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Photoluminescence from water-soluble BSA-protected gold nanoparticles

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Abstract

The photoluminescence from water-soluble gold nanoparticles, each composed of a 5.1 nm gold core and a bovine serum albumin (BSA)-protected layer, has been observed. The maximal excitation and the maximal emission wavelength are at 320 and 404 nm, respectively. The photoluminescence quantum yield is estimated as 0.053 ± 0.0070, at room temperature. The mechanism of the luminescence is hypothesized to be associated with interband transitions between the filled $5d^{10}$ band and $6(sp)^1$ conduction band. The photoluminescence is sensitive to pH, organic solvents and metal ions. These observations suggest that this nanoparticles are a viable alternative to organic fluorophores or semiconductor nanoparticles for biological labeling and imaging.

Keywords: Photoluminescence; Bovine serum albumin; Gold nanoparticle; Fluorescence quantum yield

1. Introduction

The photoluminescence quantum yield from bulk gold and copper films is very low on the order of 10^{-10} [1] and has been proven to be unimportant technologically, so luminescence from metal received only a limited amount of attention [1–6]. The reason for the rarity of luminescence from metals is quite simple: metals do not have band gaps [6]. However, recently luminescence from nanometer-sized metallic and semiconductor particles has received increasing research attention because of their optical properties [7–9]. The reason is that at sizes comparable to the Fermi wavelength of an electron, optical properties are significantly modified, and discrete nanocluster energy levels become accessible [10–13]. Such metal nanoclusters, composed of only several tens of atoms, exhibit molecule-like transitions as the density of states are insufficient to merge the valence and conduction bands [13–17].

On a larger scale, luminescence studies have been conducted on nanometer-sized gold clusters in the visible region of the spectrum, where chromatographically separated gold clusters <5 nm in diameter were shown to be luminescent while larger 15 nm clusters were not [18]. Luminescence near 440 nm was measured for most of the clusters after exciting at 230 nm, which was attributed to sp to d interband transitions with a quantum yield of $\sim 10^{-4}$ to 10^{-5} [18]. However, more impressive photoluminescence involves field enhanced emission from only a few tens of atoms gold nanoclusters with a quantum yield in the range of from 10^{-4} to 10^{-2} [6,9,12,19–23].

Key issues in the study of very small nanoparticles are the size effects and contributions from surface characteristics. These issues are interrelated, since the surface-to-volume ratio increases as size decreases. Here, we report the visible luminescence of a new water-soluble nanoparticles, i.e., BSA-protected gold nanoparticles. Based on excitation at 320 nm and detection at 404 nm, and using a quinine sulfate standard, the photoluminescence quantum yield of the BSA-protected nanoparticle is very high, about 0.053. These water-soluble and high quantum yield gold nanoparticles lend insight into the molecular nature of small metal nanoclusters and may find application as novel biocompatible fluorophores.

2. Experimental

2.1. Chemicals

$HAuCl_4 \cdot 4H_2O$ (Shanghai Chemical Reagent Corporation, China >99.95%), BSA (Shanghai Bio Life Science & Technology Co., China), sodium borohydride (Tianjin Damao Chemical Reagent Factor, China, >99%) were used as received. Ultra-pure

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water was prepared with a Milli-Q Pure system (18.2 MΩ) and used throughout. All other chemicals were analytical grade and used as received.

2.2. Syntheses of BSA-protected gold nanoparticles

Widely used albumin, NH_3-terminated and COOH-terminated BSA was utilized to stabilize and solubilize gold nanoparticles in aqueous solutions. After 670 mg of BSA (10 μmol) and 12.5 mg of $HAuCl_4{\cdot}4H_2O$ (30 μmol) was dissolved in 100 mL of ultra-pure water, the obtained yellow solution was stirred more than 2 h. And then, ~0.153 g of $NaBH_4$ (4 mmol) was slowly added into this solution with rapid stirring for ~3 h. Reduced gold atoms aggregate within the BSA to form small nanoparticles and large nanoparticles. The ruby red solution was stirred for an additional day. At last, the product was purified through centrifugation (11 000 rpm) to remove the large gold nanoparticles, leaving a clear, dark red BSA-protected gold nanoparticles solution.

2.3. Transmission electron microscopy (TEM)

The size and dispersivity of the nanoparticles were determined by a JEOL-JEM 2010FEF TEM (Japan) operated at 200 kV, for which samples were dropped onto a copper grid covered with a thin film of amorphous carbon. From the enlarged TEM pictures, the size distribution was determined by counting more than 100 particles.

2.4. Spectroscopic measurements

The absorption spectrum and photoluminescence spectrum of BSA-protected gold nanoparticles were measured at room temperature. Absorption spectra (200–800 nm, 0.5 nm resolution) were recorded by a UV-2450-PC spectrophotometer (Shimadzu, Japan). The photoluminescence spectra were collected by a F-4500 fluorescence spectrometer (Hitachi, Japan) covering a wavelength range from the visible to the infrared (200–1100 nm).

3. Results and discussion

3.1. UV–vis absorption spectra and fluorescence spectra

The molar ratio of BSA to $HAuCl_4$ was optimized. In conditions that molar ratios of BSA to $HAuCl_4$ were 1:2, 1:4, 1:5, 1:6, 1:8, 1:12 and 1:24, no photoluminescence can be detected from the obtained nanoparticles. Only in the case of molar ratio was 1:3, fluorescent nanoparticles can be produced. This fluorescent nanoparticles were mono-dispersed with an average core diameter of 5.1 nm (counted from 130 particles). Fig. 1 shows TEM images of BSA-protected gold nanoparticles.

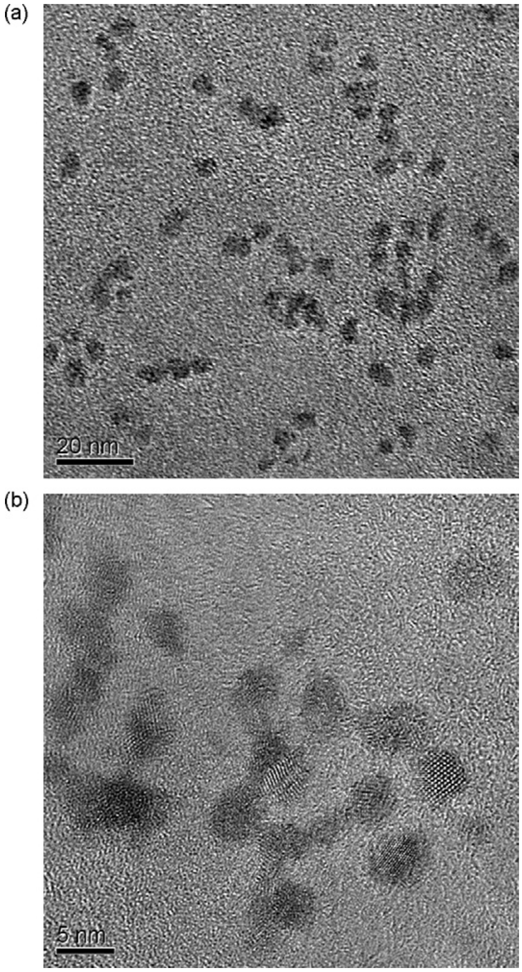

Fig. 1. TEM (a) and high resolution TEM micrograph (b) of gold nanoparticles.

Although weak as compared to the 280 nm pure BSA peak, a clear absorption spectrum of BSA-protected gold nanoparticles is obtained by subtracting the pure BSA absorption. It can be seen from Fig. 2B that a new absorption band at ~300 nm with a full width at half-maximum of ~70 nm appears in the final gold nanoparticles solutions. Contrary to the absorption spectrum of small gold nanoparticles [24,25], a weak surface plasmon absorption peak near 520 nm appeared, indicated that the nanoparticles were larger than 2 nm. This result is consistent with TEM data.

The excitation and emission maxima were at 320 and 404 nm, respectively (shown in Fig. 3). The emission from BSA was very weak under 320 nm excitation (Fig. 4). The possibility of the emission arising from some adventitious impurity from the reagents or synthesis was carefully scrutinized. BSA, $NaBH_4$, $HAuCl_4$, and the mixture of BSA and $NaBH_4$ did not fluoresce or emitted very weak fluorescence with excitation at 320 nm. Overall, the evidence is firm that the emission definitely originates from the BSA-protected nanoparticle itself. The crude BSA-protected nanoparticles is much less photoluminescent than following purification by centrifugation. It seemed that photoluminescence of BSA-protected nanoparticles was quenched by big gold particles. After centrifugate and remove the precipitate, photoluminescence intensity of product will enhance at least 10 times.

The BSA-protected gold nanoparticles are very stable in salt solution. After incubated in 3% NaCl solution for several days, no color change or UV–vis absorption change was observed. The

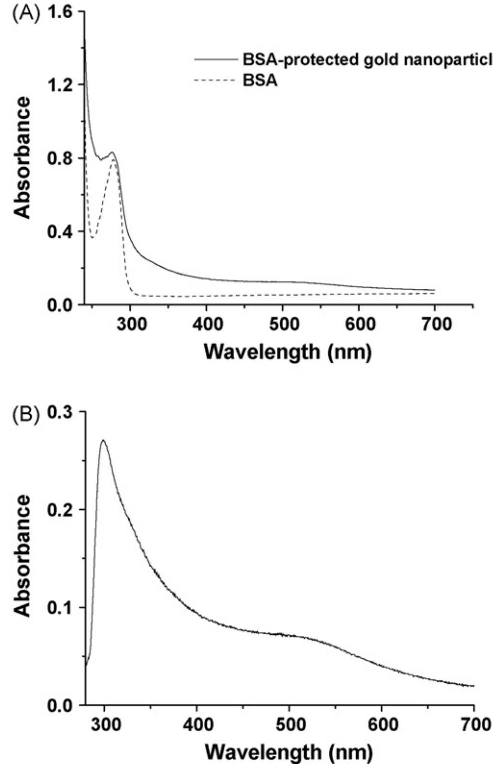

Fig. 2. UV–vis absorption spectra of BSA-protected gold nanoparticles and BSA (A), and subtraction of absorption spectra in A (B).

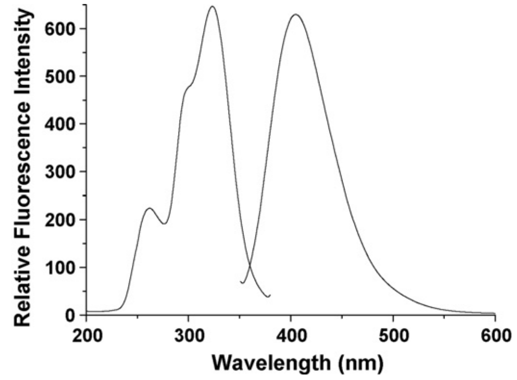

Fig. 3. Excitation and emission spectra of BSA-protected gold nanoparticles.

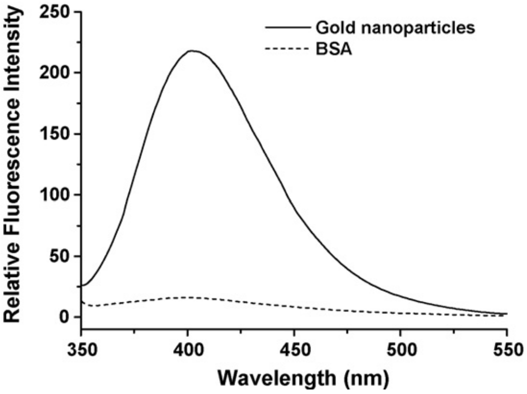

Fig. 4. Emission intensity of gold nanoparticles (solid line) and BSA (dashed line) at 320 nm excitation.

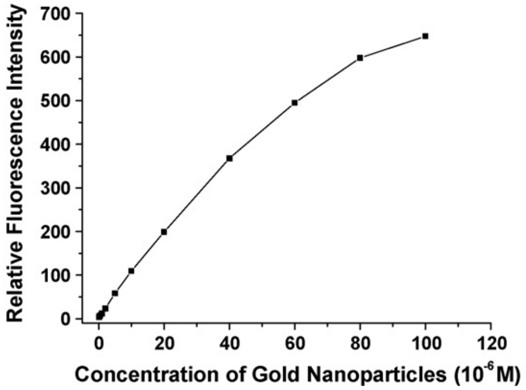

Fig. 5. Emission intensity from BSA-protected gold nanoparticles vs. concentration. The linear from 0.1 to 40 μM fit equation is $I_F = 9.2022 \times$ [gold nanoparticle] + 6.8284, $R = 0.9974$. The concentration of gold nanoparticles was given in concentration of BSA.

BSA-protected gold nanoparticles are also stable in phosphate buffer solution.

At low gold nanoparticles concentrations from 0.1 to 40 μM (in concentration of BSA), the fluorescence intensity changes linearly with concentration, as shown in Fig. 5. At higher concentrations, the emission intensity rolls over. The high-concentration effect is certainly associated with excessive absorption of the exciting beam by the dark nanoparticles solution (this has been seen [26]); there is also the possibility of self-quenching by the gold nanoparticles themselves [22].

3.2. *Quantum yield*

The intensity of the photoluminescence of the BSA-protected gold nanoparticle is comparable to those of quinine sulfate solutions at similar range of concentration. The quinine sulfate has quantum yield of 0.55 excitated at 313 nm [27,28]. We can conservatively estimate the quantum yield of gold nanoparticle to be 0.053 ± 0.0070 ($P = 0.90$). This quantum yield is approximately eight orders of magnitude greater than that of gold films.

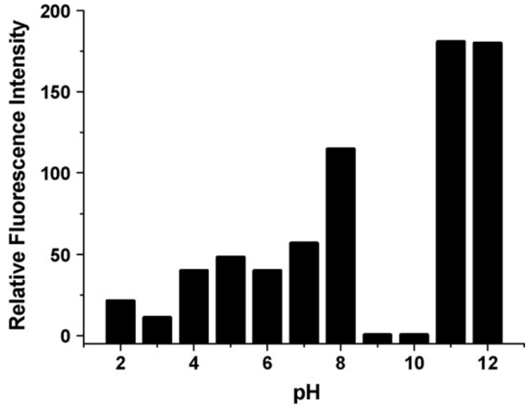

Fig. 6. pH effects on fluorescence.

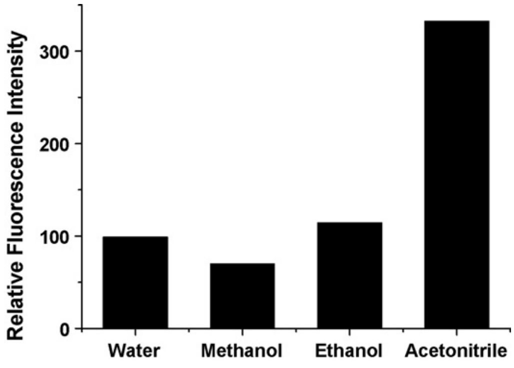

Fig. 7. Solvent effects on photoluminescence.

3.3. Factors influencing fluorescence of BSA-protected gold nanoparticles

Fig. 6 shows the pH effects on gold nanoparticles photoluminescence. The photoluminescence intensity reaches to the maximal value at pH 11 and 12, and drops sharply to minimal value at pH 9 and 10. The reason of this anomalous pH-dependent phenomenon is unknown.

The photoluminescence intensity of BSA-protected nanoparticle is also solvent-dependent (see Fig. 7). Compared to disperse in water, the photoluminescence decreased slightly as dispersed in methanol while increased slightly as in ethanol. As dispersed in acetonitrile, the photoluminescence enhanced about 3 times with changes in maximal excitation wavelength (from 320 to 308 nm) and maximal emission wavelength (from 404 to 413 nm).

Metal ion effects were also investigated. 1×10^{-5} M of Ba^{2+}, Mg^{2+} and Zn^{2+} enhanced the fluorescence slightly while Fe^{3+} and Ni^{2+} quenched the fluorescence moderately. Ag^+, Ca^{2+}, Co^{2+}, Cu^{2+} and Pb^{2+} did not affect the fluorescence (see Fig. 8). It indicated that BSA-protected gold nanoparticles maybe a potential fluorophore for metal ion sensing.

3.4. Photoluminescence mechanism

Excitated the gold nanoparticles with 320 nm illumination leads to excitation of the surface plasmon coherent electronic motion as well as the d electrons. Relaxation of these electronic motions followed by the recombination of the sp electrons with holes in the d band we believe leads to the observed emission [21]. As mentioned above photoluminescence from gold nanoparticles is thought to arise from transitions between the filled d band and sp conduction bands [9,13,21,22]. The high fluorescence quantum yield enhancement over that of differently prepared larger nanoparticles probably results from three factors. First, the lower density of states present in very small nanoparticles minimizes internal nonradiative relaxation pathways [13]. Second, the large BSA better protects these nanoparticles from quenchers in solution. Third, in the purified solutions, there are no large gold nanoparticles to quench the nanoparticles fluorescence [13,29,30].

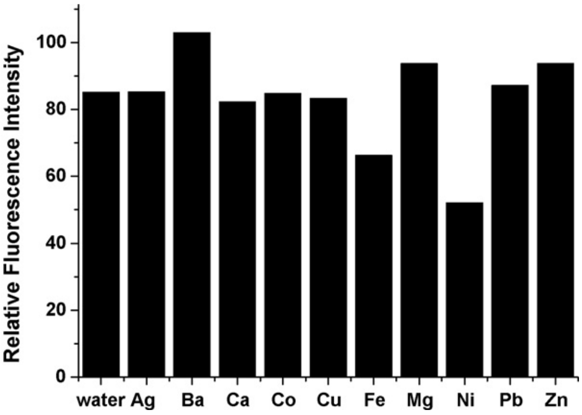

Fig. 8. Metal ion effects on photoluminescence. The concentrations of metal ions were all 1×10^{-5} M.

4. Conclusions

In summary, photoluminescence from BSA-protected gold nanoparticles was observed. The excitation and emission maxima were at 320 and 404 nm, respectively. This photoluminescence has been assigned to interband transitions between the filled $5d^{10}$ band and $6(sp)^1$ conduction band; however, the exact mechanism is unknown. We have established a conservative estimate for the quantum yield of the 5.1 nm gold nanoparticles to be 0.053 ± 0.0070, which is more than eight orders of magnitude greater than that of bulk gold, may offer advantages in applications in optical devices and biosensors.

Acknowledgments

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