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Colorimetric and fluorescent dual-mode sensing of alkaline phosphatase activity in L-02 cells and its application in living cell imaging based on in-situ growth of silver nanoparticles on graphene quantum dots

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ABSTRACT

Herein we report a new colorimetric and fluorescent dual-mode sensor for alkaline phosphatase (ALP) activity based on the specific enzyme amplification of ALP and the unique optical properties of graphene quantum dots (GQDs)/silver nanoparticles (AgNPs) hybrid. In this strategy, ALP enabled the removal of phosphate group from ascorbic acid 2-phosphate to yield ascorbic acid. Silver ions could be attached on the surfaces of GQDs via electrostatic interaction and reduced by ascorbic acid to produce AgNPs, which in-situ grew on the surfaces of GQDs, accompanied by a substantial increase in the SPR band of AgNPs and an evident fluorescence quenching of GQDs simultaneously. AgNPs acted as a "nanoquencher" to decrease the fluorescence of GQDs by fluorescence resonance energy transfer from GQDs (donor) to AgNPs (acceptor). The mechanism of ALP sensor was examined by transmission electron microscope (TEM), atomic force microscope (AFM), energy dispersive spectrometer (EDS) and elemental mapping. Under optimal conditions, the detection limits of ALP activity are as low as 0.1 U/L and 0.02 U/L by colorimetric and fluorometric method, respectively. The dual-mode sensor could discriminatively detect ALP in L-02 cell lysates with the recoveries ranging from 93.9% to 106.5%. The probe can be employed to monitor the ALP levels in L-02 cells related to different extents of alcoholic fatty liver injury. The images of confocal laser scanning microscopy reflect that the GQD-based sensor was successfully applied to intracellular imaging of ALP activity in L-02 cells due to its favorable biocompatibility and outstanding fluorescent property.

1. Introduction

Alkaline phosphatase (ALP), a group of glycoprotein enzymes, can catalyze the dephosphorylation process of nucleic acids, proteins, and some small molecules [1]. The occurrence and development of certain diseases, including hepatobiliary disease, adynamic bone disease, breast and prostatic cancer and diabetes have been revealed to be associated with the abnormality of ALP activity [2–5]. Therefore, the sensitive detection of ALP activity by using simple and low-cost assays is important in clinical diagnostics. In recent years, the signal amplification sensors based on combination of organic molecules with nanomaterials have gained increasingly importance in monitoring ALP activity with added benefits for rapid analysis and direct measurement. Several different indicators, such as metal nanoparticles [6,7], graphene quantum dots (GQDs) [8–10], Copper sulfide nanoparticle-decorated graphene sheet [11], carbon quantum dots [12,13] have been designed for the determination of ALP. However, in contrast to single readout based techniques, multi-signal sensing assays of ALP have attracted more attention because they offered more than one kind of output mode simultaneously, thus providing improved accuracy and attractive diversity [14,15]. Considering this, the exploration of a novel dual-mode sensing platform for sensitive and selective detection of ALP activity is of great significance.

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GQDs are discovered as a class of zero-dimensional graphitic nanocrystals with tiny sizes of only several nanometers, strong quantum confinement and edge effects, hence exhibiting tunable photoluminescence [16]. In addition, GQDs has been found to be overwhelmingly superior to traditional semiconductor quantum dots in terms of low toxicity, excellent solubility, high biocompatibility and robust chemical inertness. These emerging properties endowed GQDs with many potential applications, such as cellular imaging [17,18], drug delivery [19], catalysis [20], and biosensor [21–25]. So far, many researchers have devoted their efforts to explore GQD-based sensing systems for metal ions (Cu^{2+}, Fe^{3+} and Hg^{2+}) [26,27], small biomolecules (L-cysteine, dopamine and glucose) [24,28,29], enzymes [8,30,31], biomarkers [32,33], and nucleic acid sequences (miRNA, DNA) [25,34]. The widely used approaches of constructing sensors included fluorescence resonance energy transfer [24,25], electron transfer process [28,35], inner filter effect [29], and aggregation [36,37].

Silver-based nanomaterials attracted substantial research interest due to rich, intriguing, and complex optical properties originating from the excitation of surface plasmon resonances (SPR) [38,39]. In particular, its localized SPR absorption was extremely sensitive to size, composition, distance, and surrounding media [40], which offered an excellent opportunity to construct devices and assays with unparalleled functionalities for highly sensitive target analysis. Recently, the fluorescent or colorimetric single-mode sensing strategy has been developed for the detection of prostate specific antigen [32], hydrogen peroxide (H_2O_2) and glucose [41] based on GQDs/AgNPs hybrid as probe. When AgNPs was removed via oxidative etching by H_2O_2, the incorporated GQDs could be readily released, exhibiting a "turn-on" fluorescence signal of GQDs [32] or a fading absorbance response of AgNPs [41]. To the best of our knowledge, no literature reported about the in-situ growth of surfactant-free AgNPs on the surfaces of GQDs for the dual-mode sensing of ALP activity.

Intrigued by the above facts, we herein developed a new, convenient and dual-mode sensor with both colorimetric and fluorometric readout for detecting ALP activity by using ascorbic acid-phosphate (AA-P) as the substrate. ALP enabled the removal of phosphate group from AA-P to yield ascorbic acid (AA), which reduced silver ions to produce AgNPs. The construction of sensor for ALP activity was based on the in-situ growth of AgNPs on the surfaces of GQDs, accompanied by a substantial increase in the SPR band of AgNPs and an evident fluorescence quenching of GQDs simultaneously. The proposed sensor for ALP activity exhibits high sensitivity, with the detection limits as low as 0.1 U/L and 0.02 U/L by colorimetric and fluorometric measurements, respectively. Besides, the influences of potential interfering substances in cell lysate including ALT, AST, Try, GOx, HSA and BSA were performed. It was found that only target ALP could result in the formation of GQDs/AgNPs hybrid, and the potential interfering substances had no obvious effect on the absorbance/fluorescence intensity of tested solutions. The utility of sensing platform was explored by monitoring the endogenous variation of ALP levels in L-02 cells as a result of alcohol induced damage. More importantly, due to the good biocompatibility and fluorescent property of GQDs, the sensor could be applied for in vitro imaging of ALP in living cells.

2. Experimental

2.1. Materials

All chemicals were of analytical grade and used without further purification. Citric acid, alcohol and oleic acid were obtained from Simopharm Chemical Reagent Co., Ltd (Shanghai, China). Silver nitrate ($AgNO_3$) was purchased from Shanghai Shengbo Chemical industry Co., Ltd (Shanghai, China). L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate (AA-P), alkaline phosphatase (ALP), Alanine transaminase (ALT), aspartate aminotransferase (AST), Trypsin (Try), glucose oxidase (GOx), human serum albumin (HSA), Bovine serum albumin (BSA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (USA). Fetal Bovine Serum, dimethyl Sulphoxide (DMSO), phosphate buffer solution (PBS, 10 mM, pH 7.4) and Tris was provided by Biyotime Institute of Biotechnology (Shanghai, China). Human hepatic L-02 cells were purchased from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Milli-Q water (resistance >18 MΩ cm) from a Millipore system (Bedford, MA) was used throughout the experiments.

2.2. Instruments

UV–visible absorption spectra were recorded using an Agilent 8453 UV–vis spectrometer (Agilent, USA). Fluorescence measurements were performed on a Shimadzu RF-5301 PC fluorescence spectrophotometer. Transmission electron microscope (TEM) and high resolution transmission electron microscopy (HRTEM) images and Energy-dispersive spectrum (EDS) were taken on a JEOL JEM-2100F field-emission high-resolution transmission electron microscope operated at 200 kV. Scanning transmission electron microscopy (STEM) images and EDS mapping profiles were collected on a JEOL ARM-200F HAADF field-emission transmission electron microscope operated at 200 kV. Atomic force microscopy (AFM) study was performed by means of Veeco DI Nano-scope MultiMode V system. Diameter distribution and zeta potential measurement was performed by Mastersizer 2S-90 (Melvin Instrument Co., Ltd.). The cell imaging was observed by a laser scanning confocal microscope (Leica, Germany).

2.3. Preparation of GQDs

GQDs were synthesized by the pyrolysis of citric acid according to previously reported method with slight modifications [42]. Firstly, 2 g of citric acid was placed in a vial and heated to 200 °C with a heating mantle for 30 min. During this time the citric acid turned from solid to colorless liquid to pale orange, implying the formation of GQDs. And then, the orange liquid was added dropwise into 100 mL of 0.25 M NaOH solution under vigorous stirring for 10 min. Lastly, the GQDs were dialyzed for 48 h with the dialysis membrane of 1000 cutoffs and stored at 4 °C after vacuum freeze drying for use.

2.4. Procedures for sensing ALP activity

To a series of 5 mL calibrated test tubes, 50 μL of AA-P (24 mM) and 20 μL of different concentrations of ALP were mixed in 200 μL of Tris buffer (10 mM, pH 9.8, containing 1 mM $MgSO_4$) and incubated at 37 °C for 15 min. Subsequently, 50 μL of GQDs (10 mg/mL) and 100 μL of $AgNO_3$ (24 mM) was successively added to the above solution and diluted to 2 mL with ultrapure water. The mixed solution was shaken and incubated at room temperature for another 45 min. Finally, the mixed solution was transferred separately into 1 cm quartz cuvette and directly monitored by UV–vis spectrometer and fluorescence spectrophotometer, respectively. The ΔA_{415} signal ($\Delta A = A - A_0$, where A and A_0 are the absorbance at 415 nm in the presence and absence of ALP) and fluorescence quenching efficiency (Effq (%) = $(F_0 - F)/F_0$, where F and F_0 are the fluorescence intensity of sensing system in the presence and absence of ALP) were used as a criterion to appraise the performance of colorimetric and fluorescent sensor, respectively.