MECHANISTIC ASPECTS OF BIOSYNTHESIS OF NANOPARTICLES BY SEVERAL MICROBES

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Abstract:

While a large number of microbial species are capable of producing metal NPs, mechanism of nanoparticle biosynthesis is very important. The metabolic complexity of viable microorganisms complicates the analysis and identification of active species in the nucleation and growth of metal NPs. Strategies such as enzymatic oxidation or reduction, sorption on the cell wall and, in some cases, subsequent chelating with extracellular peptides or polysaccharides have been developed and used by microorganisms. Some species can control the membrane transport of heavy metals towards, or their active efflux from, the cell. Metal ion resistance via transport and passive mechanisms leading to extracellular precipitation is more characteristic for prokaryotes.\cite{1,2} in this article we study several enzymes have important role in nanoparticle synthesis by microorganisms.

Key word: nanoparticle, biosynthesis, Reduction.

1. INTRODUCTION:

Variety of techniques for making of metal nanoparticles are chemical recovery using regenerative materials, aerosol technique, electrochemical deposition, photochemical recovery, laser exposure and ... . But as the term of green nanotechnology has emerged that a lot of attention has attracted and includes a wide range of processes that reduce or eliminate toxic substances to restore environment. Green Nanotechnology also seek more effective alternatives for energy production (e.g. solar and fuel cells). In green nanotechnology, for the synthesis of nanoparticles microorganisms are used. Well known that many microorganisms, aggregate inorganic material within or outside the cell to form nanoparticles. While a large number of microbial species are capable of producing metal NPs, the mechanism of nanoparticle biosynthesis is very important. Many things about the biochemical and molecular mechanism of these processes remain unknown and should be revealed. In fact, the biochemical mechanisms referred to finding materials like enzymes, which may mediate the biosynthesis mechanism. The studies of the enzyme structure and the genes which code these enzymes may help improve our understanding of how metal nanoparticle synthesis is performed. Improvements in chemical composition, size and shape and dispersity of generated nanoparticles could allow the use of nanobiotechnology in a variety of other applications \cite{3,4} Cell walls and cell wall proteins are likely to play an important role in the reduction of metal ions \cite{5}. in this article we study enzymes are important in several microorganisms in nanoparticle synthesis process.
2. BIOREDUCTION BY OXIDOREDUCTASE ENZYMES:

One mechanism of metal nanoparticles biosynthesis by microorganisms is bioreduction [1]. In microbial bioreduction processes, myriads of proteins, carbohydrates and biomembranes are involved [6]. Nanoparticles are formed on cell wall surfaces, and the first step in bioreduction is the trapping of the metal ions on this surface. This probably occurs due to the electrostatic interaction between the metal ions and positively charged groups in enzymes present at the cell wall. This may be followed by enzymatic reduction of the metal ions, leading to their aggregation and the formation of nanoparticles [7]. The microbial cell reduces metal ions by use of specific reducing enzymes like NADH-dependent reductase or nitrate-dependent reductase [8].

2.1. Oxidoreductase in yeast:

In the yeast the membrane bound (as well as cytosolic) oxidoreductases and quinones might have played an important role in the process. The oxidoreductases are pH sensitive and work in alternative manner. At a lower value of pH, oxidase gets activated while a higher pH value activates the reductase [9,10]. Along with this, a number of simple hydroxy/methoxy derivatives of benzoquinones and toluquinones are elaborated by lower fungi (especially *Penicillium* and *Aspergillus* species) [11]. Yeast might be treasuring any other such quinine because it belongs to the same class of fungi thereby facilitating the redox reactions due to its tautomerization. The transformation seems to be negotiated at two distinct levels, at the cell membrane level immediately after addition of the TiO(OH)$_2$ solution which triggers tautomerization of quinones and low pH sensitive oxidases and makes molecular oxygen available for the transformation. Such a stress generated response had earlier been suggested in case of *Candida glabarata* cell, challenged with cadmium ion in form of elaboration of an enzyme phytochelatin synthase and a protein HMT-1 which effectively aborted the CdS nanocrystals from Cytosol [12]. Once entered into the cytosol, the TiO(OH)$_2$ might have triggered the family of oxygenases harboured in the endoplasmic reticulum (ER), chiefly meant for cellular level detoxification through the process of oxidation/oxygenation [11]. Taking use of the above-mentioned facts was earlier reported synthesis of metallic selenium [13], cadmium [14], silver [15], titanium [16], as well as antimony oxide [17].

2.2. NADH-dependent reductase

2.2.1. *Fusarium oxysporum*:

Protein assays indicate that an NADH-dependent reductase, is the main responsible factor of biosynthesis processes. This reductase gains electrons from NADH and oxidizes it to NAD$^+$. The enzyme is then oxidized by the simultaneous reduction of metal ions [18]. The enzymatic route of in vitro synthesis of silver hydrosol of 10–25 nm using α-NADPH-dependent nitrate reductase (44 kDa) from *F. oxysporum* with capping peptide, phytochelatin was demonstrated recently. The mechanistic aspect was explained by Duran et al. that apart from enzymes, quinine derivates of napthoquinones and anthraquinones also act as redox centres in the reduction of silver nanoparticles [6]. A similar finding was also reported in the reduction of gold (III) chloride to metallic gold by α-NADPH-dependent sulfite reductase of molecular mass of 35.6 kDa and phytochelatin. A dimeric hydrogenase enzyme (44.5 and 39.4 kDa) of *F. oxysporum* that showed optimum activity at pH 7.5 and 38 °C passively reduced H$_2$PtCl$_6$ to platinum nanoparticles was also reported. To date, only very few reports have been documented on optimization in biological process, a 29-kDa “gold shape-directing protein (GSP)” present in the extract of green algae Chlorella vulgaris was used in the bioreduction and in the synthesis of shape-and size-controlled distinctive triangular and hexagonal gold nanoparticles. With increase
in the concentration of GSP produced gold plates with lateral sizes up to micrometers. Such mechanistic components should be unraveled for efficient biological processes [6]

2.2.2. Aspergillus flavus:

The fungus, Aspergillus flavus also resulted in the accumulation of silver nanoparticles on the surface of its cell wall when incubated with silver nitrate solution [19]. Extracellularly produced nanoparticles were stabilized by the proteins and reducing agents secreted by the fungus. A minimum of four high molecular weight proteins released by the fungal biomass have been found in association with nanoparticles. One of these was strain specific NADH-dependent reductase. However, emission band produced by fluorescence spectra indicate the native form of these proteins present in the solution as well as bound to the surfaces of nanoparticles [20,21]. Further, the reduction of metal ions and surface binding of the proteins to the nanoparticles did not compromise the tertiary structure of the proteins.

2.3. Nitrate/nitrite reductase

2.3.1. Fusarium oxysporum:

One other important enzyme that is responsible for this reduction in some microorganisms is nitrate-dependent reductase. In Fusarium oxysporum, this enzyme is conjugated with an electron donor (quinine), reduces the metal ion, and changes it to elemental form. In the case of rapid extracellular synthesis, because the reduction happens in very few minutes, complex electron shuttle materials may be involved in the biosynthesis process[1].

2.3.2. Enterobacteriaceae:

The culture supernatants of Enterobacteriaceae (Klebsiella pneumonia, E. coli and Enterobacter cloacae) also rapidly synthesized silver nanoparticles by reducing Ag⁺ to Ag₀. With the addition of piperitone, silver ion reduction was partially inhibited, which showed the involvement of nitroreductase enzymes in the reduction process [6].

2.4. Sulfate and sulfitreductase:

2.4.1. Rhodobacter sphaeroides:

In the formation of ZnS nanoparticles by Rhodobacter sphaeroides (see Fig. 1) a series of reductase have serious roles: First, soluble sulfate enters into immobilized beads via diffusion, and later is carried to the interior membrane of R.sphaeroides cell facilitated by sulfate permease. Then, the sulfate is reduced to sulfite by ATP sulfurylase and phosphoadenosine phosphosulfate reductase, and next sulfite is reduced to sulfide by sulfite reductase. The sulfide reacts with O-acetyl serine to synthesizes cysteine via O-acetylserine thiolylase [22], and then cysteine produces S²⁻ by a cysteine desulfhydrase in presence of zinc. After this process, S²⁻ reacts with the soluble zinc salt and the ZnS nanoparticles are synthesized [22]. Finally, ZnS nanoparticles are discharged from immobilized cells to the solution. In the
2.4.2. cyanobacteria:

The mechanisms of gold bioaccumulation by cyanobacteria (Plectonema boryanum UTEX 485) from gold (III) - chloride solutions have documented that interaction of cyanobacteria with aqueous gold (III) - chloride initially promoted the precipitation of nanoparticles of amorphous gold (I)-sulfide at the cell walls, and finally deposited metallic gold in the form of octahedral (III) platelets near cell surfaces and in solutions [24]. Adding further to the mechanism, a sulfate-reducing bacterial enrichment was used to destabilize gold(I) thiosulfate complex to elemental gold and proposed that this could occur by three possible mechanisms involving iron sulfide, localized reducing conditions, and metabolism [25] The interaction of P. boryanum UTEX485 with Au(S₂O₃)₂⁻³ promoted the accumulation in membrane vesicles with 10–25 nm in size in cubic morphology, clustering inside the cell with ≤10 nm and precipitated in solution with ~10–25 nm along with admixed AuS nanoparticles of ≤10 nm. But in the presence of AuCl₄⁻ precipitation was resulted in octahedral gold platelets of 1–10 µm in solution and ≤10 nm inside the bacterial cells [6].

4. HYDROLASES IN FUNGI:

Proteins have been implicated in nanoparticle formation in a number of different studies[7] found that during the formation of zirconia nanoparticles, the fungus secreted proteins capable of extracellular hydrolyzing compounds with zirconium ions, and this was confirmed in subsequent studies with silica and titania [27]. They found that their fungus was also capable of hydrolyzing metal halide precursors under acidic conditions. Their studies indicated that the proteins involved in the reduction of metal nanoparticles were cationic proteins with molecular weights of around 21–24 kDa[28]. also suggested that a cationic protein of around 55 kDa found in extracellular extracts of Verticillium sp. might be responsible for the hydrolysis of [Fe(CN)₆]₃⁻ and [Fe(CN)₆]₄⁻ found proteins bound to the nanoparticle surface, and the presence of S atoms around the silver nanoparticles was taken to suggest an association between nanoparticles and fungal proteins[29].

4.1 Cysteine desulphydrase in Rhodopseudomonas palustris:

This enzyme belongs to the family of lyases, specifically the class of carbon-sulfur lyases. The systematic name of this enzyme class is D-cysteine sulfide-lyase. This enzyme participates in cysteine metabolism. A simple route for the synthesis of cadmium sulfide nanoparticles by photosynthetic bacteria Rhodopseudomonas palustris has been demonstrated. The purified solution yielded the maximum absorbance peak at 425nm due to CdS particles in the quantum size regime. Transmission electron microscopic analysis of the samples showed a uniform distribution of nanoparticles, having an average size of 8.01±0.25 nm, and its corresponding electron diffraction pattern confirmed the face-centered cubic (fcc) crystalline structure of cadmium sulfide. Furthermore, it was observed that the cysteine desulphydrase producing S²⁻ in the R. palustris was located in cytoplasm, and the content of cysteine desulphydrase depending on the growth phase of cells was responsible for the formation of CdS nanocrystal, while protein secreted by the R. palustris stabilized the cadmium sulfide nanoparticles. In addition, R. palustris was able to efficiently transport CdS nanoparticles out of the cell [30].
5. GLUTATHIONE:

The major molecules that contribute to the detoxification mechanisms in yeast cells are glutathione (GSH) and two groups of metal-binding ligands: metallothioneins and phytochelatins. GSH with the structure \(\text{Glu-Cys-Gly}\) is an important tripeptide involved in various metabolic processes in bacteria, yeasts, plants, and animals. The unique redox and nucleophilic properties classify this compound as a detoxificator, actively taking part in the bioreduction and defense against free radicals and xenobiotics. Metallothioneins are low-molecular-weight and it classified according to the arrangement of these residues. Although the model has been created on the basis of detoxification of \(\text{Cu}^{2+}\) ions, it is also accurate for other metals in yeast. There is evidence that metallothioneins play a similar role in some plants and yeast species, e.g. in \(\text{S. cerevisiae}\) and \(\text{C. glabrata}\). GSH is also a structural unit in phytochelatin molecules, which is one of its major functions.

Although, initially, they were described as cadmium binding peptides, phytochelatin formation is induced by a large number of elements such as \(\text{Cd}^{2+}, \text{Pb}^{2+}, \text{Zn}^{2+}, \text{Sb}^{3+}, \text{Ag}^{+}, \text{Ni}^{2+}, \text{Hg}^{2+}, \text{HAsO}_{4}^{2-}, \text{Cu}^{2+}, \text{Sn}^{2+}, \text{SeO}_{3}^{2-}, \text{Au}^{+}, \text{Bi}^{3+}, \text{Te}^{4+}\), and \(\text{W}^{6+}\), when supplemented to the medium. Phytochelatins have the general structure \(\text{Glu-Cys})_n\text{Gly}\), where \(n = 2–11\) and a multitude of structural variants has been described in the scientific literature. The enzyme phytochelatin synthase or \(\text{Glu-Cys dipeptidyl transpeptidase (EC 2.3.2.15)}\) catalyzes the reaction of transpeptidation of \(\text{Glu-Cys}\) dipeptide from a GSH molecule to a second molecule of GSH, resulting in phytochelatin PC2, or to a phytochelatin molecule, resulting in an \(n + 1\) oligomer. Phytochelatin synthesis begins within minutes after exposing yeast cells to cadmium ions and is regulated by enzyme activation in the presence of metal ions. The best activators are cadmium ions, followed by ions of \(\text{Ag, Bi, Pb, Zn, Cu, Hg, and Au}\). In yeasts, \(\text{Cd-phytochelatin complexes are formed in the cytosol but accumulate in vacuoles. Detailed studies of the fission yeast S. pombe revealed that nearly the whole cadmium and phytochelatin amounts are located in vacuoles. Compared to metallothioneins, phytochelatins feature many advantages, derived from their unique structure and especially the repeated \(\text{Glu-Cys units. For example, they have better metal-binding capacity. In addition phytochelatins can incorporate large amounts of inorganic sulfur, resulting in increased capacity of these peptides to bind cadmium. Dameron and Winge developed the model presented in Fig. 2. For crystal lattice of CdS consists of 85 CdS pairs covered by 30 (c-Glu-Cys)n-Gly peptides, with } n = 3–5 [2].

In the presence of heavy metal stress, yeast cells increase cellular pools of glutathione and glutathione-like compounds called phytochelatins. The resulting metal thiolate complex formation neutralizes the toxicity of metal ions and traps them inside the cell. Sulfide anions readily incorporated into these cadmium-glutathione complexes, resulting in the formation of nanocrystals

6. DISCUSSION AND CONCLUSION:

Today, nano metal particles have drawn the attention of scientists because of their extensive application to new technologies in chemistry, electronics, medicine, and biotechnology. Beside many physical and chemical methods which have been developed for preparing metal nanoparticles, nanobiotechnology also serves as an important method in the development of clean, nontoxic, and environmentally friendly procedures for the synthesis and assembly of metal nanoparticles. This new biotechnological method has important advantages in comparison to conventional methods. For instance, it
is an easier and cheaper procedure. To be utilized in different scientific fields, biological synthesis requires an understanding of the biochemical and molecular mechanisms of the reaction for obtaining better chemical composition, shape, size, and monodispersity. As said before in nanoparticle biosynthesis, many enzymes such as reductases, syntases, hydrolases and ..., are important and describe specific genes and characterization of enzymes involved in the biosynthesis of nanoparticles is also required. Therefore, a complete knowledge of the molecular mechanisms involved in the microbial synthesis of nanoparticles is necessary to control the size, shape and crystallinity of nanoparticles. In regard with nanoparticle biosynthesis considerable advantages, with improvement of these methods they could be the leading large-scale production method for nanoparticles in future [2].

References


