Available online at www.sciencedirect.com
ScienceDirect
Materials Letters 61 (2007) 3984–3987
materials letters
www.elsevier.com/locate/matlet

Biosynthesis of gold nanoparticles using the bacteria *Rhodopseudomonas capsulata*

Shiying He, Zhirui Guo, Yu Zhang, Song Zhang, Jing Wang, Ning Gu *

State Key Laboratory of Biolectronics, Southeast University, Nanjing, 210096, PR China
Jiangsu Laboratory for Biomaterials and Devices, Southeast University, Nanjing, 210096, PR China

Received 10 October 2006; accepted 1 January 2007
Available online 13 January 2007

Abstract

The use of microorganisms in the synthesis of nanoparticles emerges as an eco-friendly and exciting approach. In this study, the bacteria *Rhodopseudomonas capsulata* was screened and found to successfully produce gold nanoparticles of different sizes and shapes. The important parameter, which controls the size and shape of gold nanoparticles, was pH value. The *R. capsulata* biomass and aqueous $HAuCl_4$ solution were incubated at pH values ranging from 7 to 4. The results demonstrated that spherical gold nanoparticles in the range of 10–20 nm were observed at pH value of 7 whereas a number of nanoplates were observed at pH 4.

Keywords: Biosynthesis; Nanomaterials; Gold; Enzyme; Microorganism; Electron microscopy

1. Introduction

The optoelectronic and physicochemical properties of nanoscale matter are size- and shape-dependent [1–3]. So the synthesis of gold nanoparticles of different sizes and shapes is of great importance for their applications in optical devices, electronics, biotechnologies and catalysis [4–6]. Conventional synthetic methods of gold nanoparticles have involved a number of chemical methods [7–10]. There is an increasing pressure to develop clean, nontoxic and environmentally benign synthetic technologies. Microbial resistance against heavy metal ions has been exploited for biological metal recovery via reduction of the metal ions or formation of metal sulfides [11]. So the attractive procedure is using microorganisms such as bacteria and fungi to synthesize gold nanoparticles recently. An earlier study found that *Bacillus subtilis* 168 [12] were able to reduce Au^{3+} ions to gold nanoparticles with a size range of 5–25 nm inside the cell walls. *Shewanella algae* were found to reduce Au^{3+} ions forming 10–20 nm gold nanoparticles extracellularly with the assistance of hydrogen gas [13]. Fungi (*Verticillium* sp [14] and *Fusarium oxysporum* [15]) and actinomycete (*Thermomonospora* sp [16] and *Rhodococcus* sp [17]) were also used to synthesize nanoparticles intra- or extracellularly. However, the biosynthesis of gold nanoplate extracellularly is still scarce. In this study, prokaryote bacteria *Rhodopseudomonas capsulata*, recognized as one of the ecologically and environmentally important microorganisms, commonly existing in the natural environment, were investigated for reducing Au^{3+} ions at room temperature with a single step process. Especially gold nanoplates were formed under the lower starting pH.

2. Experiment

2.1. Synthesis

Photosynthetic bacteria *R. capsulata* were cultured in the medium containing purvate, yeast extract, NaCl, NH_4Cl and K_2HPO_4 at pH 7 and 30 °C. The bacteria were cultured for 72 h and separated from broth by centrifugation (5000 rpm) at 4 °C

* Corresponding author. State Key Laboratory of Biolectronics, Southeast University, Nanjing, 210096, PR China. Tel.: +86 25 8379 2576; fax: +86 25 8379 4960.
E-mail address: guning@seu.edu.cn (N. Gu).

0167-577X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.matlet.2007.01.018

for 10 min. The collected bacteria were washed five times with distilled water to obtain about 1 g wet weight of bacteria and then resuspended in 20 mL of 1×10^{-3} M aqueous $HAuCl_4$ solution in a test tube. The reactants were adjusted to neutral pH using 0.1 M NaOH solution. In other experiments, the starting pH of the 1×10^{-3} M aqueous $HAuCl_4$ solution added with biomass was adjusted to 6, 5 and 4 using 0.1 M NaOH and HCl solutions respectively in test tubes. All the experiments were conducted at room temperature for a period of 48 h.

The UV–Visible spectra of gold nanoparticles synthesized were measured on a Shimadzu spectrophotometer (model UV-3150PC). The products were dropped on a carbon-coated grid and then analyzed by transmission electron microscopy (TEM) (JEOL, JEM-200EX) and electronic diffraction (ED, JEOL, JEM-200EX) was used to determine the crystal structure of the samples. X-ray diffraction (XRD) measurements were carried out on a Shimadzu XD-3A instrument. The reaction solution was inoculated on the agar plate to detect the viability of bacteria after reacting with aqueous $HAuCl_4$ solution.

3. Results and discussion

The aqueous chloroaurate ions were reduced during exposure to the bacteria *R. capsulata* biomass. The color of the reaction solution turned from pale yellow to purple (as shown in Fig. 1A), which indicated the formation of gold nanoparticles extracellularly. The reaction was completed after 48 h of incubation indicating that it was a slow process. The color of the reaction solution remained purple without any changes, and the gold nanoparticles analyzed by UV–Vis spectra and TEM were stable after 48 h of reaction. Control experiments without biomass addition stayed pale yellow, indicating that the production of gold nanoparticles was obtained by the reduction of microorganisms indeed. Fig. 1B shows the UV–Vis absorption spectra recorded from the gold nanoparticles solution after 48 h of reaction (curve 1). The results indicate that the reaction solution has an absorption maximum at about 540 nm attributed to the surface plasmon resonance band (SPR) of the gold nanoparticles. After the reaction, the gold nanoparticles solution was filtered from the bacteria *R. capsulata* biomass and aged for three months. Then the gold NPs solution was tested for stability using UV–Vis spectra measurement. The results as shown in Fig. 1B (curve 2) indicate that the solution was stable for at least three months with only little aggregation of particles in the solution.

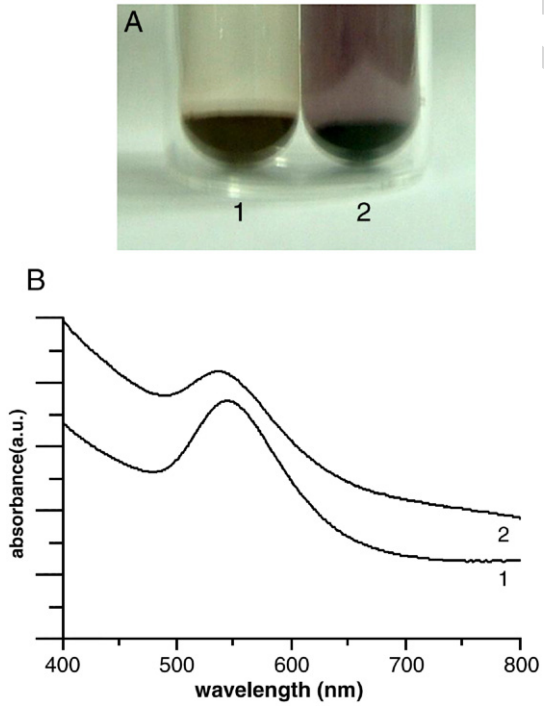


Fig. 1. (A) Picture of test tubes containing the bacteria *R. capsulata* biomass before (test tube 1) and after 48 h (test tube 2) incubation in an aqueous of $AuCl_4^-$ solution at neutral pH; (B) UV–Vis absorption spectra of gold nanoparticles after the reaction of 10^{-3} M aqueous $HAuCl_4$ solution at neutral pH with the bacteria *R. capsulata* for 48 h (curve 1) and kept for three months after reaction (curve 2).

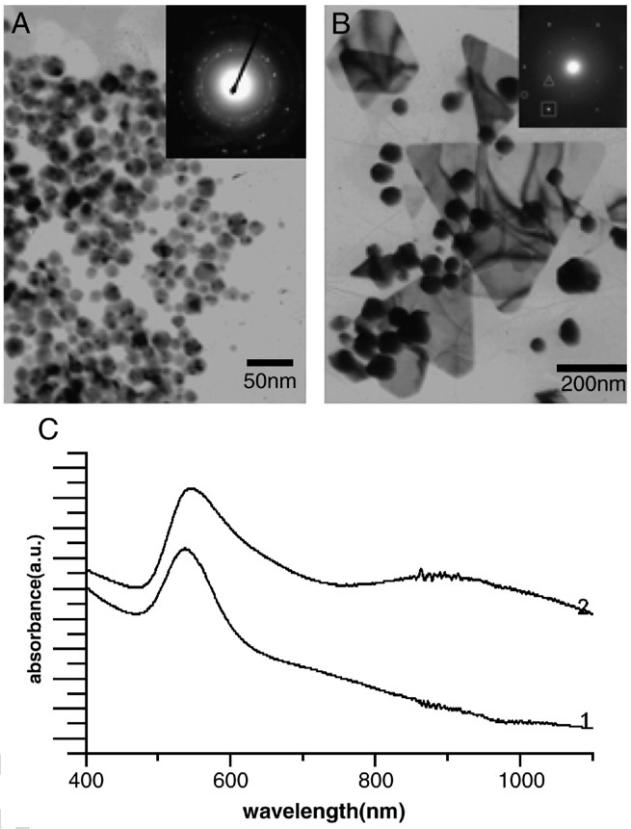


Fig. 2. (A) TEM image of the gold nanoparticles produced by the reaction of 10^{-3} M aqueous $HAuCl_4$ solution with bacteria *R. capsulata* biomass at pH 7. The inset shows their ED pattern. (B) TEM image of the gold nanoparticles produced by the reaction of 10^{-3} M aqueous $HAuCl_4$ solution with bacteria *R. capsulata* biomass at pH 4. The inset shows a typical SAED pattern of a gold nanoplate. (C) UV–Vis absorption spectra of gold nanoparticles produced at pH 7 (curve 1) and pH 4 (curve 2).

Experiments were performed to detect the viability of the bacteria to the gold ions and gold NPs. Bacteria *R. capsulata* were found to proliferate when a small drop of solution containing bacteria and gold NPs was inoculated on agar plates demonstrating that the bacteria are resistant to the gold ions and gold NPs.

Transmission electron microscopy (TEM) measurements show the various shapes and sizes of gold nanoparticles formed with the changes of pH. As shown in Fig. 2A, well-separated gold nanoparticles with occasional aggregation are mainly spherical in the size range 10–20 nm at pH 7. This is consistent with the optical spectrum (Fig. 2C). The diffraction ring, as can be seen from the electron diffraction (ED) pattern recorded from the gold nanoparticles, is consistent with

nanocrystalline gold. With the decrease of pH, gold nanoplates appeared. When the pH was adjusted to 4, there were a number of nanoplates observed in addition to the spherical gold nanoparticles in the reaction solution. Fig. 2B shows the representative TEM image of gold nanoparticles obtained. It is clearly seen that the predominantly larger nanoparticles are triangular nanoplates. The single-crystalline structure of these nanoplates was further confirmed by their corresponding selected-area electron diffraction (SAED) analysis. From many TEM images similar with Fig. 2B, statistical analysis of the gold nanoparticles was performed. The results indicate that nearly 60% of the total nanoparticle population is due to triangular gold nanoplates in the size of 50–400 nm. And most of the other nanoparticles are spherical with a size range of 10–50 nm. Fig. 2C shows typical UV–Vis–NIR spectrum of gold nanoparticles at pH 4. Two SPR bands of the gold nanoplates centering respectively at about 540 nm (band I) and 900 nm (band II) are clearly visible. Band I is a common feature corresponding to the traverse surface plasmon resonance and band II that occurs in the NIR region indicates the longitudinal plasmon resonance of the gold nanoplates.

As shown by the XRD pattern in Fig. 3, which corresponds to the gold nanoparticles in Fig. 2B, Bragg reflections are present, which could be indexed on the basis of the face-centered cubic (fcc) gold structure. No spurious diffractions due to crystallographic impurities were found. An overwhelmingly strong diffraction peak located at 38.09° is ascribed to the {1 1 1} facets of face-centered cubic metal gold structures, while diffraction peaks of other four facets are much weak. It is worth pointing that the ratio of intensity between the {2 0 0} and {1 1 1} peaks is much lower than the standard value (0.068 versus 0.53). The ratio between the {2 2 0} and {1 1 1} peaks is also much lower than the standard value (0.039 versus 0.33). These observations indicate that the gold nanoplates formed by the reduction of Au (III) by bacteria *R. capsulata* are dominated by the {111} facets, and most of the {111} planes parallel to the surface of the supporting substrate were sampled.

These results show that the starting pH could have an important effect on the size and shape of gold nanoparticles. Different pH would regulate the proton concentration resulting in the control of gold nanoparticles morphology. The main groups of the enzyme secreted by biomass that may play an important role in reducing the $AuCl_4^-$ ions include amino, sulfhydryl and carboxylic groups [14–16,18]. And the $AuCl_4^-$ ions could bind to biomass through these functional groups. These groups of the biomass might carry more positive charge at low

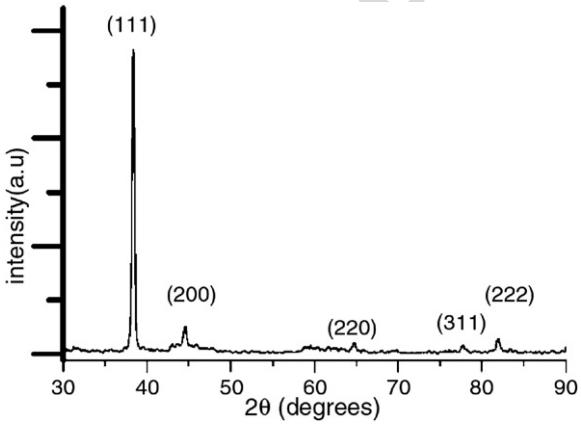
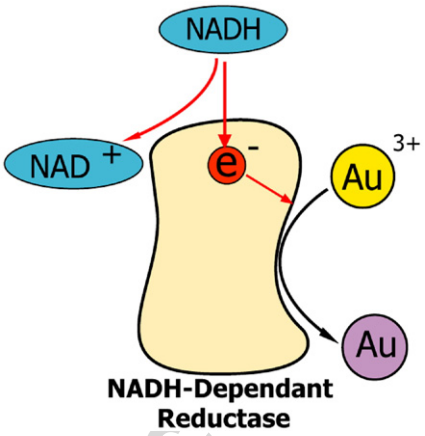


Fig. 3. Representative XRD pattern of gold nanoplates synthesized by the reaction of 10^{-3} M aqueous $HAuCl_4$ solution with bacteria *R. capsulata* biomass at pH 4.



Scheme 1. Possible mechanisms of gold ions bioreduction.

pH values, which weakens the reducing power of the biomass and allows the $AuCl_4^-$ ions to get closer to the binding sites. So the reaction rate of gold ions is very slow and Au-biomass biosorbent is strong, which would contribute to the formation of nanoplate morphologies. On increasing pH values, the reducing power and reaction rate increase correspondingly and could contribute to the formation of thermodynamic-favored spherical particles.

The possible role as shown in Scheme 1 is involved in the electron shuttle enzymatic metal reduction process. Previous studies [15,19,20] have indicated that NADH- and NADH-dependent enzymes are important factors in the biosynthesis of metal nanoparticles. Bacteria *R. capsulata* are known to secrete cofactor NADH- and NADH-dependent enzymes that may be responsible for the bioreduction of Au (3+) to Au (0) and the subsequent formation of gold nanoparticles. The reduction seems to be initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier. Then the gold ions obtain electrons and are reduced to Au (0). The exact mechanism of the reduction of gold ions is yet to be elucidated for bacteria *R. capsulata*. Our current research focuses on the biological reduction mechanism of gold ions and our findings will be reported later.

4. Conclusion

In conclusion, it has been demonstrated that the bacteria *R. capsulata* are capable of producing gold nanoparticles extracellularly and the gold nanoparticles are quite stable in solution. And this is an efficient, eco-friendly and simple process. The shape of the gold nanoparticles is controlled by pH. Work is continuing for the study of the biological mechanism for the nanoparticles formation and more detailed study of controlling the shapes and sizes of nanoparticles. The NIR absorbance of the gold nanotriangles could find interesting applications in cancer hyperthermia. This report will also lead to the development of a rational biosynthetic procedure for other metal nanomaterials such as silver and platinum with the bacteria *R. capsulata*.

Acknowledgements

The authors gratefully acknowledge the financial support given to this research from the National Natural Science Foundation of China (Nos. 60371027, 60171005 and

90406023). We are also grateful to Mr. Aiqun Xu and Xun Xiao from the Analysis and Testing Centre of Southeast University for their kind help with the measurements.

References

[1] A.P. Alivisatos, J. Phys. Chem. 100 (1996) 13226.
[2] R. Jin, Y. Cao, C.A. Mirkin, K.L. Kelly, G.C. Schatz, J.G. Zheng, Science 294 (2001) 1901.
[3] J. Aizpurua, P. Hanarp, D.S. Sutherland, M. Kall, G.W. Bryant, F.J.G. de Abajo, Phys. Rev. Lett. 90 (2003) 057401-1.
[4] C.N.R. Rao, A.K. Cheetham, J. Mater. Chem. 11 (2001) 2887.
[5] M. Moreno-Manas, R. Pleixats, Acc. Chem. Res. 36 (2003) 638.
[6] M. Han, X. Gao, J.Z. Su, S. Nie, Nat. Biotechnol. 19 (2001) 631.
[7] W.M. Tolles, Nanotechnology 7 (1996) 59.
[8] P.R. Selvakannan, S. Mandal, R. Pasricha, S.D. Adyanthaya, M. Sastry, Chem. Commun. 13 (2002) 1334.
[9] K. Okitsu, A. Yue, S. Tanabe, H. Matsumoto, Y. Yobiko, Langmuir 17 (2001) 7717.
[10] Y. Sun, Y. Xia, Science 298 (2002) 2176.
[11] J.R. Stephen, S.J. Maenaughton, Curr. Opin. Biotechnol. 10 (1999) 230.
[12] T.J. Beveridge, R.G.E. Murray, J. Bacteriol. 141 (1980) 876.
[13] Y. Konish, N. Deshmukh, T. Tsukiyama, N. Saitoh, Trans. Mater. Res. Soc. Jpn. 29 (2004) 2341.
[14] P. Mukherjee, A. Ahmad, M. Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Ramani, R. Parischa, P.V. Ajayakumar, Angew. Chem., Int. Ed. 40 (2001) 3585.
[15] P. Mukherjee, S. Senapati, A. Ahmad, M.I. Khan, M. Sastry, ChemBiochem 3 (2002) 293.
[16] A. Ahmad, S. Senapati, M.I. Khan, R. Kumar, M. Sastry, Langmuir 19 (2003) 3550.
[17] A. Ahmad, S. Senapati, M.I. Khan, R. Ramani, V. Srinivas, M. Sastry, Nanotechnology 14 (2003) 824.
[18] V. Armendariz, I. Herrwra, J.R.P. Visea, M.J. Yacaman, J. Nanopart. Res. 6 (2004) 377.
[19] A. Ahmad, P. Mukherjee, M. Mandal, S. Senapati, M.I. Khan, R. Kumar, M. Sastry, J. Am. Chem. Soc. 124 (2002) 12108.
[20] S. Senapati, A. Ahmad, M.I. Khan, M. Sastry, R. Kumar, Small 1 (2005) 517.