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Can animals too negotiate nano transformations?

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Abstract. Cockroach (*Periplaneta americana*) broth has been employed to assess its potential as a candidate source animal tissue for the synthesis of gold nanoparticles. The synthesis is performed akin to room temperature in the laboratory ambience. X-ray and transmission electron microscopy analyses are performed to ascertain the formation of nanoparticles. The synthesis of nanoparticles might have resulted due to the activity of chitin, metallothioneine and tropomyosin. A possible involved mechanism for the biosynthesis of nanoparticles has also been proposed. This work further indicates that the animal wastes too can effectively participate in nano-transformations thereby helping in controlling the environmental pollution and subsequently the different diseases.

Keywords: nanobiotechnology; biological synthesis; nano gold; nanoparticles; cockroach

1. Introduction

Synthesis of different varieties of nanomaterials using Bionanotechnological approach has gained prominence nowadays. Biosynthesis of metal, metal oxide as well as chalcognide nanoparticles have been reported in the immediate past using banana fly (Jha and Prasad 2012a), microbes (Jha and Prasad 2012b, 2010a, Prasad *et al*. 2010, Jha *et al*. 2007, 2009a, 2009b, 2010, Bansal *et al*. 2005, Sadowski *et al*. 2008, Nair and Pradeep 2002, Joerger *et al*. 2001, Klaus *et al*. 2001, Mukherjee *et al*. 2001, Shankar *et al*. 2003, Prasad and Jha 2009, 2010), plants (Agarwal *et al*. 2013, Kotakadi *et al*. 2013, Subba Rao *et al*. 2013, Jha and Prasad 2012c, 2011a, Jha *et al*. 2009c, 2009d, Arangasamy and Munusamy 2008, Huang *et al*. 2007, Chandran *et al*. 2006, Armendariz *et al*. 2004, Shankar *et al*. 2004, Shankar *et al*. 2003, Song and Kim 2009, Jha and Prasad 2010b), fruits and/or food beneficiaries (Jha and Prasad 2011b, Jha *et al*. 2011, Cruz *et al.* 2010, Jain *et al.* 2009, Li *et al.* 2007, Philip 2009a, 2009b, Ankamwar *et al.* 2005, Damle *et al.* 2005), *etc*. With an exponential growth of meat consumption in different forms has resulted in to a vast heap of slaughter house wastes leading to different forms of environmental pollution. These wastes could also be potential sources of many important metabolites (like allantoin and bile acids). Using a lower animal tissue as a potential candidate source has many fold advantages as it might act as a model animal tissue for synthesizing nanomaterials thereby opening an avenue of opportunity for utilizing the slaughter house wastes as putative mean for synthesizing

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nanomaterials. Cockroaches excrete a potent allergen, Bla g 2, that is associated with IgE production and asthma (Arruda *et al.* 1995). The thermodynamic/stereochemical agility of tropomyosin / Bla g 2 could also be a boon in the procedure as they have plenty of sulfa hydryl group in the molecule.

In this work, broth of Cockroaches (*Periplaneta americana*) have been employed to assess their potential as a model candidate animal tissue source for the synthesis of gold nanoparticles for the first time. To ascertain the formation of gold nanoparticles (abbreviated hereafter as Au NPs) was characterized by X-ray diffraction and transmission electron microscopy studies. An effort has also been made to understand the mechanism of nano-transformation of accomplishing biosynthesis.

2. Experimental details

2.1 Preparation of cockroach broth

Two healthy and taxonomically authenticated cockroaches were procured and were sacrificed in boiling 30% ethanol (100 ml) after removing their wings and lower alimentary canal, having the body weight of 3.67 gm, (in order to avoid defecation) until the body integuments get softer and show signs of detachment leading to a color change of the medium (from transparent to a dirty straw). This extract was carefully filtered after gently meshing the animal tissues, pooled and cooled for 20 minutes at room temperature. The finely meshed animal tissue was further boiled in fresh pool of sterile distilled water (200 ml) for 10 minutes. This too was cooled and filtered in order to ensure complete extraction of the probable candidate metabolites. Thereafter, both the extracts were mixed and utilized for the preparation of Au NPs.

2.2 Biosynthesis of gold nanoparticles

Now, 20 ml of 0.25 M aqueous $HAuCl_4$ was taken and added to the 200 ml of culture solution and was heated on steam bath up to 40°C for 15 minutes until reddish-brown colour appeared in the culture solution, indicating the initiation of transformation. The culture solution was cooled and allowed to incubate at room temperature in the laboratory ambience overnight after which distinctly markable coalescent dark brown clusters get deposited at the bottom of the flasks leaving faint wine red supernatant and subsequently filtered and dried.

2.3 Characterization

The formation of Au NPs was checked by X-ray diffraction (XRD) technique using a X-ray diffractometer (Rikagu Miniflex, Japan) with CuK_α radiation λ = 1.5406Å between the angles 15° and 75°. TEM micrograph and selected area diffraction (SAED) pattern of Au NPs were obtained using Bruker transmission electron microscope. The specimen was suspended in distilled water, dispersed ultrasonically to separate individual particles, and one or two drop of the suspension was deposited onto holey-carbon coated copper grids and dried under infrared lamp.

3. Results

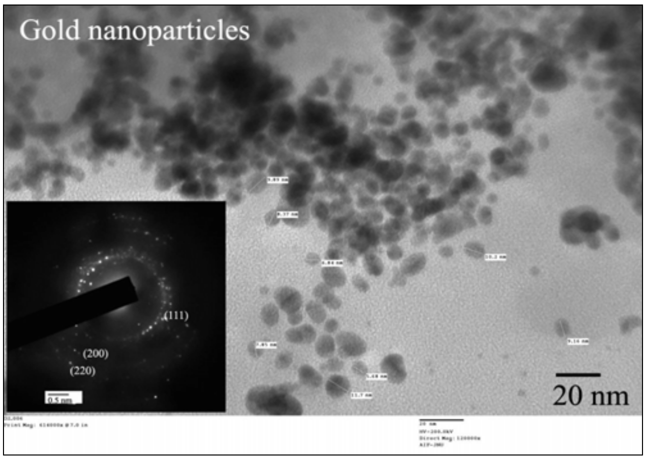

Fig. 1 TEM photograph and SAED pattern of Au NPs synthesized using *Periplaneta americana*

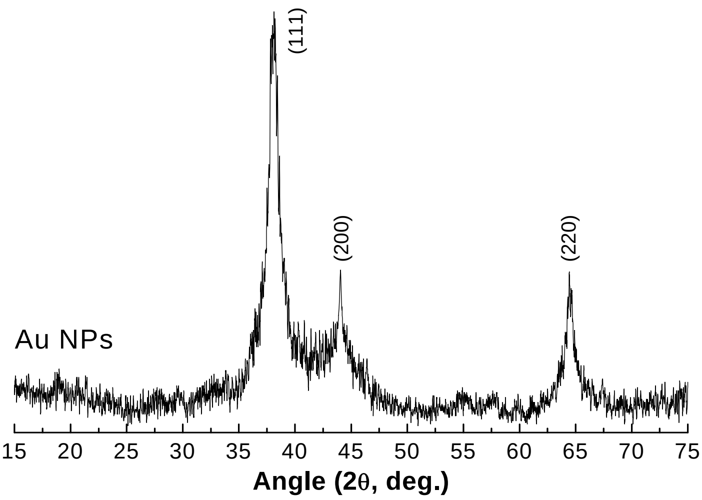

Fig. 2 X-ray diffraction pattern of Au NPs at room temperature

Fig. 1 shows the TEM micrograph of the Au NPs being formed using *Periplaneta americana*. Nearly spherical shaped particles of 7-12 nm are found. The difference in size may possibly be due to the fact that the nanoparticles are being formed at different times. Also, a combination of Scherrer rings and spotty pattern, as seen in the SAED pattern (inset Fig. 1), clearly indicate the formation nanocrystalline particles.

To ascertain the crystal structure of Au NPs, XRD study was carried out. Fig. 2 illustrates the indexed XRD profile of Au NPs, at room temperature. Three Bragg reflections corresponding to the (111), (200) and (220) sets of lattice planes of face-centered cubic (*fcc*) gold were indexed using a software "POWD". The XRD pattern showed the presence of broad peaks. The broad peaks indicate either particles of very small crystallite size, or particles are semi-crystalline in nature. The cell edge length was estimated using experimental 2θ-values of peaks to be 4.076 Å which is in agreement with the literature report (ICDD no. #04-0784). A little difference of 0.002 Å between the cell parameter of gold bulk particles (4.078 Å) and nanoparticles has been observed. Consequently, a lowering in unit cell volumes of bulk Au and Au NPs has been noticed which may be due the nanosizing (quantum sizing) effect. An estimate of the apparent particle size of the nanoparticles was made from the line broadening of the strongest reflection using the Scherrer formula: $D = 0.89\lambda / \beta_{1/2} \cos\theta$, where $\beta_{1/2}$ is the full width at half maximum. It is found that the apparent particle size (~10 nm) of the prepared NPs estimated using Scherrer technique to be in fairly good agreement with the size estimated by TEM results. Furthermore, indexing of SAED pattern (inset Fig. 1) revealed the *fcc* lattice structure, which is in agreement with XRD result.

4. Discussion

Insects, like other animals, require heavy metals in their metabolism, but suffer from metal toxicity when they are exposed to levels above their capacity for detoxification. It is argued that heavy metals played a crucial role in the origin of life itself (Arruda *et al.* 1995). Insects also have adopted heavy metals as essential elements in their metabolism. They maintain the equilibrium of their internal milieu by diffusion mechanisms, but more frequently by a substantial storage of metals in the cells of numerous organs in a structure called the spherocrystal, which originates from the endoplasmic reticulum-Golgi complex: elements precipitate on a glycosaminoglycan nucleus in a thin peripheral stratum. Some spherocrystals contain exclusively mineral compounds, frequently phosphates, whereas others may contain organic compounds such as urates. When fed additional metals found in the environment, insects such as cockroach and ant are able to stay alive and to trap the metals (Cd or Pb, for example) in the peripheral strata of spherocrystals; the cytoplasm is not altered. It seems that these insects are able to resist exposures to high levels of toxic metals. Along with all these, the lysosomes are able to retain toxic heavy metals (Cd or Hg, for example) within metallothionein-like proteins (Ballan-Dufrançais 2002).

Metallothioneins are ubiquitous, small, cysteine-rich proteins with the ability to bind heavy metals. The induction of metallothionein (MT) was studied in the housefly larvae (*Musca domestica*). Upon dietary exposure to Cd, two Cd-binding proteins were isolated from the whole body homogenates, using gel filtration and ion exchange chromatography. Mass spectrometric measurement revealed that they have high purity and the molecular weights are 9045.9Da and 11560.2Da, respectively. Amino acid analysis showed that the content of cysteine is the highest, attaining to 18.2%. However, aromatic amino acid residues such as tyrosine (2.5%) and phenylalanine (3.1%) were also detected. From insects to mammals, metallothionein genes are induced in response to heavy metal load by the transcription factor MTF-1, which binds to short DNA sequence motifs, termed metal response elements (MREs) (Selvaraj *et al.* 2005). At the gene level, the expression of *MTs* is transcriptionally regulated by metal-responsive transcription factor 1 (MTF-1), homolog to the mammalian MTF-1. Upon metal load, MTF-1 binds to the short DNA motifs termed metal-response elements (MREs) in the MT promoter, which are necessary and sufficient to mediate the transcriptional response to heavy metals (Stuart *et al.* 1984).

The body wall of cockroach is made up of chitin (a polymer of N-acetylglucosamine). Due to presence of both the amino group as well as acetyl group, it shows mildly acidic property in the

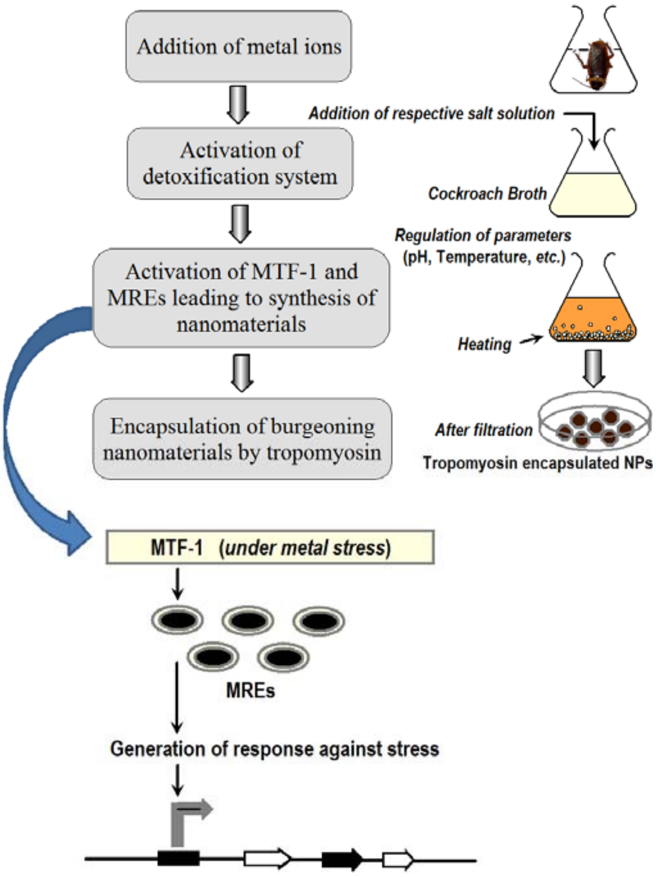

Fig. 3 Schematics for the biosynthesis of Au NPs using *Periplaneta americana*

extract solution which can be suitably manipulated to obtain desired nanomaterial (Fig. 3). Though, the cells die after boiling but the liberated metabolites help in nanomaterials fabrication. Further, cockroaches contain the proteins that trigger allergic reactions among human identified as tropomyosin. Tropomyosin is an actin-binding protein that regulates actin mechanics (Asturias *et al.* 1999). It is composed of four alpha helices, A, B, C, and D, which coil together to form the quaternary structure and is important, among other things, for muscle contraction (Asturias *et al.* 1999). In the present case this protein might also have helped in redial encapsulation of freshly burgeoned nanomaterials thereby rendering stability. The overall similarity of Bla g 2 to the structures of other well-known aspartic proteases, suggest that ligand binding could be involved in the function of this allergen (Arruda *et al.* 1995). Our investigation involving other insects and their developmental stages is still on.

5. Conclusions

In summary, Au NPs have successfully been prepared using cockroach (*Periplaneta americana*). Therefore, present biosynthetic method is capable of producing gold nanoparticles, which might have resulted due to the activity of chitin, metallothioneine and tropomyosin, indicated thereby that animals too can negotiate nano transformations. Furthermore, present work might be a step towards utilizing animal system and/or animal wastes in synthesizing different nanomaterials and consequently addressing environmental issues.

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