanoparticles can rapidly self-assemble and form aggregates in the presence of Zn^{2+}, resulting in enhanced luminescence emission. Upon introduction of PPi, stronger binding affinity between Zn^{2+} and PPi leads to disassembly of the aggregates and luminescence quenching. As a result, the fluorescent probes have selective response to Zn^{2+} or PPi. In particular, the presence of ALP causes rapid breakdown of the PPi-Zn^{2+} complexes and luminescence enhancement of the system, due to released free Zn^{2+} and the corresponding Zn^{2+}-induced assembly of the nanoparticles. The assembly-induced fluorescence enhancement is applied for monitoring ALP levels and its inhibitor in a continuous and real-time way. Furthermore, this method can be used for analyzing biological samples. Correspondingly, sensitive and simple assays for biosensing are successfully established by the assembly-enhanced fluorescence emission from functionalized nanoparticles.

2. Material and methods

2.1. Chemicals and reagents

Hydrogen tetrachloroauratetrihydrate ($HAuCl_4{\cdot}3H_2O$), L-Glutathione in the reduced form, pyrophosphate (PPi), alkaline phosphatase from bovine intestinal mucosa (ALP), and zinc chloride were obtained from Sigma-Aldrich. Yeast glutathione reductase (≥100 unites/mg protein) was bought from Calbiochem. Reduced β-nicotinamide adenine dinucleotide 2′phosphate tetrasodium salt (NADPH) was purchased from Roche. Other chemicals such as lalanine, silver nitrate, cadmium nitrate tetrahydrate, L-cysteine, and sodium sulfide were all analytical grade. All solutions were prepared using ultrapure water (Millipore).

2.2. Characterizations

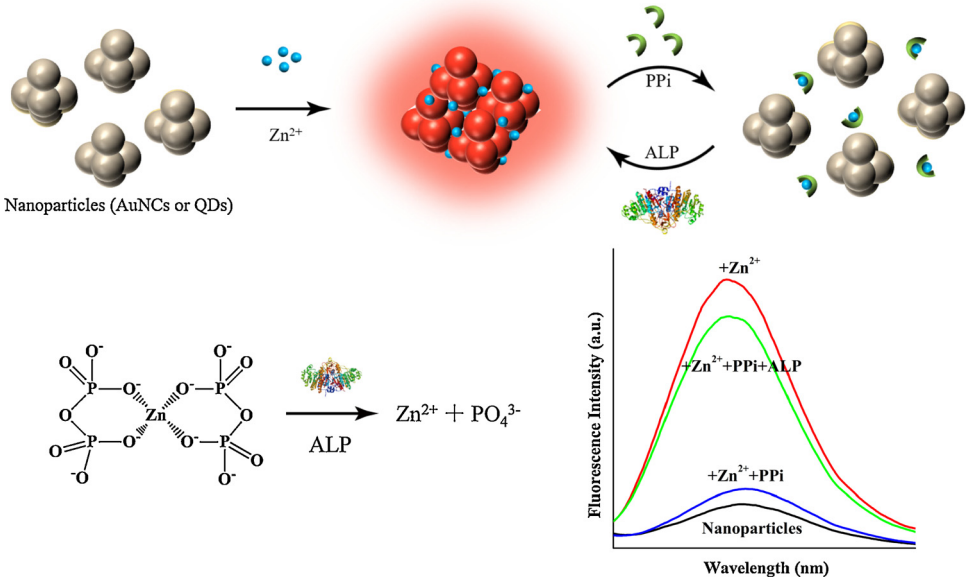
Fluorescence spectra were collected with Cary Eclipse spectrometer (Varian Inc). The fluorescence decay curves were performed using a FELIX 32 system (Photon Technology International, U. S. A.). UV–vis absorption spectra were recorded by a Shimadu UV-2600 spectrometer. The transmission-electron microscopy (TEM) images were obtained with Hitachi HT-7700 electron microscope. The atomic force microscope (AFM) images were obtained with an atomic force microscope cantilever (SCANASYST-AIR). The high resolution TEM (HRTEM) measurements were carried out by using an JEOL JEM-2100 with an accelerating voltage of 200 kV. All optical measurements were performed at room temperature.

2.3. Preparation of AuNCs

AuNCs were prepared according to a reported method [38]. Freshly prepared aqueous solutions of $HAuCl_4$ (20 mM, 0.50 mL) and glutathione (100 mM, 0.15 mL) were mixed with 4.35 mL of ultrapure water at 25 °C. The reaction mixture was heated to 70 °C under gentle stirring (500 rpm) for 24 h to get orange-emitting AuNCs. The AuNCs were precipitated by addition of isopropanol and washed with isopropanol repeatedly for three times. The product was then dispersed in ultrapure water and could be stored in the refrigerator (ca. 4 °C) for a long time with negligible changes in their optical properties.

2.4. Preparation of L-cysteine-capped CdS quantum dots

CdS QDs were prepared as follows with little modification [43]. A 0.025 mmol portion of $Cd(NO_3)_2$ and 0.025 mmol of L-cysteine (0.025 mmol) were dissolved in 25 mL of deionized water and purged with pure nitrogen gas for at least 30 min under magnetic stirring. A 0.025 mmol portion of Na_2S dissolved in 1.25 mL water was added to



Scheme 1. Schematic illustration of assembly-induced fluorescence enhancement for ALP assay.