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Water-soluble gold nanoclusters prepared by protein-ligand interaction as fluorescent probe for real-time assay of pyrophosphatase activity

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ABSTRACT

This paper reports a new and facile method for the synthesis of water-soluble thiolate-protected AuNCs *via* protein-ligand interaction. Using 3-mercaptopropionic acid (MPA) as a model ligand and bovine serum albumin (BSA) as a model protein, water-soluble AuNCs (BSA/MPA-AuNCs) with intense orange-yellow fluorescent emission (quantum yield = 16%) are obtained. Results show that AuNCs produced with this method have hydrophobic interactions with BSA. The synthetic strategy is then successfully extended to produce water-soluble AuNCs protected by other thiolates. Moreover, a sensitive and eco-friendly sensing system is established for detection of the activity of inorganic pyrophosphatase (PPase), which relies on the selective coordination of Fe^{3+} with BSA/MPA-AuNCs, the higher affinity between pyrophosphate (PPi) and Fe^{3+}, and the hydrolysis of PPi by PPase. A good linearity between the fluorescence intensity and PPase activity within the range from 0.1 to 3 U/L is found, with a detection limit down to 0.07 U/L. Additionally, the fluorescent assay developed here is utilized to assay the PPase activity in real biological samples and as well as to evaluate PPase inhibitor, illustrating the great potential for biological analysis.

1. Introduction

Metal nanoclusters (NCs) comprised of several to tens of atoms have a size approaching the Fermi wavelength of the conduction electrons, resulting in unique physical, electrical, and optical properties, including HOMO-LUMO transitions (Zhu et al., 2008), magnetism (Zhu et al., 2009), optical chirality (Zeng et al., 2013), quantized charging (Chen et al., 1998), and strong photoluminescence (Yuan et al., 2013). Such properties are substantially different from the physicochemical characteristics of corresponding larger nanocrystals. Among various metal NCs, Au nanoclusters (AuNCs) have been extensively investigated because of their high chemical stability, low toxicity, and excellent biocompatibility. Owing to the strong interaction between thiol and gold, thiolate is the most commonly adopted protector in AuNC synthesis. A number of well-defined thiolate-protected AuNCs have been reported with precise control at the atomic level (Chen et al., 2015; Das et al., 2014, 2015; Yu et al., 2014; Zeng et al., 2014). However, such nanoclusters typically include organic-soluble ligands, thus limiting their solubility to organic solvents. Water-soluble AuNCs are highly desirable in many fields, including molecular markers, sensors, catalysts, and drug delivery materials. Until now, a few small thiolate ligands, such as methionine (Deng et al., 2015b), glutathione (Negishi et al., 2004), dihydrolipoic acid (Shang et al., 2011a), 11-mercaptoundecanoic acid (Sun et al., 2013), and *N*-acetyl-*L*-cysteine (Deng et al., 2015a) have been used for the preparation of water-soluble AuNCs. However, many synthetic approaches, for example two-phase (Yuan et al., 2011), etching (Zhou et al., 2009), and ligand-exchange methods (Shichibu et al., 2005), are time-consuming and involve relatively complex procedures and post-treatment, hampering their practical applications. Moreover, thiolate ligands are very limited and terminal carboxylic acid groups (–COOH) are often required to stabilize the Au core. However, because the aqueous solubility of these clusters is usually related to solution pH, the presence of hydrogen bond-forming functional groups like –COOH and $–NH_2$ can cause particle aggregation by forming bonds between clusters.

It is well known that nanoparticles (NPs), when exposed to a biological medium, can interact with biomolecules (*e.g.*, protein,

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DNA, lipids) because of their small size and large surface-to-volume ratio. Particularly, the study of protein-NP interactions has become one of the most fascinating areas of basic and applied research (Mahmoudi et al., 2011). The formation of a "protein corona" around NPs has been shown to have an impact on their surface properties, charge, anti-aggregation properties, and hydrodynamic size (Nel et al., 2009). Considering that ultrasmall NCs have a higher surface-to-volume ratio than large NPs, protein-NC interactions have attracted some attention. The adsorbed protein layer on a NC surface was reported to markedly change the fluorescent properties, uptake behavior, and cytotoxicity of NCs (Shang et al., 2012a, 2012b; Xu et al., 2014b).

Inorganic pyrophosphatase (PPase) can specifically catalyze the hydrolysis of inorganic pyrophosphate (PPi) to orthophosphate (Pi), which provides a thermodynamic pull for biosynthetic reactions (Lahti, 1983). Thus, PPase plays a crucial role in many biological processes, such as phosphorus and carbohydrate metabolism, calcium absorption, and DNA synthesis. Besides, an abnormal level of PPase is also related to some diseases including colorectal cancer (Friedman et al., 2004), hyperthyroidism (Koike et al., 2006), and lung adenocarcinomas (Lu et al., 2004). To date, several conventional approaches based on enzymatic, chromatographic, and spectral methods have been established for the assays of PPase activity (Bloemers et al., 1970; Eriksson et al., 2001; Vance and Czarnik, 1994). Among these techniques, fluorescence detection is superior in terms of simplicity, specificity, sensitivity, rapidity, and high spatial and temporal resolution. Compared with widely used organic dyes, where practical application is limit by biotoxicity, poor photostability, and spontaneous autoxidation, AuNCs are a promising alternative in the construction of biosensors due to their ultrasmall size, low toxcity, good biocompatibility, and excellent photostabilty. Therefore, the design of AuNCs-based sensor for the detection of PPase activity should be appealing.

Herein, we converted water-insoluble AuNCs to water-soluble AuNCs *via* protein-ligand interaction. Water-soluble AuNCs with intense orange-yellow fluorescent emission were prepared with 3-mercaptopropionic acid (MPA) as a model ligand and bovine serum albumin (BSA) as a model protein. The AuNCs fabricated in this manner have an ultrasmall Au(0) core surrounded by Au(I)-MPA shells attracted to BSA through hydrophobic interactions. Diverse water-soluble AuNCs could be further produced by tuning the proteins and thiolate ligands. Furthermore, the as-prepared BSA/MPA-AuNCs can serve as an efficient fluorescent nanoprobes for sensitive and selective detection of the activity of PPase, demonstrating their great feasibility for applications in life science and environmental monitoring.

2. Experimental

2.1. Chemical and materials

All chemicals and solvents were of analytical grade and commercially available. PPase was purchased from Sigma-Aldrich (Shanghai, China). MPA, 2-mercaptoimidazole (MIZ), 1*H*-1,2,4-triazole-3-thiol (TZT), and 1-methyl-5-mercaptotetrazole (MMT) were purchased from Adamas Reagent Co. Ltd. (Shanghai, China). $FeCl_3 \cdot 6H_2O$, sodium pyrophosphate ($Na_4P_2O_7$), KF, $HAuCl_4 \cdot 4H_2O$ and proteinase K were obtained from Aladdin Reagent Company (Shanghai, China). CH_3COONa (NaAc), CH_3COOH (HAc), BSA and NaOH were bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Deionized water was used throughout the experiments. All glassware was washed with aqua regia and rinsed with water.

2.2. Apparatus and characterization

UV–vis absorption spectra and photoluminescence (PL) spectra were measured using a UV-2450 UV–vis spectrophotometer (Shimadzu, Japan) and Cary Eclipse fluorescence spectrophotometer (Agilent, USA), respectively. Circular dichroism (CD) spectra were obtained using a Jasco J-810 spectropolarimeter. IR spectra were measured at wavenumbers 400–4000 cm^{-1} using a Nicolet Avatar 360 FT-IR spectrophotometer. Absolute quantum yield measurements were carried out using an integrating sphere (Edinburgh Instruments). Time decay measurements were conducted on a F900 time-correlated single-photon-counting fluorescence lifetime spectrometer (Edinburgh Analytical Instruments, UK). Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were collected with a JEM-2100 microscope (JEOL, Japan). Height characterization was carried out by atomic force microscopy (AFM) (from Veeco with a IIIa controller) operating in tapping mode. X-ray photoelectron spectroscopy (XPS) was performed using an ESCALAB 250 XI electron spectrometer (Thermo, USA) using monochromatic Al Kα radiation (1486.6 eV) for analysis of the surface composition and chemical states of the product. Binding energy calibration was based on C 1 s at 284.8 eV. X-ray diffraction patterns were obtained with a Bruker D8 Advance diffractometer.

2.3. One-step synthesis of BSA/MPA-AuNCs

Aqueous $HAuCl_4$ (2.5 mL, 10 mM) and NaOH (0.25 mL, 1 M) solutions were added to BSA solution (2.5 mL, 50 mg/mL). MPA solution (0.25 mL, 4 M) was then introduced, and the mixture was incubated at 4 °C for 1 h. The solution changed from light yellow to colorless. After synthesis was complete, the solution was dialyzed with a 7 kDa cut-off dialysis bag for more than 48 h to remove all small molecular impurities. The resulting solution of AuNCs was stored in the dark at 4 °C for later use.

2.4. Fluorescent assay of PPase and inhibitor

PPase activity detection was realized as follows: first, 100 μL solution containing different concentrations of PPase was added into 135 μL NaAc-HAc buffer solution (50 mM, pH = 7.4) containing 70 μM Mg^{2+} and 16 μM PPi. The solution was incubated in a water bath at 37 °C for 30 min. Then, 45 μL HAc was added to adjust the pH to 3.0. After that, 20 μL as-prepared BSA/MPA-AuNCs and 200 μL Fe^{3+} (5 μM) solutions were added to the above solution and the mixed solution was incubated in a water bath at room temperature for 10 min. Finally, the fluorescence intensity at 575 nm (F_{575}) was recorded. To evaluate the inhibition effect of KF on PPase activity, PPase (4 U/L) was pretreated with different concentrations of KF. The followed detection procedure was the same as that of PPase activity detection.

3. Results and discussion

3.1. BSA-mediated formation of water-soluble AuNCs with intense orange-yellow fluorescent emissions

We found that AuNCs prepared using MPA as the reducing-cum-protecting agent (MPA-AuNCs) tended to aggregate in aqueous solution, due to carboxyl group-induced formation of hydrogen bonds between clusters. Intriguingly, when BSA was present during the synthetic procedure, MPA-AuNCs readily dispersed in water (BSA/MPA-AuNCs). Fig. 1A clearly shows that the significant photoluminescence (PL) observed in BSA/MPA-AuNCs stemmed from MPA-AuNCs, while BSA played a dominant role in