High yield synthesis of single-crystalline gold nanoplates using the metal ion-reducing bacteria

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Gold nanoplates were prepared in high yield at room temperature using the Fe(III)-reducing bacterium *Shewanella algae*. The cell extract, prepared by sonicating a suspension of *S. algae* cells, plays an important role in improving productivity of gold nanoplates. *S. algae* cell extract was capable of reducing aqueous AuCl_4^- ions into elemental gold within 60 min when H_2 gas was provided as an electron donor. Gold nanoplates with an edge length of 100 nm appeared after 6 hours, and compact gold nanoplates with an edge length of 100–200 nm were obtained after 24 hours. The prepared nanoplates had diffraction spots characteristic of single-crystalline (111)-oriented gold nanocrystal. Using *S. algae* cell extract, the yield of gold nanoplates relative to the total nanoparticle population was 60 %, which was four times higher than that obtained with a suspension of resting cells. Following centrifugal ultrafiltration of the *S. algae* extract, only the fraction containing biomolecules greater than 50 kDa successfully produced gold nanoplates.

Key words: microbial synthesis, gold nanoparticle, cell extract, eco-friendly process

INTRODUCTION

Noble metal nanoparticles have a wide range of applications such as in catalysis, optics and biosensing. The optoelectronic and physicochemical properties of nanoparticles are strongly correlated to both particle shape and size. The synthesis of gold nanoplates represents a fascinating and intellectually challenging problem due to their potential in cancer hyperthermia, as surface-enhanced Raman spectroscopy substrates, and as infrared radiation-absorbing optical coatings (1-2). Wet chemical methods have often been used to prepare gold nanoplates. Recent reports have detailed high yields of planar and triangular gold nanostructures produced with the crystal growth method and controlled by appropriate capping reagents such as cetyltrimethylammonium bromide (CTAB) (3-5) and polyvinylpyrrolidone (PVP) (6-10). These chemical methods often require heating at elevated temperatures to complete the reduction of soluble Au ions. Furthermore, expensive chemicals are required, such as capping agents to control particle shape and a large amount of reducing agents. Therefore, finding feasible methods for producing gold nanoplates at room temperature without additives such as capping agents and reducing agents is advantageous for green chemistry.

Of recent interest is a low-cost and environmentally benign method for gold nanoplate synthesis using plant extracts to deposit gold metals by reducing gold ions in aqueous solution at room temperature. The Sastry group successfully prepared a gold nanoprism using plant extracts such as *Cymbopogon flexuosus* (lemongrass) (11) or *Aloe vera* (12). Excellent shape and selective formation of single crystalline triangular gold nanoparticles was observed. The Lee group developed a biological method to produce large quantities of gold nanoplates using a brown seaweed extract (13). Recently,

our group reported on a microbial deposition of gold nanoplates using the metal-reducing bacterium *Shewanella algae* (14-15). This microbial method with metal-reducing bacteria was used to deposit metals by reducing the metal ions in an aqueous solution at room temperature and pH 3.2. This biological system provides an environmentally friendly and safe procedure for nanoparticle and nanoplate synthesis with low-energy consumption. However, the yield of gold nanoplates was relatively low and further detailed investigations are required to describe this process accurately and clearly.

In this study, we report for the first time on an eco-friendly method for the high yield synthesis of gold nanoplates using a cell extract prepared by sonicating a suspension of *S. algae* cells at room temperature without adjusting the pH of the solution. The effects of molecular weight, reaction time, and initial concentration of Au(III) ions on the particle morphology were investigated.

EXPERIMENTAL SECTION

Bacterial strain and growth conditions

S. algae ATCC51181 was obtained from the American Type Culture Collection (ATCC). Cells were cultured in ATCC medium 2 containing sodium lactate as an electron donor and Fe(III) citrate as an electron acceptor. For anaerobic conditions, 250 ml of the liquid medium was added to a 500 ml screw-cap flask and bubbled with N_2 for 30 min. The bacterial strain was grown anaerobically at 25 °C. The cells were harvested 24 h after batch inoculation, by centrifugation, re-suspension in distilled water under anaerobic gas, and re-pelleting by centrifugation. This procedure was repeated twice, and the washed cells were subsequently re-suspended in distilled water under anaerobic gas. The prepared *S. algae* suspension (10 ml) was sonicated in a

50 ml screw-cap flask in a bath sonicator (Ultrason Velvo Clear VS150H, 44 kHz) for 30 min at room temperature. The resulting *S. algae* cell extract was used for further experiments.

Experimental procedure

In a typical reduction experiment at 25 °C, 5 ml of the *S. algae* cell extract was added to 0.3 ml of aqueous chloroauric acid (HAuCl₄) solution and the volume was increased to 15 ml by adding an appropriate amount of distilled water. The gas phase was H₂-N₂ (80:20, v/v), with H₂ gas provided as an electron donor. The cell concentration in the solution was 0.9–2.2×10¹⁶ cells/m³. The pH of the mixed solution was 2.8, and no pH adjustment was required. The number of *S. algae* cells in the solution was counted in a Petroff-Hausser counting Chamber (Hausser Scientific, Horsham, PA, USA) with a microscope (BX51, Olympus, JAPAN). Fourier transform infrared spectroscopy (FTIR) measurement of the *S. algae* extract deposited on germanium cell were carried out on a Shimadzu FTIR-8700 in the diffuse reflectance mode operating at a resolution of 4 cm⁻¹. The morphology and grain size of the biogenetic particles were observed by transmission electron microscopy (TEM) using JEOL model JEM-2100FX and JEM-2010. The biogenetic particles were also characterized by nano-beam electron diffraction (n-ED). Samples for TEM analysis were prepared on a carbon-coated copper TEM grid. To ascertain the characteristics of the biomolecules responsible for the formation of gold nanoplates, the *S. algae* cells extract was fractionated by centrifugal ultrafiltration using a 50 kDa molecular weight cutoff membrane into two fractions: fraction 1 (biomolecules of MWs less than 50 kDa), and fraction 2 (biomolecules of MWs greater than 50 kDa).

RESULTS AND DISCUSSION

Figure 1(a) shows the TEM image of gold nanoplates obtained after 24 h with *S. algae* cell extract at 25 °C and pH 2.8. The concentration of *S. algae* cells was held constant at 2.2×10¹⁶ cells/m³. With *S. algae* cell extract, the biogenetic gold nanoparticles were deposited extracellularly and exhibited various morphologies, including a triangular, truncated triangular, and hexagonal shape. The prepared nanoplates had an edge length of 100–150 nm and tended to be larger than particles with other shapes (Figure 1(a)). The observed SAED pattern of the nanoplate showed diffraction spots characteristic of single-crystalline (111)-oriented gold nanocrystal (Figure 1b). Interestingly, the yield of gold nanoplates prepared using *S. algae* cell extract was larger than that prepared using *S. algae* cell suspension (Figure 1c). This higher yield of gold nanoplates suggests that the shape-control agent responsible for their production might be released from the bacterial cells into the aqueous solution of the cell extract. As a result, the shape-control agent can react with the generated gold nanoparticles more efficiently. The mechanism for gold nanoplate formation with lemongrass and *Aloe vera* extracts has been explained due to the presence of carbonyl groups which act as a shape-controlling agents adsorbing on selected facets of gold nuclei (11-13).

Figure 1. (a) TEM image of biogenetic Au nanoplates prepared using *S. algae* cell extract in the presence of H₂ gas after 24 h at 25 °C and pH 2.8. (b) n-ED spots of the biogenetic particle shown in Figure 1(a). (c) TEM image of biogenetic Au nanoplates prepared by the resting cells of *S. algae*.

To confirm the existence of such a shape-controlling agent in *S. algae* cell extract, FTIR analysis was performed (Figure 2). The observed FTIR spectrum of the *S. algae* cell extract showed a band at 1650 cm^{-1} , assigned to the existence of a carbonyl functional group in ketones and aldehydes, which is responsible for the preparation of gold nanoplates.

To ascertain which biomolecules were responsible for the formation of gold nanoplates, the two fractions of *S. algae* cells extract obtained by ultracentrifugation (low-molecular-weight (less than 50 kDa) and high-molecular-weight (higher than 50 kDa)) were individually reacted with AuCl_4^- ions in aqueous solution. Only fraction 1 was observed to cause formation of gold nanoplates (figure 3(a)). Large triangular and hexagonal gold nanoplates were seen in addition to smaller nanoparticles. On the other hand, nanoplates were not observed when fraction 2 was added to 1 mol/m^3 HAuCl_4 solution (Figure 3(b)). Thus, biomolecules with a molecular weight greater than 50 kDa play an important role in the formation of gold nanoplates.

The effect of the reaction time on particle morphology

Figure 4 shows the TEM images of gold nanoparticles produced after different reaction times with *S. algae* cell extract. The reaction time was found to be an important factor in controlling the morphology of biogenetic gold particles. In the early stages of reaction, well-dispersed spherical gold nanoparticles with a mean size d_p of 9.6 nm, σ_g of 1.34 were synthesized (Figure 4(a)). Agglomerations of nanoparticles were observed after 3 h (Figure 4(b)), and gold nanoplates 100 nm in size with a liquid fluid surface started to form after 6 h. The TEM image indicates that the nanoparticles were generated on selected surfaces of the gold nanoplates (Figure 4(c)). Similar experimental results were also obtained when gold nanotriangles were prepared using lemongrass extract (11). After 12 h of reaction time, compact gold nanoplates with an edge length of 50–100 nm were observed, and the yield of gold nanoplates increased with time (Figure 4(d)). After one day with the cell extract, 60 % of the total nanoparticle population was composed of gold nanoplates, and this was markedly higher than that obtained using resting cells.

The effect of the initial Au(III) concentration on particle morphology

Figure 5 shows the TEM images obtained after 24 h with different initial Au(III) ion concentrations ($0.5\text{--}2.0\text{ mol/m}^3$) at 25°C . The concentration of *S. algae* cells was held constant at $0.9\text{--}1.0 \times 10^{15}\text{ cells/m}^3$. At the initial Au(III) concentration of 0.5 mol/m^3 , nanoplates with an edge length of 10–80 nm were produced along with small size of nanoparticles. When the Au(III) concentration was increased from 0.5 to 1 mol/m^3 , the yield of the nanoplates increased (Fig. 5(b)). A further increase in Au(III) concentration resulted in the formation of larger nanoplates (Fig. 5(c)).

Figure 2. FTIR spectra of *S. algae* cell extract prepared by sonication of the *S. algae* suspension.

Figure 3. TEM images of biogenetic Au particles prepared in the presence of H_2 gas after 24 h with either fraction 1 (a) or fraction 2 (b) of the *S. algae* cell extract.

Figure 4. TEM images of Au nanoplates prepared after different reaction times with *S. algae* cell extract at 25°C and pH 2.8 in the presence of H_2 gas: a) 1 h, b) 3 h, c) 6 h, and d) 12 h.

Figure 5. TEM images of Au nanoplates prepared using different initial Au(III) concentrations with *S. algae* cell extract at 25 °C and pH 2.8 in the presence of H₂ gas: a) 0.5 mol/m³, b) 1.0 mol/m³ and c) 2.0 mol/m³.

CONCLUSIONS

Gold nanoplates were successfully produced at room temperature using *S. algae* cell extract without adjusting the pH of the solution. After 24 h of reaction time, gold nanoplates with an edge length of 100–200 nm were produced and the yield of gold nanoplates relative to the total number of particles formed was 60 %. Furthermore, this study revealed that the utilization of *S. algae* cell extract allowed production of higher yields of gold nanoplates compared to *S. algae* cell suspension. The bioreductive synthesis of gold nanoplates is potentially attractive as an environmentally friendly alternative to conventional methods.

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