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Green synthesis of gold nanoparticles by the marine microalga *Tetraselmis suecica*

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The application of green-synthesis principles is one of the most impressive research fields for the production of nanoparticles. Different kinds of biological systems have been used for this purpose. In the present study, AuNPs (gold nanoparticles) were prepared within a short time period using a fresh cell extract of the marine microalga *Tetraselmis suecica* as a reducing agent of $HAuCl_4$ (chloroauric acid) solution. The UV–visible spectrum of the aqueous medium containing AuNPs indicated a peak at 530 nm, corresponding to the surface plasmon absorbance of AuNPs. The X-ray diffraction pattern also showed a Bragg reflection related to AuNPs. Fourier-transform infrared spectroscopy was performed for analysis of surface functional groups of AuNPs. Transmission electron microscopy and particle-size-distribution patterns determined by the laser-light-scattering method confirmed the formation of well-dispersed AuNPs. The most frequent size of particles was 79 nm.

Introduction

Currently, there is an impressive development in the field of nanotechnology for the synthesis of nanoparticles. Nano-sized particles exhibit unique properties that arise from their larger surface/volume ratio and higher surface energy. Metal nanoparticles have wide-ranging applications in diverse areas such as chemistry, physics, and biomedical and material sciences [1]. However, in most methods for nanoparticle synthesis, the applied organic solvents and toxic reagents cause environmental pollution. Therefore there are progressive needs to omit such hazardous materials and develop eco-friendly methods for nanoparticle synthesis. Biological systems seem to be a promising and cost-efficient means for this purpose [2].

Growing interests to biomedical applications of AuNPs (gold nanoparticles) have been reported. AuNPs absorb light in the visible region of the electromagnetic spectrum due to their surface plasmon resonance and convert it into heat. Therefore, AuNPs could be used for photothermal therapy and imaging [3]. In the field of biomedicine, AuNPs are used for several purposes e.g. leukaemia therapy [4], biomolecular immobilization [5], biosensor production [6,7] and labelling for contrast enhancement in cryoelectron microscopy [8].

Some prokaryotic organisms, such as *Actinobacter* spp. [2], *Rhodopseudomonas capsulata* [9] and *Pseudomonas aeruginosa* [10], and eukaryotes, for example *Fusarium oxysporum* [11], have been used as nanofactories for green synthesis of AuNPs. They were all shown to have the capability of reducing either intracellular or extracellular metal ions into their corresponding nanometals, depending on the location of their reducing agents [4,11]. Additionally, AuNPs could be prepared by different parts of plants, such as alfalfa roots [12] and neem (*Azadirachta indica*) leaf broth [13]. Methanol extracts of some medicinal plants were also screened for the preparation of monodisperse AuNPs [14]. Natural polymers, such as chitosan, have a residue that can enable a reductive reaction of $HAuCl_4$ for preparation of AuNPs [15]. The exposure of aqueous $HAuCl_4$ to a powder prepared from a marine alga, *Sargassum wightii*, resulted in the conversion of Au^{3+} into Au^0 after 12 h [16].

Microalgae (photosynthetic prokaryotes and/ or eukaryotes) have been shown to have potential for conducting safe reactions in biotransformation processes [17] and for the removal of heavy metals from the environment [18].

Key words: green synthesis, gold nanoparticle (AuNP), marine microalga, *Tetraselmis suecica*.

Abbreviations used: AuNP, gold nanoparticle; CSIRO, Commonwealth Scientific and Industrial Research Organization; FTIR, Fourier-transform infrared; TEM, transmission electron microscopy; XRD, X-ray diffraction.

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The present study shows the capability of *Tetraselmis suecica* for rapid synthesis of AuNPs following the incubation of $HAuCl_4$ solution with fresh algal-cell extracts. To the best of our knowledge, there is no previously published report on the synthesis of highly stable AuNPs by *T. suecica*.

Experimental

Chemicals, organism and culture conditions

All reagents and solvents were of analytical grade and purchased from Merck and Sigma–Aldrich.

An axenic culture of *T. suecica* (Kylin) Butcher [culture code CS-187; CSIRO (Commonwealth Scientific and Industrial Research Organization) Microalgae Culture Collection, CSIRO Marine Research, Hobart, TAS, Australia] was grown and maintained in sterile f/2 seawater-medium [19] agar slants and subcultured freshly before use in later experiments. The composition of f/2 medium ($g \cdot l^{-1}$ in filtered seawater unless otherwise stated) was as follows: $NaNO_3$, 0.075; $NaH_2PO_4 \cdot H_2O$, 0.005; $NaSiO_3 \cdot 9H_2O$, 0.03; trace-element solution, 1 ml; distilled H_2O up to 1 litre. The trace-element solution contained (in $g \cdot l^{-1}$) Na_2EDTA, 4.36; $FeCl_3 \cdot 6H_2O$, 3.15; $MnCl_2 \cdot 4H_2O$, 0.18; $CuSO_4 \cdot 5H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.022; $CoCl_2 \cdot 6H_2O$, 0.01; $NaMoO_4 \cdot 2H_2O$, 0.006; distilled water up to 1 litre. The pH was adjusted to 7.0 with 1 M HCl and/or NaOH prior to autoclaving at 121 °C for 20 min. Thiamine (100 μg), biotin (0.5 μg) and vitamin B_{12} (0.5 μg) were also added to the medium after sterilization using a 0.22-μM sterile filter [18]. For short-term storage, the microorganism was maintained at 4 °C on f/2 medium.

To prepare adequate algal cell mass, the microorganism was inoculated into two 2-litre Erlenmeyer flasks containing 500 ml of sterile f/2 liquid medium enclosed with cotton plugs. Flasks were illuminated at 60 μmol of photons$\cdot m^{-2} \cdot s^{-1}$ supplied by three fluorescent tubes under a continuous-light photoregime from all sides at 25 °C and bubbled with sterile air without shaking.

Preparation of AuNPs

Microalgal cultures were harvested in logarithmic phase (1.2×10^6 cells$\cdot ml^{-1}$) on day 6 and centrifuged at 2000 ***g*** for 10 min at 4 °C to reach a packed-cell volume of 5 ml [20]. The supernatant was then removed, and the biomass was washed with sterile 0.9% NaCl and centrifuged at 2000 ***g*** for 10 min. A 5-ml packed-cell volume of *T. suecica* was transferred into a sterile mortar and liquid N_2 was added until the whole cells were frozen. The cells were completely disrupted using a sterile pestle. Disrupted cells were then centrifuged at 2000 ***g*** for 10 min at 4 °C and the supernatant containing the cell extract was collected. The formation of AuNPs was investigated by adding various amounts of *T. suecica* cell extract to 4 ml of aq. 10^{-3} M $HAuCl_4$ (chloroauric acid) prepared with deionized water. The mixtures were incubated at different temperatures between 30 and 90 °C in a water bath. Conversion of the yellow colour of the $HAuCl_4$ solution into a ruby-red colour indicated the formation of AuNPs [14].

Analysis

The UV–visible spectrum of the reaction mixture was recorded using a Labomed Model UVD-2950 UV-Vis Double Beam PC Scanning spectrophotometer, operated at a resolution of 1 nm in the range 200–700 nm. For monitoring, a solution containing the appropriate volume (1 ml) of *T. suecica* cell extract and 4 ml of deionized water was used as a blank. In the next step, the UV–visible pattern for aq. 10^{-3} M $HAuCl_4$ after exposure to different temperatures was monitored while deionized water was used as a blank.

Samples for TEM (transmission electron microscopy) analysis were prepared by drop-coating the AuNP solution on to carbon-coated copper TEM grids. Following removal of excess water using blotting paper, the films on the TEM grids were allowed to stand for 2 min to allow the grids to dry. TEM measurement was performed using a Zeiss Leo 910 instrument operated at 100 keV.

XRD (X-ray diffraction) analysis of the extract of *T. suecica* that reduced AuNPs was carried out on films of the respective solution drop-coated on to glass substrates (Rich Seifert) using a P3000 instrument (Rich Seifert) operating at a voltage of 40 kV and a current of 30 mA with Cu $K\alpha_1$ radiations.

For FTIR (Fourier-transform infrared) spectroscopy analysis, dried powder of AuNPs was prepared. For this purpose, the solution was centrifuged at 20 000 ***g*** for 20 min after the formation of AuNPs using a Mikro 200 instrument (Hettich). The pellet was redispersed and washed four times consecutively with ethanol, acetone, diethyl ether and distilled water to remove any biological residue. Finally, the pellet was oven-dried at 37 °C for 48 h and the dried pellet was used for FTIR spectroscopy with a PerkinElmer Spectrum One instrument at a resolution of 4 cm^{-1} in a KBr pellet.

The particle-size-distribution pattern of AuNPs was measured by laser-light scattering using a Malvern Mastersizer instrument (MS2000, Ver. 5.30).

Results and discussion

Chemical reduction in an aqueous solution of $HAuCl_4$ is one of the most widely practical methods for the preparation of AuNPs. However, it is notable that some chemical reducing agents are toxic and hazardous [16]. In the present study, a rapid green synthesis of AuNPs using fresh cell extract of the marine microalga *T. suecica* was examined to overcome the this problem.

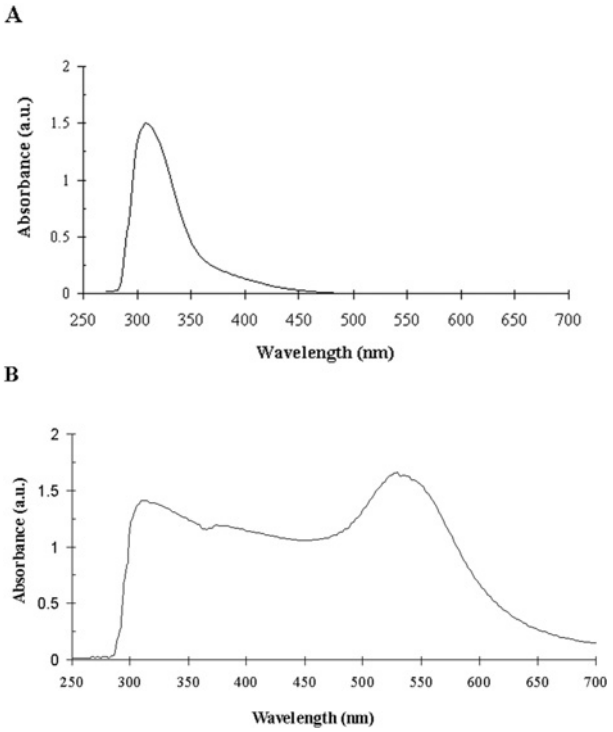

Figure 1 UV–visible spectra of aq. 10^{-3} M $HAuCl_4$ (A) and the mixture of *T. suecica* fresh cell extract with aq. 10^{-3} M $HAuCl_4$ (B) after incubation at 90°C

a.u., arbitrary units.

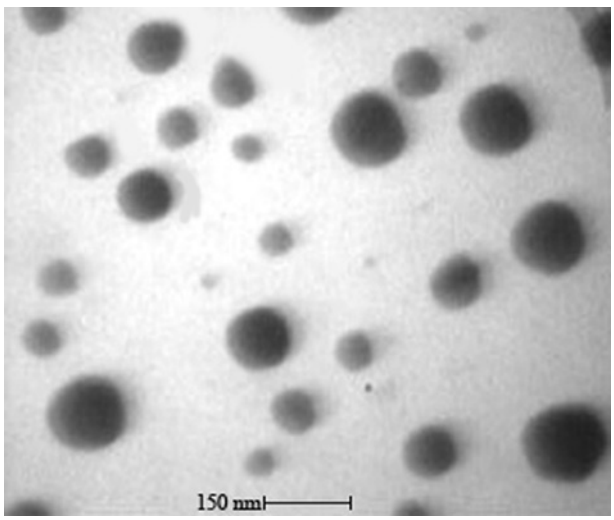

Figure 2 Transmission electron micrograph recorded from a small region of a drop-coated film of $HAuCl_4$ solution treated with the *T. suecica* fresh cell extract

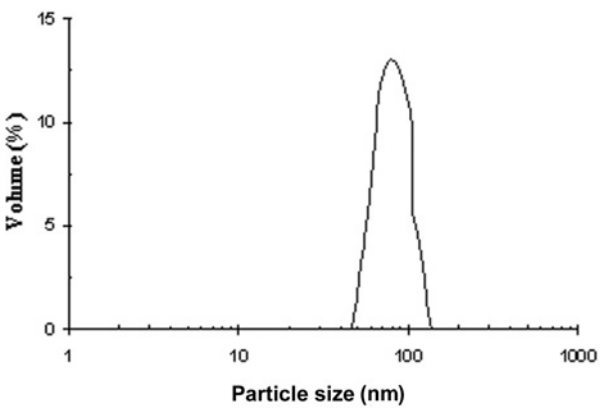

Figure 3 Particle-size-distribution pattern of AuNPs synthesized by *T. suecica* fresh cell extracts

It is well known that AuNPs display a reddish colour in aqueous medium related to excitation in their surface plasmon vibrations and scattering of visible light due to particle size [21]. Formation of AuNPs was observed only in a mixture containing 1 ml of cell extract after 5 min of incubation at 90°C, and AuNPs were not synthesized in other mixtures and thermal conditions (results not shown). It is very important to understand the biochemical mechanism of nanoparticle synthesis by algal-cell extract in order to achieve better control over size and shape of the AuNPs. In this regard, enzymes such as NADH-dependent reductase [11] are not responsible for reduction of $HAuCl_4$ because *T. suecica* is not a thermophilic microorganism containing heat-tolerant enzymes, and AuNPs were not produced at any of the incubation time periods and at thermal conditions below 90°C. However, the rapid formation of AuNPs at these thermal conditions might be due to the production of reducing agents in the mixture following the incubation period, which results in a colour alteration from limpid yellow to red. Substances such as polyols and water-soluble heterocyclic compounds have been proved to be responsible for the reduction of silver or chloroaurate ions and stabilization of nanoparticles respectively [22]. More studies and purification steps are needed to understand the chemical structures involved in the formation of AuNPs using the fresh cell extract of *T. suecica*.

Formation of AuNPs is detectable by UV–visible spectroscopy. Light absorbance is performed within a narrow range of wavelengths for metallic nanoparticles shorter than the wavelength of light [23]. For very small particles (e.g. < 25 nm in diameter for gold), the shift of surface plasmon band peak position is rather short. For larger nanoparticles (> 25 nm in diameter for gold), the surface plasmon peak shows a red-shift [23]. The interaction between AuNPs and light with shorter wavelength than the size of particles leads to polarization of the free conduction electrons and gives rise to an absorption band at 510–540 nm [23]. The UV–visible spectrum for aq.

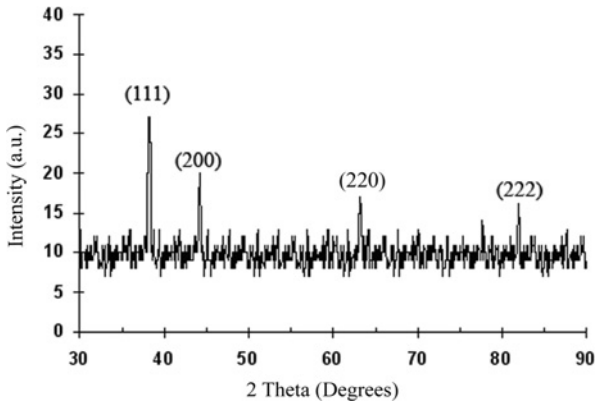

Figure 4 XRD pattern of AuNPs prepared by reduction of $HAuCl_4$ in the presence of *T. suecica* fresh cell extracts

a.u., arbitrary units.

10^{-3} M $HAuCl_4$ showed a clear band at 308 nm (peak in Figure 1A), and after adding the cell extract followed by incubation at 90 °C, a clear band became apparent at 530 nm, corresponding to the formation of AuNPs (Figure 1B).

A transmission electron micrograph of AuNPs showed well-separated spherical nanoparticles without any aggregation (Figure 2). AuNPs were synthesized in the diameter range 51–120 nm (Figure 3). The specific surface area for AuNPs was 57.9 $m^2 \cdot g^{-1}$. Based on the results obtained, AuNPs provide a comprehensive surface that could be applied for specific applications [24]. Singaravelu et al. [16] reported the formation of 8–12-nm-diameter AuNPs after 12 h by *S. wightii* without any thermal incubation. Our present study reports the preparation of AuNPs using *T. suecica*'s cell extract after 5 min with incubation at 90 °C, and particle sizes of 79 nm were the most frequent. The difference may arise from the various reducing agents that exist in the two algal species or from the difference between the methods applied.

The detailed properties of AuNPs formed with the applied reduction method were investigated by XRD-spectrum analysis (Figure 4). In the XRD pattern, it was observed that AuNP formation was based on four peaks positioned at $2\theta = 38.2^\circ$,44.6°, 63.1° and 76.6° due to (111), (200), (220) and (222) lattice planes respectively. The XRD pattern agreed with a previously determined Bragg reflection associated with AuNPs [25]. Meanwhile, the XRD pattern clearly indicated the crystalline structure of the obtained AuNPs. In the cell extract of *T. suecica*, while the crystalline layers of AuNPs were grown under the reductive reactions, some unknown compounds entrapped within the layers and their functional groups were exposed in the surface of AuNPs. FTIR measurements of AuNPs were applied to identify the functional groups located on the surface of nanoparticles (Figure 5). AuNPs showed main peaks at 3431.58 and 1675.11 wave numbers·cm^{-1}. The strong peak at 3431.58 cm^{-1} could correspond to O–H or N–H, indicating an alcohol or N–H-containing amide. Although carbonyl absorption was usually observed at 1690–1760 cm^{-1}, the strong peak at 1675.11 cm^{-1} in Figure 5 could be related to a carbonyl group. No difference was observed between FTIR spectra of AuNPs with different synthesis times (results not shown). Each of the above-mentioned functional groups could be used for bioconjugation of the various active compounds or immobilization of enzymes on the surface of the AuNPs [24].

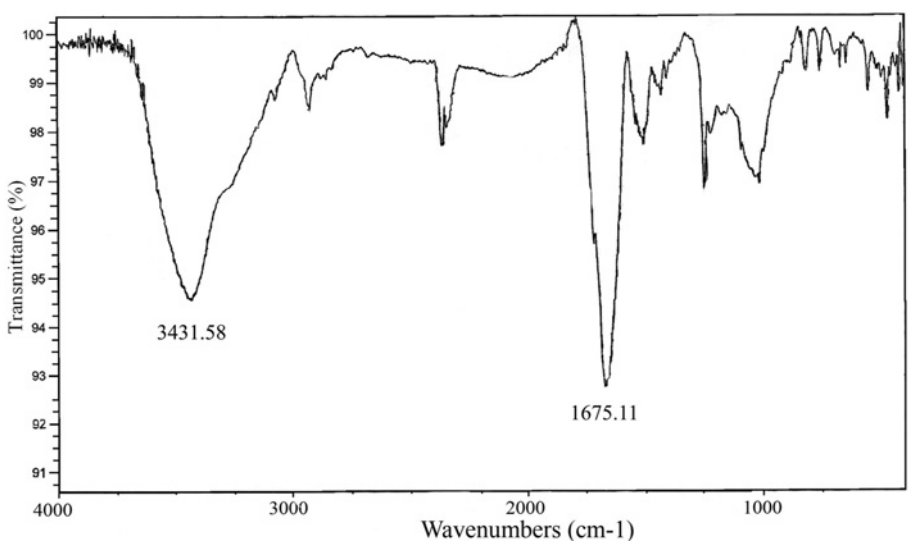

Figure 5 FTIR spectrum of AuNPs produced by adding *T. suecica* cell extracts to $HAuCl_4$ solution

In conclusion, the extract from *T. suecica* showed the capability of reducing Au^{3+} ions to gold nanoparticles by a simple method. UV–visible, TEM and XRD techniques were applied to confirm the reduction of the Au^{3+} ions to polydisperse and crystalline AuNPs and their characterization. According to our knowledge and a thorough literature survey, this is the first report on the synthesis of AuNPs using this marine microalga. Furthermore, the rapid extracellular synthesis applied makes the process cheaper and easier for downstream processing in the preparation of AuNPs. It is also important to understand the biochemical mechanism of nanoparticle synthesis by algal-cell extracts in order to achieve better control over size, shape and dispersity of AuNPs.

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