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Enhanced stability and fluorescence of mixed-proteins-protected gold/silver clusters used for mercury ions detection

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ABSTRACT

We have synthesized gold/silver nanoclusters (NCs) under the protection of both bovine-serum-albumin (BSA) and lysozyme (Lyz) and found that the NCs protected by the mixed-proteins give rise to largely enhanced stability and fluorescence, enabling promising applications for chemosensing and bioimaging. The enhanced fluorescence of Au NCs was found to be exclusively quenched at the presence of Hg^{2+} ions with an ultralow detection limit up to ~0.7 nM. It is also interesting to find that the BSA-Lyz-Au NCs are nontoxic and available for fluorescence imaging of Hg^{2+} detection in MCF-7 cells. The low cytotoxicity, good penetrability and fluorescence sensitivity of BSA-Lyz-Au NCs suggest promising biological applications. Similar observations were also addressed for the Ag system, indicating general applicability of this facile strategy based on BSA-Lyz mixed proteins for the protection of metal NCs.

1. Introduction

Heavy metal pollution has attracted much attention as it can cause severe health problems [1], among which mercury is one of the most toxic and dangerous chemicals in view of the wide existence of ore cinnabar in nature and the use as pigment vermilion and detoxification/anticorrosive medicines, as well as the use of mercury fulminate detonator in explosives. In particular, mercury can be introduced and accumulated by the food chain in the human body leading to permanent damage for the central nervous and endocrine systems [2–9]. The oxidized form (i.e., Hg^{2+}) is a source of contamination by forming a multitude of toxic binary compounds [10]. Therefore, trace detection of Hg^{2+} ions has long been a crucial subject in order to avoid unintentional intake which, however, give rise to preclinical harmfulness to the human body. It is notable that, mercury is also not allowed aboard an aircraft because a very small amount of mercury can seriously corrode aluminum by forming mercury-aluminum amalgam which readily destroys the surface-protective aluminum oxide layer.

Because of the unique electron configuration that all the 1s-6s subshells are filled up, mercury displays distinctive stability and strong resistance of electron detachment similar to noble gases. In view of this, trace detection of mercury via facile titration reactions is not available; instead, a few methods including inductively-coupled plasma mass spectrometry (ICPMS) [11–13], atomic absorption spectrometry (AAS) [14–16], atomic emission spectrometry (AES) [17], UV–vis absorption spectroscopy [18,19], have been successfully developed. However, these methods need sophisticated equipment and/or complicated sample preparation, hindering far-range and quick detections. In comparison, fluorescence chemo-sensing has found a convenient and effective approach for trace detection of mercury ions taking advantages of rapid response time, high sensitivity and good selectivity [2,20–24]. Numerous fluorescent probes and sensors (e.g., carbon nanodots [25], and graphene quantum dots [26], etc.) have been reported showing applications in the fields of biochemical sensing, imaging and cancer therapy. In the recent decade, luminous metal nanoclusters (NCs) have received substantial attention owing to the advantages of facile preparation, high fluorescence quantum yield, favorable photo-stability and excellent biocompatibility [27–45]. Owing to strong quantum confinement of free electrons, fluorescent metal NCs with sizes being small enough to approach the De Broglie wavelength of conduction electrons possess discrete electronic states and exhibit interesting molecule orbitals and size-

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dependent properties, giving rise to uprising research interest in a wide scientific community [46,47].

Biomolecule-protected metal nanoclusters (or Bio-NCs in short) have been found to be a smart design to gain good biocompatibility [48–50]. Protein-protected Au/Ag NCs could be similar to the naturally occurring metalloproteins or proteins with metal ion cofactors. Several proteins, such as bovine serum albumin (BSA) [45,51], insulin [52], horseradish peroxidase [53], pepsin [54], lactotransferrin [55], and lysozyme [7,56] have been employed as templates for the preparation of fluorescent metal NCs, showing new biomedical functions to improve their performance applied in chemosensing, bioimaging, and therapeutics. For example, glutathione-capped gold nanoclusters have been used for the detection of Cu^{2+} ions [57]; human-serum-albumin (HSA) stabilized gold nanoclusters have been applied for the detection of free bilirubin in blood serum as fluorometric and colorimetric probe [58]. A colorimetric detection system for glucose was developed based on the peroxidase-like activity of apoferritin-paired gold clusters [59]. Recently Xie et al. [24] found that the BSA-stabilized gold nanoclusters are one of the best candidates for sensing Hg^{2+}, and concluded that a key of fluorescence chemosensing for detecting ions such as Hg^{2+} is to employ effective fluorescents which are selectively sensitive enough (quenching or enhancing) to the presence of aim ions.

In this study, we have synthesized highly fluorescent Au and Ag NCs by using two different-sized proteins (namely BSA and Lyz) as stabilizer and reducer. We present a new label-free technique for the detection of Hg^{2+}, which relies on the metallophilic Hg-Au (or Hg-Ag) interactions to quench the fluorescence of the NCs. This one-step method is simple, fast, highly selective, and ultrasensitive. Together with discussion of the sensing mechanism, toxicity of the Au (and Ag) NCs is tested illustrating an application for cancer cell imaging of MCF-7 cells.

2. Material and methods

2.1. Synthesis of gold/silver nanoclusters

Bovine serum albumin (BSA) was purchased from Amresco (Ohio, USA). Chloroauric acid trihydrate ($HAuCl_4{\cdot}3H_2O$) and lysozyme were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH) and chloride salts including Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Al^{3+}, Cr^{3+} and Fe^{3+} were acquired from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All chemicals were used directly after purchase without further purification.

All glasswares for the experiments were cleaned with aqua regia (HCl:HNO_3 volume ratio = 3:1), and extensively rinsed with ultrapure water. The fluorescent BSA-Lyz-Au NCs are synthesized through BSA- and Lyz-mediated reduction of $HAuCl_4$ based on the previously reported method [60]. Briefly, 2 mL Lyz solution (20 mg/mL, 37 °C) was added to BSA solution (2 mL, 50 mg/mL, 37 °C) under vigorous stirring. After 10 min, 3 mL aqueous $HAuCl_4$ solution (10 mM, 37 °C) was added to this mixture and stirred for 15 min. Then, 1.0 M NaOH solution was introduced until the white precipitate completely disappeared (pH = 12), and the reaction was allowed to proceed under vigorous stirring at 37 °C for 12 h to get a clear brown colored solution. The product was dialyzed against ultrapure water for 24 h using a 1 kDa dialysis membrane, then concentrated by freezing (−80 °C) and drying under vacuum. The solid of Au NCs was dissolved to 15 mg/mL with water, and stored at 4 °C for further use.

Silver clusters were prepared keeping the protein and Ag concentration exactly the same as those used for the Au cluster synthesis. As revealed by previously reported investigation [61], gold can be reduced by tyrosine residues of proteins at basic pH, but due to lower reduction potential silver requires a stronger reducing agent. In this regard, sodium borohydride ($NaBH_4$) was used as the external reducing agent, and the solution was kept at 0 °C in an ice bath.

2.2. Characterization

UV–vis absorption spectra were recorded using a Shimadzu UV-3600 spectrophotometer. Fluorescence spectra were collected using a Horiba Scientific Fluoromax-4 spectrophotometer equipped with a quartz cuvette of 1.0 cm path length with a xenon lamp as the excitation source. Both the excitation and emission slit widths were set to 5 nm, and the measurement was done at 20 °C. The excited wavelength was 470 nm. Each spectrum was recorded after the average of three scans and the background spectrum was subtracted. The size distribution of particles was analyzed using dynamic light scattering (DLS) equipment Zetasizer Nano ZS ZEN3600 of Malvern Instruments Ltd. Transmission electron microscope (TEM) images were obtained on a JEOL JEM-2100F system with an accelerating voltage of 200 kV. The samples for TEM characterization were prepared by dropping the aim solution on a carbon-coated copper grid and allowing it to dry completely at room temperature.

2.3. Ions detection experiments

In a typical process, Hg^{2+} solutions of different concentrations were obtained by stepwise dilution of a concentrated stock solution (20 mM). 60 μL volume of BSA-Lyz NCs (15 mg/mL) was added to 300 μL of Tris–HCl buffer solution (50 mM, pH 7.4), followed by the addition of different concentrations of Hg^{2+} solutions, and then, the volume of the mixtures was adjusted to 3 mL with ultrapure water. The mixture solution was completely mixed with a vortex mixer at room temperature for a few seconds to accelerate the chelation reaction. The mixtures were equilibrated at room temperature for 15 min before the fluorescence spectra were recorded.

To evaluate the selectivity of Au and Ag clusters for Hg^{2+} detection, a series of competitive metal ions, including Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Al^{3+}, Cr^{3+} and Fe^{3+}, at a final concentration of 20 μM, were tested following the same experimental procedure.

2.4. Toxicity test and bioimaging

The in vitro cytotoxicity was measured using the methyl thiazolyl tetrazolium (MTT) assay in MCF-7 cell lines. Cells in log-growth phase were seeded into a 96-well cell-culture plate at 1×10^4 per well in 100 μL DMEM (Dulbecco's modified Eagle's medium). BSA-Lyz-Au NCs were added to the wells (100 μL per well) of the treatment group, and the final concentration ranged from 0 to 50 μg/mL. The cells were incubated for 24 h at 37 °C under 5% CO_2. A combined MTT/PBS solution (100 μL, 0.5 mg/mL) was added to each well of the 96-well assay plate, and incubated for an additional 2 h. An enzyme-linked immunosorbent assay reader (μQuant, Bio-Tek, USA) was used to measure the OD570 (absorbance value) of each well referenced at 690 nm. The formula used to calculate the viability of cell growth is: viability (%) = (mean absorbance value of treatment group/mean absorbance value of control) × 100.

Prior to the bioimaging experiment, MCF-7 cells were cultured in DMEM. Cells were incubated with 50 μg/mL BSA-Lyz-Au NCs at 37 °C for 2 h. After washing with PBS three times to remove remaining BSA-Lyz-Au NCs, the cells were then incubated with an appropriate concentration of Hg^{2+} solution for 30 min at room temperature. The incubated cells were washed with PBS and mounted