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Spirogyra submaxima-A Green Alga for Nanogold Production

Piya Roychoudhury and Ruma Pal
Department of Botany, University of Calcutta, 35, Ballygunge Circular Road,
Kolkata 700019, India,E-mail: piyaroychoudhury2@gmail.com; rpalcu@rediffmail.com

Abstract

Biogenic synthesis is an important technique to synthesize eco-friendly metal nanoparticles. In a thorough screening program of gold nanoparticles (AuNPs) production using algae as bioreagent, the present study demonstrates the efficacy of a chlorophycean micro-alga, *Spirogyra submaxima* for nanogold synthesis from auro -tetrachlorate solution. The algal biomass turned purple in color after 24 h of exposure in aqueous Au (III) solution due to formation of AuNPs by bioconversion of Au (III) to Au (0) at intracellular level. The AuNPs were extracted from the algal biomass by sodium citrate solution as capping agent and subjected to different experimentation for their characterization. The specific plasmon band of the extracted suspension was observed at 532 nm by UV-vis spectroscopy. Spherical, triangular, hexagonal structures of the AuNPs with 2 nm to 50 nm in diameter were identified by performing Transmission Electron Microscopy (TEM). Dynamic Light Scattering (DLS) study revealed the average hydrodynamic diameter of the particles distributed in citrate solution. Four major peaks at 38.2^0, 44.5^0, 65.6^0 and 78.6^0 obtained from X-ray powder diffraction (XRD) which confirmed the presence of elemental gold. Charges around the particles (-11.9 mV) were observed by zeta potential study. Overall this technique requires low energy and low manufacturing costs.

Key Words: algae, bioconversion, gold nanoparticles, microscopy, spectroscopy

Introduction

Green synthesis of nanoparticles is a significant process for production of precious metals such as gold, silver, platinum, and palladium due to their various applications in material sciences. Among these noble metal nanoparticles, especially gold nanoparticles play an important role in various catalytic reactions due to their unique and tunable surface plasmon resonance, high surface-to-volume ratio and high surface energy properties (EI-Sayed, 2001). This kind of nanoparticles have multiple applications in drug delivery, gene transfer and as bioprobes in cells and for tissue analysis in visualization of micro- and nano-objects, for observation of biological processes at nanoscale (Deplanche and Macaskie, 2008) to enhance electroluminescence and quantum efficiency in organic light emitting diodes (Park et al. 2004).

The synthesis of nanoparticles utilizing biological materials could be an improved alternative to toxic chemicals and the expensive physical methods. Physical methods are often difficult to achieve and time consuming and still under development and in chemical methods different chemical reagents are used which have toxic effect leading to undesirable functional aberrations in target cells, especially in medical applications. Both are hazardous and expensive. Green nanotechnology requires less energy, low manufacturing costs and environmentally safe (He et al. 2007).

In general, biological organisms have been explored in AuNPs synthesis such as bacteria (Narayanan et al. 2010), algae (Parial et al. 2012), fungi (Mishra et al. 2011) and higher plant (Chandran et al. 2008). Algae are potential bioreagent for nontoxic nanoparticle synthesis as they grow rapidly, producing large biomass at lower cost with high metal uptake capacity. There are a few reports available on algae based gold nanoparticle production like brown seaweeds *Sargassum wightii* (Singaravelu et al. 2007), *Turbinaria conoides* (Vijayaraghavan et al. 2011), *Laminaria japonica* (Ghodake et al. 2011).

In the present work, algae mediated green synthesis of AuNPs is described using green chlorophycean member, *S.submaxima*. The nanogold production was confirmed by the UV-visible spectroscopy, and the nanostructure and size were investigated by TEM. The crystalline structure was examined by the XRD technique. The surface charges of the particles were determined by zeta potential study.

Material and Methods

Synthesis of gold nanoparticles

The chlorophycean alga, *S. submaxima* was collected from Sunderbans, India and left overnight in betadine solution to remove surface contaminants and then repeatedly washed by distilled water and used as experimental material. Healthy growing algal biomass (1mg FW) was exposed to Aqueous Au (III) (prepared from HAuCl4·xH2O; MW 339.79) (SRL, INDIA) solution (25 ppm, pH 4). The experimental set was kept at room temperature for 24 h.The gold nanoparticles were separated out by the sonication of purple colored biomass with 7.5 mM sodium citrate solution by a Hielscher UP100H ultrasonic processor (Teltow, Germany). Then the sonicated biomass was

centrifuged at 3000 rpm for 5 min in a C-24 BL Remi cooling centrifuge (Maharashtra, India). The supernatant was collected and utilized for characterization.

UV–visible spectroscopy

Absorption spectrum of the algal extract was determined with a Thermo Evolution 300 UV-visible spectrophotometer (Waltham, USA) by scanning the range of 250nm-1100nm wavelength.

Hydrodynamic size and stability

The hydrodynamic size of the particles distributed in citrate solution was measured by dynamic light scattering experiment using a nano size particle analyzer, Nano ZS (Malvern). Zeta potential of the suspension was determined using the same instrument.

X-ray diffraction (XRD) analysis

Gold loaded purple colored biomass of *S. submaxima* was air-dried, made it into powder using mortar and pestle and used for powder XRD analysis.The spectra were recorded from 5° to 100° 2θ angles with a PW 3040/60, DY 2501PANalytical X-ray diffractometer (Netherland) using Cu Kα (k 1.54443) radiation operated at 40 kV and 30 mA.

Transmission Electron Microscopy (TEM)

A drop of nanoparticle extract was dried on a carbon coated copper grid and the morphology and size analysis of biosynthesized AuNPs was carried out by JEOL JEM 2100 HR-TEM.

Results

Green algal biomass showed a time dependent color change due to the reaction with aqueous Au (III) solution. After 3h of reaction, the biomass of *S. submaxima* started to turn pinkish in color and after 24 h the whole biomass turned dark pink or purple in color which was independent of temperature variation. The purple color did not change with increasing of incubation time (Fig 1). From the microphotograph of control and treated filament (Fig 1), it is revealed that the treated algal filament showed comparatively smaller cell with highly condensed chloroplast which indicated the high rate of cell division without cell elongation as a stress response in gold exposure. Interestingly, it was observed the cell wall and the chloroplast were the sites of AuNP production.

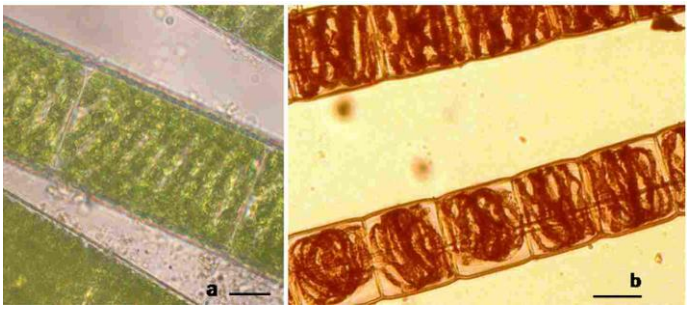

Fig 1 Microphotographs of control (a) and gold loaded (b) filament of *Spirogyra submaxima*.Scale bars 50 µm.

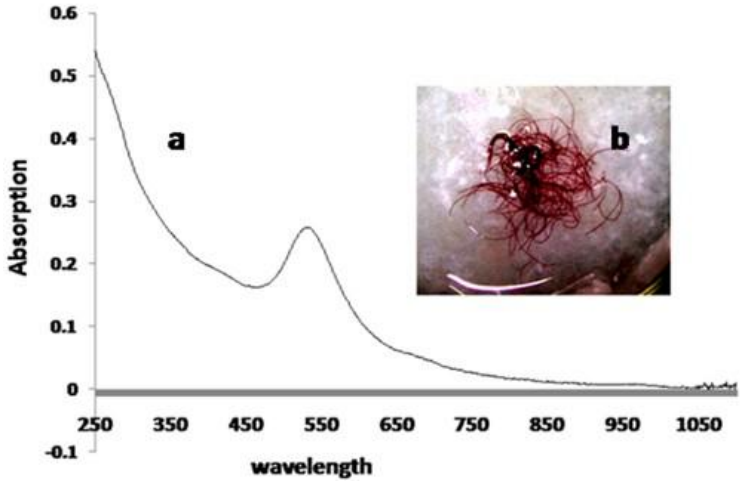

Fig 2 UV-vis spectra (a) of the AuNPs suspension and gold loaded biomass(b)

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The spectral analysis showed that the absorption peak of extracted gold particle appeared at 532nm which was specific for gold nanoparticles (Fig 2). The DLS measurement revealed that the average hydrodynamic size of gold particles was 214 nm in diameter. Zeta potential of the gold nanoparticles distributed in citrate solution was -11.9 mV. The crystallographic analysis of treated biomass by XRD showed the 2θ values at 38.2°, 44.5°, 64.8°and 77.8° which were indexed at (111), (200), (220) and (311) lattice planes (Fig 3). The diameter range of the AuNPs obtained from TEM study was 2 nm to 50 nm (Fig 4).TEM study also revealed that well dispersed gold nanoparticles were variable in size and spherical, triangular and hexagonal in nature.

Discussion

The green colored biomass turned pink in color after the exposure to gold solution (25 ppm) due to the reduction of Au (III) to Au (0) and production of gold nanoparticles at intracellular level. The color of the gold loaded biomass varies from light pink to purple depending upon the concentration of the particles (Saha et al. 2011). Therefore the selected chlorophycean alga is very efficient in bioconversion of Au (III) to Au (0).

The characteristic purple color of the extracted suspension was due to the excitation of the surface plasmon of elemental gold which provided a convenient spectroscopic absorbance band (Verma et al. 2011). This specific surface plasmon resonance arises due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field (Govindraju et al. 2008). The significant band for gold nanoparticles usually has a range of 500–550 nm in aqueous solutions, depending upon the shape and size of nanoparticles (Shankar et al. 2004). In our study also the absorption peaks observed at 532 nm indicating the synthesis of gold nanoparticles and it was confirmed by XRD and TEM study also.

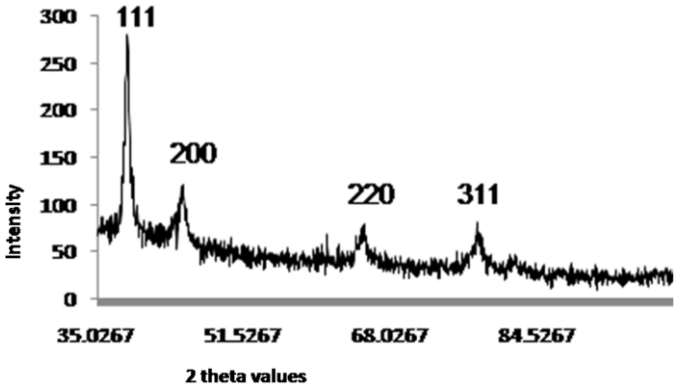

Fig 3 XRD patterns showing 4 peaks of elemental gold

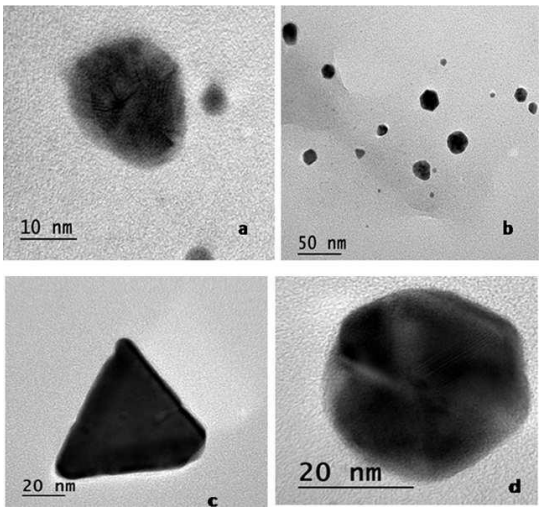

Fig 5 TEM micrograph showing triangular and spherical (a), triangle, spherical, hexagonal mixture (b), triangular (c) and hexagonal (d) shaped gold nanoparticles synthesized by *S. Submaxima*

The XRD analysis was performed to confirm the reduction of Au (III) to Au (0) and the crystalline nature of AuNPs. The Bragg reflections or 2θ values appeared at (38.2^0, 44.5^0, 65.6^0 and 78.6^0) and exhibited four diffraction peaks which can be assigned to the [111], [200], [220] and [311] reflections planes respectively. Therefore the Bragg reflections obtained from this study clearly corresponded to the fcc crystalline structure of gold which indicated the purple colored biomass of *S.submaxima* was fully loaded with pure crystalline gold (International Center for Diffraction Data, ICDD No.04–0783) (Verma et al. 2011) and reported for the structure of standard gold metal (Au0).
The DLS study gives us the information about the hydrodynamic diameter of nanoparticles. This diameter is the inorganic core of the AuNP along with coating material and the solvent layer attached to the particle as it moves under the influence of Brownian motion. For this reason the size of the particles obtained from DLS study is greater than actual dry size. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in a suspension. Zeta potential mediates interparticle interactions. High positive and negative charges cause high repulsion among the particles. Here the negatively charged particles showed moderate stability which resists aggregation upto 7 days.
The TEM images showed the biosynthesized gold nanoparticles are with variable in sizes and shapes where most of them are spherical with occasional triangular and hexagonal structure. The different size and shape of particles was due to aggregation of particles by metallic interaction. These differences in shape and size of nanoparticles synthesized by biological systems are common (Binupriya et al. 2010).
In summary, this study can be considered as a simple, rapid bioprocess for the synthesis of AuNPs by green filamentous commonly occurring alga, *S. submaxima.* The advantage of using this protocol over other methods is that the nanoparticles are pure, easily extractable. This study may therefore lead to the development of a green pathway for rapid bioreduction of chloroauric acid. The produced nontoxic goldnanoparticles can be applied in biomedical applications.

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