Microbial-mediated Au@biolayer Core-shell Nanoparticles: Synthesis and Application

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ABSTRACT

Gold nanoparticles (Au NPs) bio-synthesized through microbial activities create biocompatible and non-toxic nanomaterials that may have great applications. Previous studies have hypothesized that such biogenic Au NPs are covered by a layer of proteins. However, because of the high electron density of the Au core, the electron-opaque leaflet biolayers are hard to discern. Herein, we demonstrated that *Pantoea* sp. IMH can generate Au@biolayer core-shell NPs. Multiple complementary identification and characterization results suggest that the ~3 nm thin layer was coated on the spinous Au NPs. This unique composition enables the application of the biogenic Au@biolayer NPs in the removal and detection of dyes using surface enhanced Raman scattering (SERS).

Keywords: Au@biolayer NPs, biosynesize, *Pantoea* sp. IMH, dye removal, SERS

1 INTRODUCTION

Microorganisms have adapted to the presence of toxic heavy metals by developing a variety resistance mechanisms including reducing soluble toxic inorganic ions to insoluble metal nanoparticles (NPs). Gold (Au) NPs produced through microbial activities create biocompatible and non-toxic nanomaterials that have great applications in analysis, catalysis, and biomedicine.[1-3] The biogenic Au NPs are hypothesized to be covered by a layer of proteins.[4-6] However, because of the high electron density of the Au core, the electron-opaque leaflet biolayers are hard to discern. Investigating the existence of biolayers is important to understand the composition of microbial-mediated Au NPs, to study the biomineralization mechanism, and to explore its application.

Core-shell nanoparticles greatly break application restrictions of metallic nanoparticles which are easily and rapidly oxidized and dissolved. Organic shell coated on the Au NPs could not only protect the Au core, but also offer modification sites for target molecules, resulting in adsorption and detection of pollutants and biomolecules. Au NPs coating with surface materials has been reported to be synthesized through a variety of chemical fabrication methods. However, its widespread use is also restricted due to the chemical pollution and complicated procedure. Thus, the eco-friendly and simple method to synthesis Au@organic molecules core-shell NPs also motivated our research.

The objectives of this study were 1) to confirm the existence of biolayer on the Au nanoparticles synthesized by *Pantoea* sp. IMH (JX861130), with the formation of microbial–mediated Au@biolayer core-shell nanoparticles, and 2) to explore the application potential of microbial-mediated Au@biolayer core-shell NPs in adsorption and detection of dyes with surface enhanced Raman scattering (SERS) technique.

2 EXPERIMENTAL SECTION

2.1 Materials

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and all chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Microbiological media and ingredients were procured from Oxoid Ltd. Basingstoke Hampshire (England). Ultrapure Millipore water (18.2 MΩ) was used in all experiments. The stock solution of $HAuCl_4$ (100 mM) was prepared by dissolving $HAuCl_4 \cdot 4H_2O$ in Milli-Q water and stored in a brown bottle at 4 ºC.

2.2 Synthesis of biogenic Au@protein NPs by Pantoea sp.IMH

Pantoea sp.IMH (JX861130) from our laboratory was maintained in Luria−Bertani (LB) broth. The organism was cultivated by inoculating freshly grown bacteria in a liquid medium with LB broth. The cultures were aerobically grown to late-log phase at 30 ºC for 150 rpm. Cells were then harvested by centrifugation at 10,000 g (5424, Eppendorf, Hamburg, Germany) for 3 min, washed with PBS buffer solution (pH 7.4) for three times, and used for Au@protein nanoparticles synthesis. The harvested cells were resuspended in phosphate buffer (pH 7.4). Then, Au (III) was added to 10 mL of the above cells suspension and the final concentration of Au (III) reached to 1.8 mM. The mixture was incubated at 30 ºC under shaking (150 rpm). Control experiments without cells were also performed. After 8h, the cells and nanoparticles were harvested by centrifugation at 10,000 rpm for 10 min. All of the experiments were performed in triplicate.

The distribution, morphology, crystal structure, and chemical composition of biogenic Au@protein NPs by the *Pantoea sp.* IMH were characterized through HRTEM-

SAED-EDS (Tecnai G^2 F20 S-Twin, FEI), FESEM-EDS (SU 8020, Hitachi), and XRD (X'Pert PRO MPD, PANalytical) analysis. Gold nanoparticles were analyzed using XANES at beamline14W1 at the Shanghai Synchrotron Radiation Facility (SSRF), China. Infrared spectra of bio-Au NPs were recorded on Thermo-Nicolet Nexus 6700 FTIR spectrometer equipped with a liquid-nitrogen-cooled mercury−cadmium−telluride (MCT) detector.

2.3 Adsorption of dyes by biogenic Au@protein NPs

The biogenic Au@protein NPs (30 mg) were added to 100 mL of crystal violet, malachite green, rhodamine 6G, and congo red solutions (0.05 mM), each taken in separate 250 mL Erlenmeyer flasks. The mixture was stirred at room temperature for 20 min with the magnetic stirrer. The samples were collected at regular intervals up to 20 s in the first 2 min and then at 30 s interval during the rest of adsorption experiment. Then the solution was filtered through a 0.22 µm membrane and the dye concentration was measured using a spectrophotometer (UV-2550 Shimadzu). Each experiment was performed five times.

2.4 SERS detection of dyes on biogenic Au@protein NPs

To obtain the SERS spectrum of capping protein on Au NPs, biogenic Au@protein NPs were transferred whole to silicon wafer, and exposed to the laser for 10 s to collect the SERS signal. The samples of crystal violet, malachite green, rhodamin 6G and congo red were prepared by diluting the stock solution in DI water to reach a final concentration of twice detection concentration. The samples (0.5 mL) were mixed with the biogenic Au@protein NPs (0.5 mL). Then about 10 µL above mixture was placed on the silicon wafer, and exposed to the laser for 5 s to collect the SERS signal. Raman spectra were obtained using a portable Raman spectrometer (Enwave Optronics, Inc. USA) with a 4 cm^{-1} resolution at the excitation energy of 785 nm.

3 RESULTS AND DISCUSSION

3.1 Microbial-mediated synthesis of Au@biolayer core-shell NPs

Incubation of *Pantoea* sp. IMH with $HAuCl_4$ solution resulted in color change of the bacterial suspension with time from pale white to purple. After 12 h, a peak in UV-vis spectrum of the purple solution was at ~541 nm due to the surface plasmon resonance (SPR) of Au NPs, indicating the formation of Au NPs. The formation of Au NPs was confirmed by characterizing thin sections of strain IMH incubated with Au (III) using high-resolution transmission electron microscope (HRTEM).

The energy-dispersive X-ray analysis (EDXA) shows the appearance of Au peaks (Figure 1). Meanwhile, the dominant feature of particles in X-ray absorption near edge structure (XANES) spectra (Figure 1) had an absorbance edge (11,919 eV) in accord with zero valent Au. Both selected area electron diffraction (SAED) and XRD analysis (Figure 1) shows the Scherrer ring patterns characteristic of Au NPs at (111) and (220).

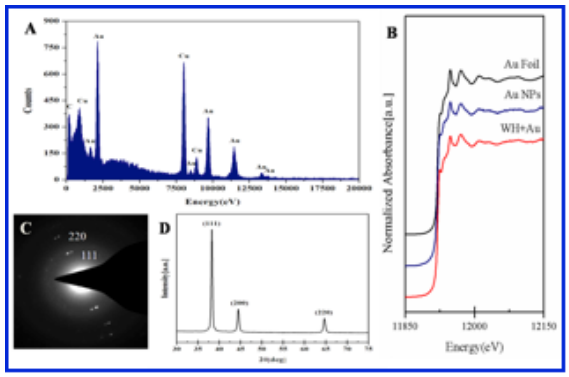

Figure 1: (A) EDS spectrum of nanoparticles observed in HRTEM images. (B) Au L-edge XANES spectra of nanoparticles in microbial environment (red line), Au NPs synthesized with chemical method (blue line), and Au foil as reference (black line). SAED (C) and XRD patterns (D) of Au NPs.

To confirm the presence of biolayer on the biogenic Au NPs, biogenic Au NPs in ordinary sample was measured through HRTEM. Figure 2 shows that a thin layer (~2.5 nm) constituted by amorphous substance was coated on the biogenic Au NPs. Au NPs were ensured through the lattice fringe spacing of 0.225 nm, corresponding to the distance between the Au (1 1 1) crystal planes. The SEM results show that clusters of biogenic Au NPs wires on the cell surface were coated by organic substance due to the sample conductivity. The EDS spectrum confirmed capping substance on the Au NPs was organic matter as a result of the appearance of C and O peaks. And the organic matter should be biomolecules owing to the biological system.

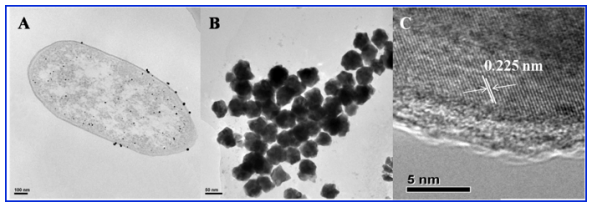

Figure 2: (A and B) HRTEM micrographs of thin section of cells with Au(III) treatment.(C) HRTEM image of nanoparticles formed in microbial medium containing 1.8 mM Au(III).

Therefore, Au NPs synthesized by bacteria were directly proved to be coated by the thin biolayer, with the formation of Au@biolayer core-shell NPs by the strain sp. IMH. Amine group and carboxyl group of nanoparticles as detected by FTIR could form peptide bond between adjacent NPs. As a result, nanoparticles would tend to form wirelike assemblies driven by Brownian motion and short-range interaction. The structure of biogenic Au@biolayer NPs was similar with that of magnetosome synthesized by several magnetotactic bacteria under iron ion stress. Thus, capping biolayer on Au NPs would be generated in the bioreduction and pumping out process by the bacteria under Au(III) stress. Biosynthesis Ag nanoparticles using *Shewanella oneidensis* were reported to be stable and speculated to be coated by protein/peptide. According to the phenomenon of biosynthesis Fe_3O_4, Au, and Ag nanoparticles, the existence of biolayer including lipid, proteins and other biomolecules would be prevalent in the biosynthesis nanoparticles by bacteria under metal stress.

3.2 Adsorption of dyes by biogenic Au@biolayer NP

Our adsorption experimental results (Figure 3) show that the removal efficiency of crystal violet, malachite green, and rhodamine 6G on biogenic Au@biolayer NPs rapidly reached equilibrium within 2 min at 98.3%, 59.2%, and 97.7%, respectively. The fast adsorption rate reflects good accessibility of the binding sites of the biogenic Au@biolayer NPs to dye molecules. While the adsorption of congo red was only 3.6% nearly could be ignorable. According to the structure of dyes, crystal violet, malachite green, and rhodamine 6G are cation-type dye, while congo red is anion-type dye. The adsorption of crystal violet, malachite green and rhodamine 6G on biogenic Au@biolayer NPs was much more than that of congo red on Au@biolayer NPs, consistent with the dyes structure. Therefore, biogenic Au@biolayer NPs were indicated to be negatively charged because of electrostatic adsorption interaction. Moreover, FTIR results that organic groups coated on Au NPs with negatively charge further testified our conclusion. Carboxylic group was reported to combine with anionic amino groups on the dyes. It was indicated that crystal violet, malachite green and rhodamine 6G were rapidly removed through adsorption mechanism involving the reaction between carboxyl group on the proteins and amino group on the dyes.

In addition, biogenic Au@layer NPs had higher adsorption ability to crystal violet than to malachite green even these two dyes are categorized as triphenylmethane dye, resulting from the structure difference of crystal violet and malachite green. The hydrophilic property of crystal violet is better than that of malachite green due to the molecular structure and biogenic Au@biolayer NPs is also hydrophilic due to the capping amine group, carboxyl group and other hydrophilic groups. Thus the hydrophilic-hydrophilic interaction may cause the adsorption difference between crystal violet and malachite green on biogenic Au@biolayer NPs.

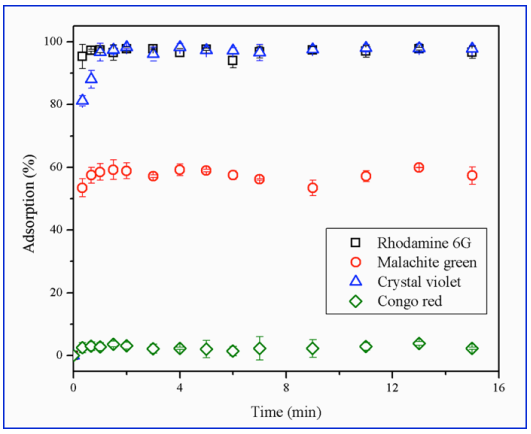

Figure 3: Adsorption kinetic of crystal violet, malachite green, rhodamine 6G, and congo red on biogenic Au@protein nanoparticles.

The adsorption of crystal violet, malachite green, and rhodamine 6G on biogenic Au@biolayer NPs were significantly higher than those on other three adsorbents as shown in Figure 4, while the adsorption of congo red on four adsorbents had no obvious difference. High adsorption capacity to dyes suggested that biogenic Au@biolayer NPs could be applied in the removal of dyes due to core-shell structure—spinous Au NPs with capping proteins

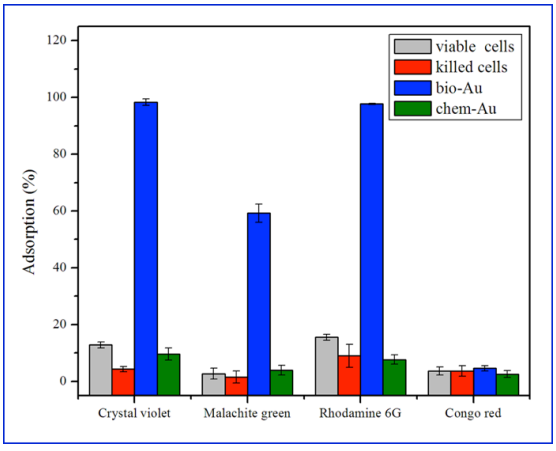

Figure 4: Adsorption of four dyes on the visible cells, dead cells, biogenic Au@protein NPs, and Au NPs synthesized with chemical method.

3.3 SERS detection of dyes on biogenic Au@biolayer NPs

Surface-enhanced Raman scattering (SERS) provides an alternative method for environmental analyses mainly due to its substantial electromagnetic enhancement induced by local surface plasmon resonance. Biogenic Au@biolayer

NPs were confirmed to be SERS-active and the capping biolayer was constituted by amine, carboxyl, phosphate, and other organic groups according to peak assignments, in accordance with FTIR results. Biogenic Au NPs by *Aspergillus nidulans* colonies are determined to be SERS-active through spectra from an extended scan map and only one genuine SERS spectrum was obtained when examined with Raman microscopy. SERS spectra based on our biogenic Au@biolayer NPs as SERS substrate are stable and could be easily acquired by a portable Raman spectrometer. Therefore, biogenic Au@biolayer NPs have the great application potential for SERS detection.

To justify our hypothesis that biogenic Au@biolayer NPs could be applied in SERS detection as Au NPs synthesized by chemical method, four dyes, including crystal violet, malachite green, rhodamine 6G, and congo red, were analyzed with SERS. Their spectra are shown in Figure 5. For comparison purposes, spectra of the dyes standards and blank substrate were also collected. Nevertheless, the background peaks would not interfere with the detection of the four dyes. In line with previous reports and our DFT calculations, the observed SERS spectra agreed well with the corresponding Raman spectra of dye standards. The lack of change in SERS peaks from the Raman spectra implies that the enhancement may be mainly due to the electromagnetic mechanism. The biogenic Au@biolayer NPs, with the spinous structure, could form "hot spots" between adjacent nanoparticle, resulting in pronounced SERS enhancement for molecules for them.

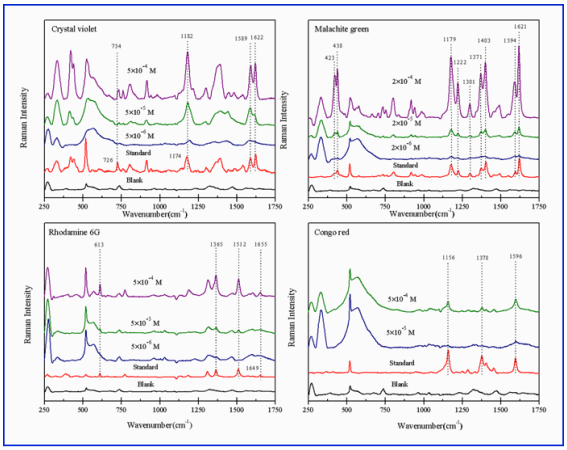

Figure 5: SERS spectra of four dyes with different concentrations at 10^{-4} M, 10^{-5} M, and 10^{-6} M based on biogenic Au@protein NPs as SERS substrate.

Figure 5 shows that the detection limit of crystal violet, malachite green and rhodamine 6G was about 10^{-6} M, while the lowest detection concentration of congo red was only 5×10^{-5} M. Therefore, it was revealed that biogenic Au@protein NPs tend to adsorb more molecules of crystal violet, malachite green and rhodamin 6G than that of congo red, according with the adsorption results of dyes on biogenic Au@biolayer NPs (Figure 8). SERS experiment demonstrated that biogenic Au@biolayer NPs exhibit surface plasmon resonance and could be used as substrate in SERS detection of organic pollutants such as dyes.

Upon successful adsorption removal and SERS detection of dyes, biogenic Au@biolayer NPs, with the special structure-spinous shape and capping biolayer, could be used in dye contaminated water treatment. And it has the possibility that fast adsorption removal of dyes, with the simultaneous monitoring dye concentration, provides a nanotechnology-based one-step green approach for water purification and improvement of environmental sustainability.

4 CONCLUSION

In conclusion, we have demonstrated that *Pantoea* sp.IMH can generate Au@biolayer core-shell nanoparticles. Examination of *Pantoea* sp.IMH incubated in $HAuCl_4$, using UV-Vis spectroscopy, HRTEM-ED-EDS, XRD, XANES, SEM-EDS, and FTIR, provided complementary identification and characterization. Microbial-mediated Au@biolayer NPs were one-step synthesized core-shell nanoparticles that thin (~2.5 nm) layer was coated on the spinous Au nanoparticles. In biogenic Au@biolayer NPs — dyes system, capping biomolecules could be combined with dye molecules, resulting in high adsorption efficiency and good SERS enhancement to dyes. With the high adsorption capacity, the biogenic Au@biolayer NPs could be applied for the removal of dyes due to the special structure of nanoparticles. Biogenic Au@biolayer NPs exhibit surface plasmon resonance and can be used as substrate for SERS detection of dyes. Our results will help to understand the transformation of metal ions to nanoparticles in nature, the gold biosynthesis mechanism, and the development of green approach for dyes-contaminating water treatment.

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