Biosynthesis of gold nanoparticles utilizing marine sponge *Acanthella elongata* (Dendy, 1905)

D. Inbakanand a,b,c, R. Venkatesan c, S. Ajmal Khan b

a Centre for Ocean Research, Sathyabama University, Rajiv Gandhi Salai (OMR), Chennai 600 119, India  
b Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608 502, Tamil Nadu, India  
c National Institute of Ocean Technology,否定Sanavas, Chennai 600 036, India

**Abstract**

The growing trend of exploring bacteria, fungi, actinomycetes and plant materials for the biosynthesis of nanoparticles is considered as eco-friendly and a green technological approach. In this backdrop the present study reports the synthesis of gold (Au) nanoparticles from gold precursor using the extract derived from the marine sponge, *Acanthella elongata* (Dendy, 1905) belonging to the primitive phylum Porifera. Water-soluble organics present in the marine sponge extract were mainly responsible for the reduction of gold ions to nano-sized Au particles. The sponge extract added to 10−3 M HCl aqueous solution at 45 °C changed to pinkish red color solution and confirm the bioreduction within 4 h with continuous stirring. UV-visible spectrum of the aqueous medium containing gold nanoparticles showed a peak around 526 nm. High-resolution transmission electron micrograph (HR-TEM) confirmed the monodispersed and spherical shaped with the size ranges from 7 to 20 nm, however a maximum number of particles were in 15 nm diameter. Through Fourier transform infrared spectroscopy (FT-IR) analysis, the reducing agent in the marine sponge extract was identified which is attributed for the biosynthesis of gold colloids. The XRD analysis respects the Bragg’s law and confirmed the crystalline nature of the gold nanoparticles.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Preparation of nanoparticles using green technologies is advantageous over chemical agents due to their environmental consequences. Green synthetic procedures include mixed-valence polynucleomelates [1], polysaccharides [2], tollens [3], irradiation [4], and biological methods [5]. In the biological method, extracts from living organisms may act both as reducing and capping agents in synthesis of nanoparticles. The reduction of metal ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides, and vitamins is environmentally benign, yet chemically complex. Living organisms have huge potential for the production of nanoparticles having wide applications. By using the organisms from simple bacteria to highly complex eukaryotes in the reaction mixture, the production of nanoparticles with desired shape and size can be obtained [6]. Rapid and green synthetic methods using biological extracts have shown a great potential in nanoparticle synthesis. However, understanding the mechanism of involvement of biomolecule is lacking. Better understanding will give new green paths in the development of controlled shape and size. Thus tailoring of materials at the atomic level to attain unique properties, which can be suitably manipulated for the desired applications, is a major task in nanotechnology [7].

Synthesis of nanoparticles using biological entities such as bacteria, actinomycetes, yeast, fungi and plants were reported and reviewed [8–10]. Literatures revealed that synthesis of nanoparticles using marine sponges or Porifera has been unexplored, which aroused our interest. The oceans are the source of a large group of structurally unique natural products which are mainly accumulated in invertebrates such as sponges, tunicates, bryozoans, and molluscs. Several compounds show pronounced pharmacological activities and are interesting candidates for new biotechnological applications. Marine sponges have been considered as a gold mine during the past 50 years, with respect to the diversity of their secondary metabolites. The biological effects of new metabolites from sponges have been reviewed [11]. Considering the chemical richness of marine sponges [12] as a valuable key we have explored and synthesized stable gold nanoparticles by the reduction of aqueous AuCl₄⁻ using extract of marine sponge, *Acanthella elongata*. Interestingly, this is the first report in synthesis of highly stable gold nanoparticles using marine sponge *A. elon-
gata. A wide-range of screening processes involving a number of marine sponges has led us to the species *A. elongata* as a dexterous candidate for the synthesis of gold nanoparticles.

2. Materials and methods

2.1. Collection of marine sponges

Marine sponges were collected from intertidal and subtidal regions (1–5 m deep, using SCUBA) of Gulf of Mannar, South Tamil Nadu, India. Samples were collected in bulk depending on the abundance of individual organisms and washed with freshwater to remove adhering debris and associated biota. Collected samples were stored in a refrigerated box and transferred to the lab. Further the sponge samples are labeled properly and stored in –70°C. The taxonomic identification of the organisms was done using spicules separated using nitric acid following standard identification keys [13,14].

2.2. Preparation of bio-extracts

Five gram of sponge was weighed and ground in 30 ml of water using sterile pestle and mortar. After a systematic grinding the crude extract was filtered using Whatman No. 1 (42 μm) filter paper and the residue was again ground with 20 ml of water and filtered. The filtrate (50 ml) or the crude filtered extract of sponge sample was stored in –70°C for further analysis.

2.3. UV-visible spectroscopy

From the stored filtrate, 10 ml was added to 100 ml of 10−3 M HAuCl₄ aqueous solution and kept at 45°C. The 95% of the bioreducing of AuCl₄⁻ ions occurred within 4 h with continuous stirring. A color change to pinkish ruby red of the medium was noted by visual observation confirming the bioreduction. Aliquots of the reaction solution were removed and absorptions were measured using a UV-1601 Shimadzu spectrophotometer operated at a resolution of 1 nm.

2.4. Electron microscopy

Samples for high-resolution transmission electron microscopic (HR-TEM) analysis were prepared by drop coating Au nanoparticles solutions onto carbon coated copper TEM grids. The films on the TEM grids were allowed to stand for 2 min following which the extra solution was removed using a blotting paper and the grid was allowed to dry, prior to the measurement. HR-TEM measurements were performed on a JEOL 3010 instrument operated at an accelerating voltage of 300 kV.

2.5. X-ray diffractometry

X-ray diffraction (XRD) measurement of the marine sponge reduced Au nanoparticles was carried out on films of the respective solutions drop coated onto glass substrates on a Enraf-Nonius CAD-4 MV31 single crystal X-ray diffractometer instrument operating at a voltage of 50 kV and a current of 40 mA with Cu K radiations.

2.6. Fourier transform infrared spectroscopy

For FT-IR measurements, both the sponge extract and the reduced colloidal solution were analyzed on a Perkin-Elmer FT-IR instrument in the diffuse reflectance mode at a resolution of 1.0 cm⁻¹. In order to obtain good signal/noise ratio, 512 scans were recorded.

2.7. Statistical analysis

The mean value of the sizes of the nanoparticles and number of the nanoparticles distributed (manually observed from the enlarged TEM micrographs) were useful to plot the histogram (Fig. 3) and also subjected to one-way analysis of variance (ANOVA) to determine the significance of individual differences at p < 0.05 level. Significant means were compared by the Kruskal–Wallis one-way analysis of variance on ranks. All pairwise multiple comparison procedures were done by Student–Newman–Keuls method. All statistical analyses were carried out using SigmaPlot for windows Version 11.0 (SYSTAT software Inc.).

3. Results

3.1. Biosynthesis of nanoparticles

The complete biosynthesis of gold nanoparticles by utilizing the extract of marine sponge *A. elongata* was reported here. A conical flask containing the extract of marine sponge *A. elongata* and 10−3 M aqueous HAuCl₄ was kept at 45°C in continuous stirring condition. After 4 h of the reaction it was observed that the color of the solution in flask was turned to pinkish ruby red. It confirmed the formation of gold nanoparticles in flask and the color change was due to excitation of surface plasmon vibrations in the metal nanoparticles. This important observation indicates the reduction of the Au⁺ ions and the biosynthesis of gold nanoparticles. This observation was confirmed by UV–visible spectrum and XRD analysis.

3.2. UV–visible spectrum

UV-visible spectral analysis confirmed the formation and stability of the biosynthesized gold nanoparticles using the extract of marine sponge *A. elongata*. A band observed in UV–visible spectrum (Fig. 1) corresponding to the surface plasmon resonance occurs at 526 nm and clearly indicates the formation of gold nanoparticles in solution as the exact position of absorbance depends on a number of factors such as the dielectric constant of the medium and size of the particle. It is observed that the crystallite shape of the biosynthesized gold nanoparticles dependent on the Au⁺ ion in solution and the biomolecules or factors from the extract of marine sponge *A. elongata*.
3.3. Transmission electron micrographs

A quick preparation by the deposition of sample containing the gold nanoparticles (reduced with the help of the A. elongata sponge extract) onto support copper grids or drop coated films using HREM revealed the size and shape of the gold nanoparticles. The gold nanoparticles that got reduced with the help of the A. elongata sponge extract had dimensions small enough to be electron transparent and imaged as polydispersed spherical nanoparticles with variable diameter ranging from 7 to 20 nm (Fig. 2).

3.4. X-ray diffraction pattern

The XRD patterns of 2θ = 38.15°; 44.3°; 64.5°; 77.35° and 81.7° of the sample are in agreement with the JCPSD (No. 65-2870) of bulk gold, which further proves the formation of crystalline Au. The crystalline peaks were identified as gold nanoparticles according to PCPDFWIN software, version 2.1 (International Centre for Diffraction Data, Newtown Square, PA). The intense peaks of reflected radiation (known as Bragg peaks) were produced at (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes by XRD analysis are assigned to the diffraction lines of the face-centred-cubic (fcc) gold, respectively (Fig. 4).

3.5. Fourier transform infrared spectrum

FT-IR spectrum of the sponge extract of A. elongata shows peaks at 3255, 2039, 1628, 1006 and 662 cm⁻¹, whereas the FT-IR spectrum of gold nanoparticles biosynthesized by sponge extract of A. elongata shows peaks at 3345, 2078, 1634, 1031 and 627 cm⁻¹. Curve of the sponge extract of A. elongata resulted a strong band at 3255 cm⁻¹ corresponding to O–H broad stretching of high concentration of alcohols or phenols; the band at 2039 cm⁻¹ corresponding to C–N stretching of any R–N=C=S; the medium band at 1628 cm⁻¹ corresponding to stretching of C=O; the strong band at 1006 cm⁻¹ corresponding to C–X stretching of fluoroalkanes; the medium band at 662 cm⁻¹ corresponding to C–X stretching of chloroalkanes. Curve of gold nanoparticles biosynthesized using the sponge extract of A. elongata resulted a strong band at 3345 cm⁻¹ corresponding to N–H stretching vibration of primary amines; the band at 2078 cm⁻¹ corresponding to C=N stretching of any R–N=C=S; the medium band at 1634 cm⁻¹ corresponding to similar conjugation effects to C=O; the strong band at 1031 cm⁻¹ corresponding to C–N stretching and overlapping of aliphatic amines; the medium band at 627 cm⁻¹ corresponding to C–X stretching of chloroalkanes. A band shift from 3255 cm⁻¹ corresponding to O–H broad stretching of high concentration of alcohols to 3345 cm⁻¹ corresponding to N–H stretching vibration of primary amines, or a shift from the strong band at 1006 cm⁻¹ corresponding to C–X stretching of fluoroalkanes to strong band at 1031 cm⁻¹ corresponding to C–N stretching and overlapping of aliphatic amines were the cause for the bioreduction of gold salt to gold nanoparticles (Fig. 5).

4. Discussion

4.1. Application of gold nanoparticles

Colloidal Au nanoparticles possess a lot of interesting properties that make them useful for biological applications. So far there is no indication of Au particle corrosion, and Au particles are inert, which make them relatively biocompatible [15]. Their unique features such as tunable core size, monodispersity, large surface to volume ratio, and easy functionalization with virtually any molecule or biomolecule allow targeting, transport, and tuning of delivery processes [16]. Yong et al. reviewed the use of colloidal gold nanoparticles for fabrication of anisotropic and multicomponent nanoparticles [17]. Malkov et al. developed gold nanoparticles functionalized with a valine-derived formamidine as catalysts for the reduction of ketimine 1 with trichlorosilane in toluene [18]. Ma et al. deposited gold nanoparticles onto the surface of indium–tin oxide electrode surface used for the amperometric sensing of glucose at alkaline and neutral solutions [19]. Sharma et al. denoted the assembly of nanoparticles into three-dimensional (3D) architectures which allow greater control of the interactions between gold nanoparticles with biomolecules [20]. Wang et al. reported that biogenic gold nanoparticles could facilitate the electron transfer between p-nitrophenol and glassy carbon electrode (GCE) by immobilizing on the electrode [21]. Huang et al. proved the versatility of AuNP applications in the direct or competitive surface plasmon resonance kinetic assay of the interaction between small molecule inhibitors and their target proteins with a high sensitivity [22]. Li et al. used gold nanorods with different localized surface plasmon resonance based quenching process of quantum dot (QD) emission to be efficient in DNA detection [23]. Kim et al. achieved the multimodal delivery of antibody-conjugated PEGylated gold nanoparticles enhancing the contrast in in vivo optical coherence tomography images of oral dysplasia in a hamster model [24]. Phillips et al. reported about the microbiological intelligence of gold nanoparticles when conjugated with poly para-phenylethenylthylene to identify three different strains of E. coli in minutes [25].

4.2. Biosynthesis of gold nanoparticles

The breakthrough on the biosynthesis of gold nanoparticles using marine alga, Sargassum wightii Greville [26] and the electrocatalytic applications of gold nanoparticles [27] made us to explore the marine living resource for metal colloidal biosynthesis. Our previous attempt on marine resources to find secondary metabolites to get a marine natural antifoulants [28] gave an idea to explore the Poriferans extract for the bioproduction of metal colloids. Thus we screened several marine sponges with different solvents to produce gold, silver, platinum, bimetallic alloys, etc. Interestingly while attempting extraction with marine sponge A. elongata with water it reduced the gold precursor resulting in spherical shaped monodisperse gold nanoparticles. Though biosynthesis of nano-gold was reported recently in Barbatell Skellicup herb [21], Penicillium sp. [29], tropical marine yeast Yarrowia lipolytica NCIM 3589 [30], Bacillus megatherium D01 [31], Bacillus licheniformis [32], Aspergillus niger [33], Stenaphrophomonas malphisilia [34], Magnolia lobus and Diaspyros kaki leaf extracts [35], edible mushroom extract [36], Pear fruit [37], etc., we are proud to report for the first time of biosynthesis of gold nanoparticles using marine sponge A. elongata.

4.3. Characterization by UV-visible spectrum

In metal nanoparticles such as in gold, the conduction band and valence band lie very close to each other and through these electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band, occurring due to the collective oscillation of electrons of gold nanoparticles in resonance with the light wave. Classically, the electric field of an incoming wave induces polarization of the electrons with respect to much heavier ionic core of gold nanoparticles. As a result a net charge difference occurs, which in turn acts as a restoring force. This creates a dipolar oscillation of all the electrons with the same phase. When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, a strong absorption takes place, which is the origin of the observed color, which was pinkish ruby red in our observation. This absorption strongly depends on the particle size, dielectric medium and chemical surroundings. Small
Fig. 2. (a–e) HR-TEM images of gold nanoparticles formed by reduction of Au⁺ ions using the extract of Acrostella elongata. (a) 50 nm scale, (b) selected area diffraction pattern, (c) 20 nm scale, (d) 5 nm scale and (e) 5 nm scale.
spherical nanoparticles (<20 nm) exhibit a single surface plasmon band. The UV/vis absorption spectra of the gold nanoparticles dispersed in marine sponge extracts is shown in Fig. 1. The absorption peak (SPR) was obtained in the visible range at 526 nm [38-41].

4.4. Characterization by electron micrographs

High-resolution transmission electron micrographs was employed to visualize the size and shape of the gold nanoparticles formed. The TEM images were (Fig. 2a, c, d and e) the representation of the gold nanoparticles biosynthesized using the extract of marine sponge A. elongata. The particles were formed in different sizes, ranging from 7 to 20 nm in diameter, poly dispersed, small and large spherical shape. The histogram (Fig. 3) obtained using the enlarged TEM graphs revealed almost 25% of the particles were 15 nm in diameter. The selected area diffraction pattern [42,43] from one of the gold in Fig. 2b supports the crystalline nature which confirmed by the circular ring pattern. Biomolecules from the extract of marine sponge A. elongate, such as secondary metabolites, are expected to interact with the crystal faces differently, thereby changing the surface energies of the latter in due course. Based on the experimental results the surface had interacted stronger with the biomolecules extracted from the marine sponge, resulting in crystal growth biased by the inhibition of Au atom accumulation of the surface.

4.5. Characterization by X-ray diffraction

The observations by UV-visible spectral analysis is reconfirmed by verifying the sample of the gold nanoparticles biosynthesized with the use of the extract of marine sponge A. elongate by XRD pattern. Fig. 4 shows the XRD patterns obtained for biogenic gold nanoparticles using marine sponge extract. XRD analysis showed three distinct diffraction high peaks at 38.15°, 44.3° and 64.6°, which indexed the planes 1 1 1, 2 0 0 and 2 2 0 of the cubic face-centered gold followed by a couple of low peaks at 77.35° and 81.2° indexed the 3 1 1 and 2 2 2 planes. A number of Bragg reflections corresponding to the lattice planes are observed which may be indexed based on the cubic face-centered structures of gold matched with the database of Joint Committee on Powder Diffraction Standards [44]. The XRD pattern thus clearly shows that the gold nanoparticles formed by the reduction of Au⁺ ions by the extract of marine sponge A. elongata are crystalline in nature.

4.6. Characterization by FT-IR spectrum

FT-IR-spectroscopy from the absorption of IR radiation through resonance of non-centro symmetric (IR active) modes of vibration and is a useful tool for quantifying secondary structure in metal nanoparticle-biomolecules interaction [45]. Fig. 5 confirms that the N-H stretching vibration of primary amines and C-N stretching and overlapping of aliphatic amines has the stronger ability to bind metal, so that the secondary metabolites from the extract of marine sponge A. elongata could most possibly form a coat covering the metal nanoparticles (i.e. capping of gold nanoparticles) to prevent agglomeration of the particles and stabilizing in the medium. This evidence suggests that the biological molecules could possibly perform the function for the formation and stabilization of the gold colloids in aqueous medium. The exact mechanism leading to the reduction of metal ions is yet to be elucidated for marine sponge A. elongate.

5. Conclusion

In conclusion, the marine sponge reduction of auric chloride by A. elongate yielded uniform gold nanoparticles. The present investigation indicates extracellular synthesis of highly stable gold nanoparticles by biotransformation using various species of marine sponges. This simple procedure for the biosynthesis of gold nanoparticles has several advantages such as cost-effectiveness,