Biosynthesis of anisotropic gold nanoparticles using Maduca longifolia extract and their potential in infrared absorption

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\textbf{ABSTRACT}

Metal nanoparticles, in general, and gold nanoparticles, in particular, are very attractive because of their size- and shape-dependent properties. Biosynthesis of anisotropic gold nanoparticles using aqueous extract of Maduca longifolia and their potential as IR blockers has been demonstrated. The tyrosine residue was identified as the active functional group for gold ion reduction. These gold nanoparticles were characterized by UV-Vis spectrophotometer, FTIR, TEM and HRTEM. The presence of proteins was identified by FTIR, SDS-PAGE, UV-Vis and fluorescence spectroscopy. The micrograph revealed the formation of anisotropic gold nanoparticles. The biologically synthesized gold nanotriangles can be easily coated in the glass windows which are highly efficient in absorbing IR radiations.

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1. Introduction

The field of nanotechnology has recently witnessed spectacular advances in the methodology of nanomaterials fabrication and utilization of their extraordinary physical and optoelectronic properties [1]. With the development of new protocols based on chemical or physical methods, very much concern for environmental contamination is also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous by-products. Thus, there is a need for “green chemistry” that includes a clean, nontoxic, and environment-friendly method of nanoparticles synthesis [2]. As an alternative to conventional methods, biological methods are considered safe and ecologically sound for the nanomaterials fabrication [3]. One of the prime confinements for researchers in biosynthetic method is the systematic size and shape control of inorganic metal nanoparticles. Control over the shape and size of metallic nanoparticles enables tuning of their optical [4], electronic [5], magnetic [6] and catalytic [7] properties. Biosynthetic methods employing either microorganisms or plant extracts have emerged in recent years as an effective, simple and viable alternative to chemical and physical synthetic methods. Following the initial reports on intracellular silver nanoparticles formation in Pseudomonas stutzeri [8], many reports on the synthesis of metal [9–13] and semiconductor nanoparticles [14–16] using fungi or bacteria have appeared. Fungi [2], actinomycetes [10], and plant extracts were used for the synthesis of silver and gold nanoparticles [17]. Recently, excellent shape-selective formation of single crystallite triangular gold nanoparticles was observed using the extract of lemongrass plant (Cymbopogon flexuosus) [3]. These biogenic metal nanoparticles are extensively used in drug delivery [18], biosensors [19], bio imaging [20], antimicrobial activities [21], food preservation [22], etc. by exploiting their unique physical chemical and biological properties.

In present study anisotropic gold nanoparticles were synthesized using Maduca longifolia leaf extract and demonstrated their potential in absorption of infrared rays.

2. Experimental

2.1. Preparation of plant extract

20 g of thoroughly washed and finely cut plant leaves were taken in a 2 L beaker and washed with 15 HNO\textsubscript{3}, rinsed with ultrapure water to remove adsorbed impurities and dispersed in 1000 ml of ultrapure water. The supernatant of plant extract was separated from the biomass 15 days later by filtration and the filtrate was frozen and then defrosted to remove the precipitated polysaccha-
rides and finally filtered through 0.45 μm sterile nylon filters. The plant extract was concentrated by lyophilization and used for dialysis.

2.2. Separation of proteins in the plant extract

The raw plant extract (10 mL) was dialyzed in membrane tubing with a molecular weight cutoff of 12 kDa against 1 L of continuously stirred ultrapure water at 4 °C. After 24 h and three water changes (at 8 h intervals), the tubing content (the high-molecular-weight components) was collected and labeled as plant proteins (Pp). The optical density of the Pp solution at 280 nm (OD_{280}) was 2.5 and this was used as an indirect measure of the protein concentration.

2.3. Synthesis of gold nanoparticles using plant extract

1 mL of 10 mM HAsCl₄ was added to 9 mL of plant extract, and the reaction was allowed to proceed under a dark condition at 303 K. The pH of the reaction medium was adjusted to 2 and 10 by adding 1 M HNO₃ and 1 M NaOH solution, respectively.

2.4. Purification of gold nanopleatelets

The purification of the gold nanopleatelets based on size was conducted by centrifugation. Larger sized particles are separated from the solution by centrifugation at 1000 x g for 10 min; medium sized particles by centrifugation at 3000 x g for 15 min and the smallest sized particles by centrifugation at 10,000 x g for 20 min. The pellets formed after each centrifugation is dispersed in double distilled water and taken for further characterization.

2.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was used to identify the number of proteins and their molecular weight in the plant extract. The aqueous plant protein was concentrated by lyophilization and then dialyzed thoroughly against MilliQ water using 12 kDa of cutoff dialysis membrane. This concentrated aqueous plant extract containing protein was analyzed by SDS-PAGE.

2.6. Instruments for characterization

UV-Vis spectrophotometer was performed on Perkin Elmer Lambda 35 B instruments operating at 1 nm resolution. Characterization of size and morphology of nanoparticles were performed using JEOL 200 FX MARK II microscope operating at 200 kV and TECNAI F-30 microscope operating at 300 kV (for high resolution TEM imaging). Identification of plant proteins present in the aqueous plant extract using Fourier transform infrared spectroscopy (FTIR) was carried out by diffuse reflectance mode at a resolution of 4 cm⁻¹. Fluorescence emission spectrum was measured with a Perkin Elmer using an excitation wavelength of 280 nm.

The experiment carried out in triplicates to measure the temperature rise inside the wooden box with 1 cm x 1 cm window, which is exposed to commercially available IR lamp placed at a distance of 12 cm from the window.

2.7. Statistical analysis

All values are expressed as means ± standard deviation. The results were analyzed using one-way analysis of variance (ANOVA), and the differences among the formulation means were analyzed using the Tukey-Kramer multiple-comparison test. The software GraphPad InStat was employed for the statistical analysis.

![Fig. 2. FTIR spectrum of plant protein from the plant extract before (A) and after (B) reaction with gold ions.](image)

![Fig. 3. Fluorescence emission spectrum of plant protein (A) before and (B) after the reaction with gold ions.](image)
3. Results and discussion

3.1. Identification of components in the plant extract

The reduction of gold ions and resulting formation of gold nanoparticles might have been driven by some active components in plant extract. In this study, the plant proteins (Pp) separated from the plant extract was found to be primarily responsible for the reduction of gold ions and formation of gold nanoparticles. The presence of protein in the plant extract was confirmed by UV-Vis spectroscopy and SDS-PAGE (Fig. 1a and b).

The SDS-PAGE analysis showed (Fig. 1b) molecules weighing in the range of 14–90 kDa. The proteins in the plant extract were first separated by dialysis according to molecular weight (MW). The raw plant extract divided into low molecular weight fraction (MW < 12 kDa) and high molecular weight fraction (MW ≥ 12 kDa referred as the plant protein or Pp) using dialysis membrane with molecular weight cut off of 12 kDa. Each fraction was then individually tested for reaction with gold ions in aqueous solution. The UV–Vis spectrum recorded for 1 mM HAuCl₄ aqueous solution after reaction with the low MW fraction showed no absorption in the 400–1000 nm regions, whereas the high molecular weight

Fig. 4. (a–e) 2-Dimensional gold nanostructure formed using plant extract at pH 2 (303 K). Bend contours characteristics of thin platelets are clearly seen. Various sizes of triangular nanoplates which give the key information about layer by layer formation mechanism of platelets. (e) Spherical gold nanostructure formed at pH 10 (303 K).
fraction (Pp) produced a noticeable increase in absorption in the same period of reaction time, due to the SPR of the gold nanoparticles. The absorption band centered at 525 nm was clearly indicating formation of gold nanoparticles (data not shown).

3.2. The confirmation of tyrosine residue involved in the gold nanoparticles synthesis

The nature of amino acid residue in the plant protein responsible for gold ion reduction revealed by FTIR and fluorescence analysis of protein structure before and after reaction with metal ions is shown in Fig. 2. The FTIR spectrum of plant protein (Fig. 2a) showed the bands at 1658 cm⁻¹, 1532 cm⁻¹, which are characteristic of amide I and amide II bands of protein. The band at 1164 cm⁻¹ is due to the phenolic group of tyrosine residues which was noticeably absent after reaction with gold ion. The disappearance of 1164 cm⁻¹ band due to depletion of phenolic residues was accompanied by appearance of carbonyl stretch at 1735 cm⁻¹ traceable to the formation of phenoxide structure from the oxidation of a tyrosine phenolic group [23]. The involvement of tyrosine residue in the reduction of gold ion was further confirmed by using fluorescence spectroscopy. The fluorescence of tyrosine is sensitive to oxidation [24]. In Fig. 3 the fluorescence of tyrosine residue in the plant protein was lost after reaction with gold ion indicating the complete oxidation of phenolic group tyrosine residue.

The finding in the study provides a basis for understanding the biological activities by identifying the tyrosine residue as an important and common reducing source in the system. The hydroxyl group of tyrosine was active in the reduction of metal ions and also proved that in the three custom designed tripeptides containing the different number of tyrosine residues (GGG, GGY, and GYY), the tripeptide GYY containing high tyrosine was found to be more active than GGY and GGG showed no apparent reactivity in the Ag ions reduction [23]. From the results, it is confirmed that tyrosine hydroxyl group is involved in the gold ion reduction which has been a supporting evidence in the biological system including bacteria [10,25], fungi [2,16], yeast [26] and plant leaves [3,17].

3.3. UV-Vis and TEM characterization

The plant extract was light yellow liquid, there was no colour change upon mixing the chloroauric acid solution with the plant extract at 303 K (pH = 2), although the colour gradually changed with time. The colour of the mixture after 2 h was slightly changed in to reddish brown colour and become progressively darker with time. Such colour changes are usually indicating changes in the metal oxidation state. In this case Au (III) was reduced to Au (0) by plant protein. Fig. 4a–d shows that TEM images of 2D nanostructures of gold nanoplates. The plates are formed very thin as is evident to the fact that they are electron transparent and exhibit bend contours characteristic of thin metal plateslets. Figure shows various size triangular nanoplates which gives key information about the mechanism of formation of the plateslets. The TEM images show 7 nm to 3 μm triangular nanoplates indicating the Au plateslets grow from the nuclei and do not form by aggregation of nanoparticles. Fig. 5 shows UV-Vis spectrum of gold nanoplatelet’s peak at 925 nm which is characteristic of anisotropic gold nanoplates [3]. The effect of pH plays a very important role on the morphology of the products. The reaction carried out at pH 10 (303 K) did not form any 2D nanostructures and only spherical gold nanoparticles were observed from the TEM image shown in Fig. 4e.

The energy in the solar system is primarily centered on the visible and infrared electromagnetic spectrum. The construction of buildings in high temperatures, geographical areas it is required for glass coating which in transparent but infrared opaque. Such a coating drastically reduces the rise in temperature within an enclosed room. We have carried out NIR absorption studies on the gold nanotriangle coated glass windows. The prolonged incubation of gold nanoparticles during synthesis leads to the lateral growth of the nanoparticles, which forms large sized gold nanoplates which shows absorption maxima around 1275 nm, which is shown in Fig. 6. The strong absorption in the NIR range makes them potentially interesting materials for IR absorption windows. Fig. 7
illustrates the difference in the temperature rise inside the box in a case of a plain glass window and a window that is coated with the gold platelets indicating the potential of the coated glass for IR absorption windows. The synthesis of gold nanotriangles using plant based protocol and use in infrared absorption would be an economically viable approach.

4. Conclusion

The plant proteins are found to be active biomolecules involved in synthesis of gold nanoparticles. The hydroxyl group in tyrosine residue was found to be responsible for reduction of noble metal ions. By the simple variation in the experimental conditions, it is easy to change the shape of the gold nanoparticles. The NIR absorption of gold nanotriangles has important application as IR blocker in optical coating in building windows.

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References