Kinetics of Synthesis of Gold Nanoparticles by *Acinetobacter* sp. SW30 Isolated from Environment

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Abstract Cell biomass and metal salt concentration have great influence on morphology of biosynthesized nanoparticle. The aim of present study was to evaluate the effect of varying cell density and gold salt concentrations on synthesis of nanoparticles and its morphology, which has not been studied in bacteria till now. When cells of *Acinetobacter* sp. SW30 were incubated with different cell density and gold chloride concentrations, tremendous variation in color of colloidal solution containing gold nanoparticles (AuNP) was observed indicating variation in their size and shapes. Surprisingly, monodispersed spherical AuNP of size ~ 19 nm were observed at lowest cell density and HAuCl_4 salt concentration while increase in cell number resulted in formation of polyhedral AuNP (~ 39 nm). Significance of this study lays in the fact that the shape and dispersity of AuNP can be customized depending up on the requirement. FTIR spectrum revealed shift from 3221 to 3196 cm^{-1} indicating the presence and role of amino acids in Au^{3+} reduction while possible involvement of amide I and II groups in stabilization of AuNP. The rate constant was calculated for cell suspension of 2.1×10^9 cfu/ml challenged with 1.0 mM HAuCl_4 , incubated at 30°C and pH 7 using the slopes of initial part of the plot $\log (A_\infty - A_t)$ versus time as $1.99 \times 10^{-8}\text{ M}$.

Also, this is the first study to report the kinetics of gold nanoparticle synthesis by *Acinetobacter* sp. SW30.

Keywords *Acinetobacter* · Cell density · Gold chloride concentration · Fourier transform infrared · Kinetics

Introduction

Now a days, biological systems are extensively used in the field of nanoscience giving rise to new term nanobiotechnology, which is tailoring of the entities in $1\text{--}100$ nm range using biological system or for the benefit of biological system [1]. Synthesis of nanomaterials by biological source is mediated by micro organisms, plant extracts and various biomolecules [2, 3]. There are many bacteria, the most abundant organisms in biosphere, reported to produce metal nanoparticles extra and intracellularly [2, 4].

Acinetobacter are a diverse group of organisms and ubiquitous in nature [5–8]. They have high ability to form biofilm and can withstand the extreme conditions [9, 10]. This group is known to be resistant to various metal ions and antibiotics [11–13]. *Acinetobacter* are also known to contain many proteins and enzymes responsible for metal nanoparticle synthesis [14].

The gold nanoparticles (AuNP) can be synthesized by many bacteria in various forms and shapes [2] and their applications highly depend on its size and shape. For large scale synthesis AuNP production needs to be optimized. The effect of single parameters such as culture age, cell density, salt concentration, temperature and pH of reaction on synthesis and morphology of AuNP are reported in our previous study [15]. However, the synthesis of AuNP by varying the proportion of cell density and gold salt concentration to control the size and shape of AuNP is not

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studied. It is important to optimize the cell number and gold salt concentration for economical production of AuNP in industries. Also, the kinetics of synthesis of AuNP must be studied to understand the process and rate of reaction of synthesis.

The aim of present study was to optimize and evaluate the effect of varying cell density and gold salt concentration on nanoparticle synthesis and its morphology, which is not studied in bacteria so far. In present study we first time report the kinetics of gold nanoparticle synthesis by bacteria.

Materials and Methods

Synthesis of AuNP Using *Acinetobacter* sp. SW 30

Acinetobacter sp. SW 30 isolated from fresh activated sewage sludge, was inoculated in Luria Bertanii broth and incubated at 30 °C and 180 rpm for 24 h. After 24 h of incubation, cells were harvested by centrifugation at $19,230 \times g$ for 10 min at 10 °C, they were washed with sterile distilled water thrice and then were suspended in sterile distilled water. These suspended cells were adjusted at different cell density corresponding to $<0.3, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4$ and 2.7×10^9 colony forming units/ml (cfu/ml) as per McFarland's standards [16]. Cells adjusted at each McFarland standard were challenged with different concentrations of gold chloride salt (HAuCl₄ procured from SRL, India) viz 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM. They were incubated at 30 °C at 180 rpm in dark. Synthesis of AuNP was monitored by observing the change in color of suspension and also recording the UV–visible spectrum (UV–Vis) from 200 to 800 nm using UV–Vis spectrophotometer (Jasco V-530, USA). Synthesized AuNP showing maximum SPR was observed by transmission electron microscopy (TEM) to understand the effect of cell density and HAuCl₄ concentration on the morphology of AuNP. The samples for TEM was prepared by drop coating the AuNP suspension on carbon coated copper grid followed by air drying.

Kinetics of AuNP Synthesis

Kinetic measurements of AuNP synthesis were carried out by incubating 1.0 mM of gold chloride salt with cells at 30 °C and pH 7. The progress of reaction was recorded spectrophotometrically at definite time intervals by pipetting aliquots of reaction mixture. The absorbance of product was measured at 540 nm and reaction was monitored for 120 h. Reaction rate constant K was calculated from slope of initial part of the graph $\log (A_\infty - A_t)$ versus time

where A_∞ is the final absorbance and A_t is absorbance at different time intervals [17–19].

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

To study the functional groups present on cell surface which were involved in nanoparticles synthesis, FTIR analysis of culture of *Acinetobacter* sp. SW30 challenged with (test) and without (control) HAuCl₄ concentration was performed. These cultures were air dried and FTIR spectrum was recorded from 380 to 4000 cm⁻¹ at resolution of 2 cm⁻¹ using Bruker tensor 37 FTIR spectrophotometer.

Results and Discussion

Synthesis of AuNP

Cell density and gold chloride concentration has an effect on synthesis and morphology of AuNP which can be revealed by change in color of cell suspension challenged with gold salt. The beautiful color shades of light pink, blue and dark purple were obtained. Cell suspension of *Acinetobacter* sp. SW30 $< 0.3 \times 10^9$ cfu/ml could synthesize AuNP with 0.1 mM HAuCl₄ concentration which showed light pink color with surface plasmon resonance at 540 nm [Fig. 1a(i)]. However, higher concentrations of gold chloride hampered the synthesis of AuNP. It may be because the concentration of cell surface biomolecules/proteins that were required for synthesis of AuNP were not adequate to reduce HAuCl₄ at higher salt concentration. Under TEM monodispersed spherical AuNP were observed (Fig. 1b). The cell suspension with 0.3, 0.6 and 0.9×10^9 cfu/ml could synthesize spherical, triangular and polyhedral well dispersed AuNP at 0.5 mM HAuCl₄ concentration [Fig. 1c(ii), d, e(ii), f, g(ii) and h] with SPR peak at 530 nm. The gold salt concentration other than 0.5 mM didn't show any synthesis of AuNP. Bacterial cell suspension with 1.2×10^9 and 1.5×10^9 cfu/ml could produce AuNP at 0.5 and 1.0 mM HAuCl₄ concentration with maximum synthesis at 1.0 mM gold chloride (Fig. 1j, k). At 1.0 mM HAuCl₄ concentration, polydispersed AuNP were observed by both the cell suspensions (Fig. 1j, l).

Increase in cell density led to increased synthesis of AuNP in higher salt concentration up to 2 mM. Cell suspension with 1.8×10^9 cfu/ml was able to produce AuNP from 0.5 to 2.0 mM HAuCl₄ concentrations (Fig. 2a). At 1.0 mM salt mostly spherical and very few triangles of AuNP were observed (Fig. 2b). The cell suspension with 2.1 and 2.4×10^9 cfu/ml could produce AuNP from 0.5 to 1.5 mM HAuCl₄ (Fig. 2c, e). It was observed that cell

Fig. 1 UV–Vis spectra with visual color change in inset (i) 0.1, (ii) 0.5, (iii) 1.0, (iv) 1.5, (v) 2.0, (vi) 2.5, (vii) 3.0 mM and TEM images of AuNP synthesized by *Acinetobacter* sp. SW 30. **a** UV–Vis spectra of $<0.3 \times 10^9$ cfu/ml incubated from 0.1 to 3.0 mM HAuCl_4 and **b** TEM image of AuNP synthesized at 0.1 mM HAuCl_4 . **c** UV–Vis spectra of 0.3×10^9 cfu/ml incubated from 0.1 to 3.0 mM HAuCl_4 and **d** TEM image of AuNP synthesized at 0.5 mM HAuCl_4 salt. **e** UV–Vis spectra of 0.6×10^9 cfu/ml incubated from 0.1 to 3.0 mM

HAuCl_4 and **f** TEM image of AuNP synthesized at 0.5 mM HAuCl_4 salt. **g** UV–Vis spectra of 0.9×10^9 cfu/ml incubated from 0.1 to 3.0 mM HAuCl_4 and **h** TEM image of AuNP synthesized at 0.5 mM HAuCl_4 salt concentration. **i** UV–Vis spectra of 1.2×10^9 cfu/ml incubated from 0.1 to 3.0 mM HAuCl_4 and **j** TEM image of AuNP synthesized at 1.0 mM HAuCl_4 salt. **k** UV–Vis spectra of 1.5×10^9 cfu/ml incubated from 0.1 to 3.0 mM HAuCl_4 and **l** TEM image of AuNP synthesized at 1.0 mM HAuCl_4 salt

suspension with 2.1 and 2.4×10^9 cfu/ml could synthesize irregular and monodispersed polyhedral AuNP at 1.0 mM HAuCl_4 concentration respectively (Fig. 2d, f).

Similar studies were carried on the tropical marine yeast *Yarrowia lipolytica* NCIM 3589 in which with increasing salt concentrations and a fixed number of cells, the size of the nanoparticles progressively increased. On the other hand, with increasing cell numbers and a constant gold salt concentration, the size of nanoparticles decreased [20]. Such trend was not observed in case of *Acinetobacter* sp. SW30. However, AuNP synthesis was also observed in higher salt concentration with increasing cell density. Surprisingly, at lowest cell number of $<0.3 \times 10^9$ cfu/ml with lowest HAuCl_4 salt concentration (0.1 mM) monodispersed spherical AuNP were observed and at highest cell number of 2.4×10^9 cfu/ml with 1.0 mM HAuCl_4 salt monodispersed polyhedral AuNP were produced. The concentration of reducing agent has profound effect on the morphology of AuNP [20]. Since the nanoparticle synthesis is bacteria-mediated, increase in

their cell number increases the number of reducing biomolecule entities, which may affect the shape of AuNP at different cell densities. In *Verticillium* sp. small uniform AuNP were obtained at 250 and 500 mg/l HAuCl_4 and at higher concentration (2500 mg/l) very large and irregular AuNP were synthesized [21]. In *Coriolus versicolor*, increased salt concentration, led to increase in rate of nanoparticle synthesis without affecting the morphology [22].

In the present study, maximum synthesis was noticed at 0.5 and 1.0 mM HAuCl_4 salt concentration which is same as with *Geotrichum candidum* [23] while in *Streptomyces hygroscopicus* maximum synthesis of AuNP was obtained at 10^{-3} and 10^{-4} mM HAuCl_4 concentration [24]. In case of *Acinetobacter* sp. SW30 synthesis of AuNP increases with increasing cell number. It may be due to large number of biomolecules available from high cell density for reduction of gold salts. In contrast, biomass of *Geotrichum candidum* from 25 to 250 mg/ml when challenged with HAuCl_4 , 50 mg/ml was found to give maximum synthesis

Fig. 2 **a** UV–Vis spectra of 1.8×10^9 cfu/ml incubated from 0.1 to 3.0 mM HAuCl₄ and **b** TEM image of AuNP synthesized at 1.0 mM HAuCl₄ salt. **c** UV–Vis spectra of 2.1×10^9 cfu/ml incubated from 0.1 to 3.0 mM HAuCl₄ and **d** TEM image of AuNP synthesized at

1.0 mM HAuCl₄ salt. **e** UV–Vis spectra of 2.4×10^9 cfu/ml incubated from 0.1 to 3.0 mM HAuCl₄ and **f** TEM image of AuNP synthesized at 1.0 mM HAuCl₄ salt. **g** FTIR analysis of **a** *Acinetobacter* SW30 cells without HAuCl₄, and **b** with HAuCl₄

of AuNP [23]. Variation in color of synthesized AuNP was observed under different cell densities and gold chloride salt concentrations because plasmon frequency of AuNP is sensitive to refractive index of surrounding medium. Any changes in surroundings of these particles (surface modification, aggregation, medium refractive index, etc.) lead to colorimetric changes of the dispersions [25].

From our previous study, in which individual parameters were optimized [15] and present study it was concluded that overall physiological and physicochemical parameters have profound effect on AuNP synthesis and its morphology in *Acinetobacter* sp. SW30.

Kinetics of AuNP Synthesis

The effect of cell density from 0.3 to 2.4×10^9 cfu/ml was studied at constant $[\text{Au}^{3+}]$ of (1.0 mM) at 540 nm. The absorbance increases with increase in cell density, it reaches a maximum after which it decreases. This observation is depicted graphically in Fig. 3a as an absorbance [cell density] profile. The hypochromic shift is observed due to the attachment of particles to the cells. However, the sudden increase in absorbance at 2.1×10^9 cfu/ml may be due to the release of particles into the solution. For all the further calculations 2.1×10^9 cfu/ml was considered as optimum because it is the concentration where maximum particles are synthesized and also released into the solution. Kinetics of silver nanoparticles are studied in detail with respect to *Fusarium Oxysporum* [26].

Figure 3b shows effect of $[\text{Au}^{3+}]$ from 0.1 to 3.0 mM at 2.1×10^9 cfu/ml cell density. The absorbance increases with increase in $[\text{Au}^{3+}]$ till 1.0 mM then the decrease was observed as the concentration was increased, it may be due to the higher concentration of gold salt exhibited toxicity on the cells leading to decreased synthesis rate. The plot of absorbance at 540 nm versus time clearly indicates that formation of gold nanoparticles starts at 0 h and increases rapidly at 12 h in beginning of the reaction Fig. 3c, further the absorbance changes very slowly as the reaction goes on and reaches to the maximum at 72 h thereafter remains constant. Figure 3d represents the changes in the plots of $\log (A_\infty - A_t)$ versus time which does not obey linearity. The rates of the most biological synthesis reactions depend on the cell density, culture age, temperature, pH and substrate concentration. Therefore, calculation of rate constant of the reaction is a crucial problem due to more number of variables. Thus, the rate constant was calculated for cell suspension of 2.1×10^9 cfu/ml challenged with 1.0 mM HAuCl₄ concentration incubated at temperature 30 °C and pH 7 using the slopes of initial part of the plot $\log (A_\infty - A_t)$ versus time as 1.99×10^{-8} M.

FTIR Analysis

The presence of peak at 1512 and 1620 cm^{-1} revealed amide I and II while peak at 3220 cm^{-1} indicates presence of amino acids/amines. Peak at 1030 cm^{-1} suggest C–O stretching of primary alcohol. After addition of gold

Fig. 3 **a** Effect of cell number of *Acinetobacter* sp. SW30 on surface plasmon absorbance of AuNP at 1.0 mM HAuCl₄ concentration, **b** Effect of HAuCl₄ concentration on surface plasmon absorbance of AuNP by 2.1×10^9 cfu/ml. **c** The plot of absorbance versus time for

formation of AuNP at 540 nm. **d** Plot of $\log(A_{\infty} - A_t)$ versus time for the formation of gold nanoparticles during the reduction of Au³⁺ by *Acinetobacter* cells incubated at 30 °C

chloride few new peaks were observed at 457 cm^{-1} for chloride and C–Cl stretching. Shift from 3221 to 3196 cm^{-1} indicated possible role of amino acids in reduction Au³⁺ while amide I and II groups may have involvement stabilization of AuNP. Many studies have shown involvement of amide I–III groups in reduction of gold chloride. Such reports in *Brevibacterium casei* indicated the presence of strong bands at 1728.1 , 1668 , 1573 and 1247 cm^{-1} [27]. Related amide groups were observed in case of other bacteria such as *Geobacillus stearothermophilus*, *Shewanella oniedensis*, fungi *Rhizopus oryzae* and also in Actinomycetes *Thermomonospora* sp. [28–31]. It has been proposed that free amine groups of proteins bind to AuNP resulting in its stabilization [32]. Also, cell surface bound proteins are responsible for reduction and stabilization of AuNP [31].

Conclusions

In conclusion, cell density and gold chloride concentrations have tremendous effect on synthesis and morphology of AuNP. Surprisingly, at lowest cell density of

$<0.3 \times 10^9$ cfu/ml with lowest HAuCl₄ salt concentration (0.1 mM) monodispersed spherical AuNP of size ~ 19 nm were observed and at highest cell number of 2.4×10^9 cfu/ml with 1.0 mM HAuCl₄ salt concentration monodispersed polyhedral AuNP of size ~ 39 nm were produced. It is important to note that the present results are specific to *Acinetobacter* strain and may vary with different bacterial species. Amino acids are involved in reduction of gold salt while amide groups may help in stabilization of AuNP. The rate constant of cell suspension of 2.1×10^9 cfu/ml when challenged with 1.0 mM HAuCl₄ concentration was found to be 1.99×10^{-8} M. Significance of this study lays in the fact that the shape and dispersity of AuNP can be customized depending up on the requirement. This is very important for large scale production of monodispersed nanoparticles in medicinal, industrial and environmental applications.

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