Protozoa: As Emerging Candidates for the Synthesis of NPs

8

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Contents

Abstract

The nanomaterials/nanoparticles (NPs) created huge impact in every facet of human life. The NPs are used in different fields of biomedical research, as anti-inflammatory, antibacterial and anticancer agents, as a drug delivery system and even as quantum dots. These remarkable features of NPs are ascribed to their nano scale size, specific shape and morphology. The toxicity of conventionally synthesized NPs always posed great threat to human health. Consequently, the nanotechnologists have been exploited different systems, such as bacteria, fungi, algae, etc., for the synthesis of human friendly nanoparticles. In spite of the available methods; the new avenues could still be explored for the production of NPs with premium biocompatibility. In this direction, researchers are also probing the feasibility and usability of protozoan as a suitable model for the synthesis of NPs.

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Being the ancestors and owing to the animal-like attributes, the protozoa can yield better biocompatible NPs. The high bioaccumulation of heavy metals by protozoa have already proved its suitability in case of quantum dots production. Therefore, they could be exploited as an emerging candidates for the synthesis of desired NPs. In this review, we discussed some basic concepts for the synthesis of nanoparticles in microorganisms, and how could the free living nonpathogenic protozoa use their detoxification and antioxidant machinery for heavy metal reduction and subsequently nanoparticle formation.

Keywords

Biocompatible · Microorganism · Protozoa · *Tetrahymena* · *Leishmania* · Anti-oxidant · Nanoparticles, Biosynthesis · Mechanism · Metallothionein · Detoxification · Heavy metals

8.1 Introduction

Nanotechnology is the production of materials at nanoscales. The biological activities of nanoparticles (NPs) ingrained in their smaller size, specific shape, surface charge and morphology. The uses of NPs are expanding day by day, owing to their unique functional properties, such as anti-inflammatory, antibacterial, anthelmintic, anticancerous, as a drug delivery system and the fluorescence and photocatalytic behaviour (Alomary and Ansari 2021; Anandan et al. 2019; Ansari and Asiri 2021; Ansari et al. 2019, 2020; Balasamy et al. 2019; Cui et al. 2019; Khan et al. 2015, 2019; Musarrat et al. 2015; Patra et al. 2018; Prasad et al. 2020; Rajakumar et al. 2020). Nonetheless, the biocompatibility of NPs has remained a major concern for the nanotechnologists. The conventionally synthesized NPs always pose toxicity to human beings. Therefore, researchers are developing new approaches for the synthesis of exceptionally biocompatible nanomaterials. This issue has been managed up to certain extent by using different microorganisms (Naahidi et al. 2013). These microorganisms, e.g., protozoa, bacteria, algae, and yeasts have certain innate behaviours, like heavy metal detoxification and free radical scavenging by the anti-oxidant system. Both these innate processes, possess many metal quinching macromolecules, such as metal binding proteins, reducing enzymes and sugars. These macromoecules reduce and stabilize heavy metal ions into nontoxic insoluble metals, which can act as precursor elemnets for the synthesis of NPs (Gahlawat and Choudhury 2019; Cui et al. 2016; Juganson et al. 2013; Li et al. 2011).

The nanotechnologists have been successfully produced several metal NPs, such as silver (Ag), gold (Au), silver–gold alloy, tellurium, platinum, copper, zinc, selenium (Se), palladium, silica, zirconium, magnetite, and alginate by using microorganisms, like bacteria, actinomycetes, yeast, fungi, algae, etc. (Jalal et al. 2018; Ovais et al. 2018; Salem and Fouda 2021; Shobha et al. 2020; Sumanth et al. 2020). In spite of the available methods, researchers are still exploring new biological systems for the production of superiorly biocompatible NPs. In recent

past, scientists have begin to explore free living nonpathogenic (nonparasitic) protozoa as emerging candidates for the synthesis of NPs. The protozoa (singular; protozoan) are unicellular, eukaryotic organisms, having plasma membrane as an outer covering. They are considered as the ancestors of animals. The protozoa have typical internal structures like animal cells, such as membrane bound nucleus and other organelles. This unicellular organism performes all animal-like activities viz. heterotrophic mode of neutrition (exception: *Euglena,* which is also autotroph), intracellular digestion, reproduction, locomotion, respiration and excretion.The locomotory organales in free living nonpathogenic protozoa are: flagella (e.g.*Euglena*), cilia (e.g. *Paramecium*) and pseudopodia (e.g. *Amoeba*). These locomotory organs are absent in parastic forms. Because of the structural and functional intricacies like animals, the nonparasitic free living protozoa could be the astounding approach for the synthesis of NPs. This chapter focuses on the brief discussion about the intracellular and extracellular syntheses of NPs, researches conducted on protozoa mediated formation of NPs, and possible mechanisms adopted by the protozoa for synthesis of NPs.

8.2 Biosynthesis of Nanoparticles (NPs)

The different microorganisms synthesized NPs by using bottom up approach where NPs are formed through oxidation and reduction processes by secreted biomolecules, such as enzymes, proteins, sugar, etc. (Fig. 8.1). In contrast to top down approach (which uses harsh chemical and physical methods for the metal reduction and stabilization), bottom up approach yields comparatively nontoxic NPs by the process of self-assembly of metal ions (Ahmed et al. 2016). In this process, the type of microorganism and the environmental conditions have major impact on the shape, size, and morphology of NPs. Therefore, the optimization of different environmental conditions, such as pH and temperature, chemical analysis of biological biomass filtrate, are required for the synthesis of desired NPs (Singhal et al. 2011). Unfortunately, the exact mechanism of biosynthesis of NPs in microorganisms is not completely understood. However, it has been reported that the they can use the intracellular or extracellular modes for nanoparticle's synthesis, which at many instances are species specific (Gahlawat and Choudhury 2019). Furthermore, the researchers also exploited the living cell extract of different microorganisms including protozoa, for the biosynthesis of different metal NPs (Gahlawat and Choudhury 2019; Juganson et al. 2013).

8.2.1 The Intracellular and Extracellular Synthesis of Nanoparticles (NPs) by Microorganisms

The microorganisms are in direct contact with their environment and materials can move in and out of these microorganisms. Henceforth, they can provide a suitable biotemplate for synthesis of NPs, in their cell interior as well as exterior. In this

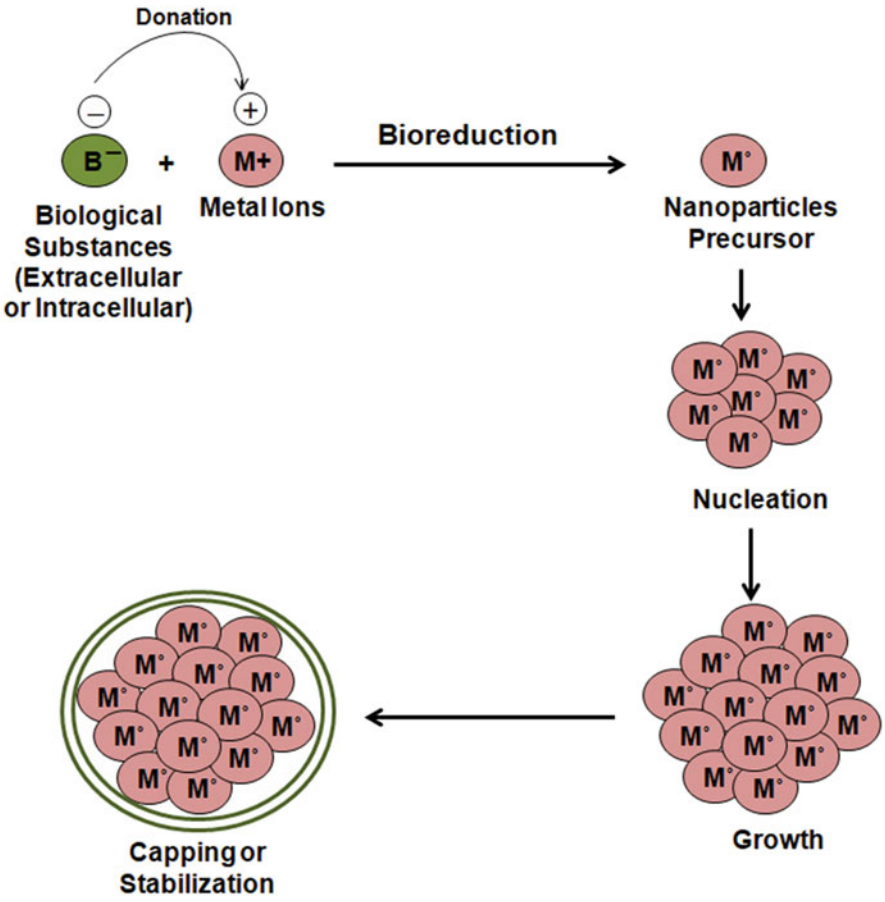

Fig. 8.1 A general concept of biosynthesis of nanoparticles. The metal ions (M^+) are reduced by the biological substances., such as enzyme, proteins, polysaccharides, etc. The reduced metal ions act as the precursors of nanoparticles (M°) that lead to the nucleation of nanoparticles. Now the nanoparticles grow in size and stabilize by the capping molecules

segment, we will give a generalized account on the intracellular and extracellular syntheses of NPs by microorganisms.

The different metallic and magnetic nanoparticles have been synthesized by the intracellular and extracellular mechanisms in bacteria, fungi, actinomycetes, algae and protozoa. In 1984, Haefeli reported that a bacterial strain, *Pseudomonas stutzeri* AG259, isolated from a silver mine, was resistant to the high concentration of silver ions. This resistant behaviour was achieved by the inactivation of toxic Ag^+, possibly with a molecule analogous to metallothioneins (MTs). Probably, this analogous molecule might sequestrate the silver ions, which eventually converted into nontoxic silver nanocomposite (Haefeli et al. 1984). Since then, extensive research have been carried out on the biosynthesis of NPs by bacteria (Guilger-Casagrande

and Lima 2019; Khandel and Shahi 2018; Li et al. 2011; Zielonka and Klimek-Ochab 2017). The intracellular and extracellular syntheses of NPs by fungi are also evident (Guilger-Casagrande and Lima 2019; Zielonka and Klimek-Ochab 2017; Khandel and Shahi 2018). The easy handling of fungi and the numerous proteins present, make them excellent source for the production of large number of NPs. The actinomycetes, which share the properties of both bacteria and fungi are also involved in the synthesis of various metal NPs (Manimaran and Kannabiran 2017; Kumari et al. 2020). It has been revealed that actinomycetes secrete four different proteins with molecular masses between 10 and 80 kDa. Because of the discrete nature of these proteins and varied strength of interaction with metal ions, actinomycetes yield NPs of different sizes, shapes, and monodispersity (Khandel and Shahi 2016).

The intracellular and extracellular syntheses of NPs depend upon different types and forms of microorganisms. The cell wall plays pivotal role for both intracellular and extracellular syntheses of NPs. The influx of different ions is facilitated by the specific ion transport system present in cell wall of these microorganisms. Moreover, the inner and outer surface of the cell wall in bacteria, fungi and algae has a strong negative charge, which creates electrostatic interaction with positively charged metal ions. Such interaction can also occur with other negatively charged macromolecules (like; proteins, enzymes and polysaccharides) present within the cell. Thus, the negatively charged inner surface of the cell wall and various intracellular macromolecules can reduce the positively charged heavy metal ions into the insoluble metal precursors of NPs. Thereafter, these insoluble metals will be capped, by these macromolecules, to form stable nanoparticles inside the cell (intracellular synthesis) (Khandel and Shahi 2016). Finally, these NPs will be diffused or actively transported out of the cell. Whereas, the extracellular synthesis of NPs is assisted by negatively charged outer surface of the cell wall and cellular exudates that contain various proteins, enzymes like nitrate reductase in various fungi, polysaccharides, etc (Fig. 8.2). A few representative microorganisms, which are involved in intracellular and extracellular syntheses of metal nanoparticles are briefed in Table 8.1.

8.3 Protozoa for the Synthesis of Biocompatible Nanoparticles (NPs)

The biological entities are in continuous interaction with their environment. These entities evolved various physico-chemical processes, including mineralization (for skeletal support) (Clark 2020) and detoxification (for toxins release), to sustain their life on earth. The detoxification machinery and the presence of numerous reducing enzymes, proteins and polysaccharides in free living nonparasitic protozoa can create suitable conditions for the synthesis of NPs. In fact, the production of NPs by different microorganisms is an adaptation to avoid undesirable events that caused by noxious metals. The protozoa also have such adaptation, where they can detoxify heavy metals by intracellular and extracellular mechanisms. Therefore, the synthesis of NPs can also occur at both the locations. However, the limited information on

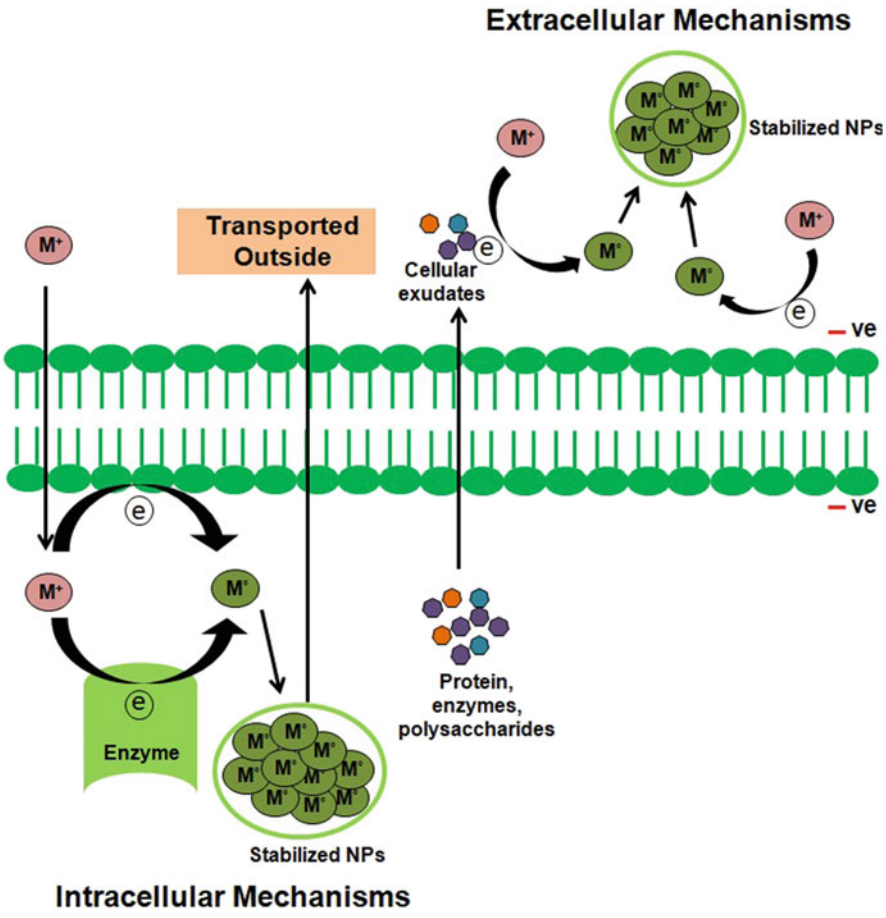

Fig. 8.2 The extracellular and intracellular mechanisms for the biosynthesis of nanoparticles (NPs). M^+: Heavy metal ion, M°: NP precursor

protozoa mediated synthesis of NPs, makes incapable to explain exact mechanism(s). Just a couple of studies on the synthesis of NPs are carried out using *Tetrahymena* spp. and soil inhabiting *Leishmania* sp. The first study for extracellular synthesis of NPs in a protozoan was reported by Ramezani et al., in 2012. He used a nonpathogenic *Leishmania* sp., isolated from soil, for the quick synthesis of Ag NP (10 and 100 nm) and Au NP (50 and 100 nm) of polyspersed spherical shape, subsequently declining the claim that the protozoa took time for the NPs' synthesis (Ramezani et al. 2012). This study further confirms the presence of amine bonds, C=O, N=O, C=N, and COOH bonds of proteins as a capping or stabilizing agents on the surface of NPs. The *Tetrahymena* spp. are capitalized for the production of different nanoparticles, such as Ag NPs, Au NPs, Se NPs and cadmium nanodots. The nonpathogenic *Tetrahymena* sp. releases acid hydrolases in

Table 8.1 A few representative microorganisms, which use intracellular and/or extracellular mode(s) for the synthesis of NPs

Sources	Nanoparticle type	Size (nm)	Location	Shape	Ref.
Bacteria					
Pseudomonas aeruginosa	Au	15–30	Extracellular	_	Husseiny et al. (2007)
Bacillus subtilis	Ag	10–20	Extracellular	Multishaped	Corradini et al. (2010)
Desulfovibrio desulfuricans	Pd	10–15	Extracellular	Spherical	Parikh et al. (2008)
Escherichia coli	CdS	2–5	Intracellular	_	Kowshik et al. (2002)
Escherichia coli	Pd, Pt, CdS	20–40	Extracellular	Semipentagonal, hexagonal	Deplanche et al. (2010)
Bacillus cereus	Ag	5	Extracellular	Spherical	Ganesh Babu and Gunasekaran (2009)
Bacillus subtilis	Ag and Au	5–10	Intra and extracellular	_	Reddy et al. (2010)
Actinobacter sp.	Fe	10–40	Extracellular	Spherical	Li et al. (2007)
Pseudomonas stutzeri	Ag	>25	Extracellular	Quasi-spherical	Lengke et al. (2006)
Enterobacter cloacae	Ag	2–25	Extracellular	Spherical	Venkataraman et al. (2011)
Cyanobacteria					
Oscillatoria willei	Ag	100–200	Extracellular	Spherical	Ganesh Babu and Gunasekaran (2009)
Spirulina platensis	Au-Ag	7–16	Extracellular	Spherical	Govindaraju et al. (2008)
Actinomycetes					
Thermomonospora sp.	Au	12–20	Extracellular	Spherical	Sastry et al. (2003)
Rhodococcus sp.	Au	5–10	Intracellular	Spherical, rods	Ahmad et al. (2003)
Virus					
Tobacco mosaic virus (TMV)	SiO2, CdS, PbS, Fe2O3	45–80	Intra- and extracellular	_	Klaus et al. (1999)
M13 *bacteriophage*	CdS, ZnS	10–25	Extracellular	Spherical	Sweeney et al. (2004)

(continued)

Table 8.1 (continued)

Sources	Nanoparticle type	Size (nm)	Location	Shape	Ref.
Tobacco mosaic virus (TMV)	Si, CdS, PbS	<30	Extracellular	Multishaped	Kashyap et al. (2013)
Fungi					
Aspergillus flavus	Ag-Au, Ag	>120	Extracellular	Spherical	Chen et al. (2003)
Penicillium fellutanum	Ag	5–25	Extracellular	Spherical	Kathiresan et al. (2009)
Fusarium solani	Ag	5–35	Extracellular	Spherical	Maliszewska et al. (2009)
Rhizopus oryzae	Au	10	Cell surface	Nanocrystalline	Gericke and Pinches (2006)
Fusarium semitectum	Au, Au-Ag	18–80	Extracellular	Multishaped	Dasaratrao Sawle et al. (2008)
Coriolus versicolor	Ag, Au-Ag	10	Extracellular	Spherical	Sanghi and Verma (2009)
Yeast					
Torulopsis	CdS	2–5	Intracellular	_	Kowshik et al. (2002)
Schizosaccharomyces pombe	CdS	200	Intracellular	Spherical	Kowshik et al. (2002)
Candida albicans	Ag	50–100	Extracellular	Spherical	Li et al. (2011)
MKY3	Ag	2–5	Extracellular	Spherical	Kathiresan et al. (2009)
Algae					
Cladosiphon okamuranus	Au	9–20	Extracellular	_	Justin Packia Jacob et al. (2012)
Spirulina platensis	Au	7–16	Extracellular	Spherical	Govindaraju et al. (2008)
Sargassum wightii	Au	18–12	Extracellular	Spherical	Singaravelu et al. (2007)
Gelidiella acerosa	Ag	12–15	Extracellular	Spherical	Vivek et al. (2012)
Diatoms					
Navicula atomus	Au	9-`2	Extracellular	Spherical	Seshadri et al. (2011))
Diadesmis gallica	Au/Si	15–25	Extracellular	Spherical	Sneha et al. (2011)

their vicinity and secretes approximately 30 different proteins under starving condition (Juganson et al. 2013). This study manifests that the optimization of different conditions, viz. pH, temperature, micronutrient in the media, etc., are utmost important for the production of desired NP. Furthermore, the alterations in culture conditions (which may act as stimuli) may change the physiology of protozoa. These stimulated protozoa may produce stimulus-specific array of biomolecules, which can help the researchers to manipulate the forms and features of NPs. The extracellular synthesis of Ag NPs was also reported by Katre Juganson et al. in 2013. He used *Tetrahymena thermophila* cell free exudates along with $AgNO_3$. The proteins present in the exudates progressively biotransformed the $AgNO_3$ into Ag NPs with hydrodynamic size of 70 nm. Moreover, the intracellular synthesis of selenium nanoparticles in the culture medium containing 150 μM Na_2SeO_3 and *T. thermophila* (SB210) at its late log phase was investigated by Cui et al. (Cui et al. 2016). He reported the overexpressed glutathione (GSH), metallothionein-1 and cluster binding related gene in *T. thermophila* might be responsible for the reduction of selenite to selenium nanoparticles (size: 50–500 nm, coexited with irregular nano selenium). The *Tetrahymena pyriformis* has also been used for the synthesis of cadmium quantum dots (QDs) with size of 8.27 nm ± 0.77 nm (Cui et al. 2019). The QDs are nanocrystalline materials with fluorescence and photocatalytic capacities. They are widely used as probes in bioimaging and biomedical fields by virtue of their brilliant properties including size-tunable fluorescent emission, broad absorption with narrow photoluminescence spectra, long fluorescent lifetime, and high resistance to photobleaching. The better accumulation capacity of Cd_2^+ by *Tetrahymena* could make it a better alternative for the production of cadmium nanodots (Cui et al. 2019).

8.3.1 Advantages of Protozoa for Biosynthesis of Nanoparticles (NPs)

The selection of a proper microorganism for the synthesis of NPs is the most critical aspect. The nanotechnologists need to consider many factors, such as easy availability, trouble free culture, inexpensive culture conditions, short multiplication time, relatedness with animals, for the synthesis of highly biocompatible NPs. The protozoa are unicellular eukaryotes enclosed in a unit membrane called as plasma membrane. Some are parasitic, while others are nonparasitic free living. Generally, the main objective of nanotechnologists is used to be the production of myriad NPs with minimum time and effort, without any risk. Given to the high safety/risk ratio, nonparasitic free living protozoa could be the potential tools for the biosynthesis of NPs. However, researches on protozoa mediated NPs formation are at very early stage and the investigators have a long way to go for the validition of free living nonpathogenic protozoa as the best model. Notwithstanding the fact, this could still be hypothesised that protozoa, because of their animal-like features, may yield comparatively more human friendly NPs. These animal-like features in protozoa are: (a) eukaryotic in nature, (b) similar structural and functional complexity,

(c) presence of plasma membrane, membrane bound nucleus and other organelles, (d) similar metabolic pathways that require same types of inorganic and organic compounds, (e) absence of cell wall is the most striking feature in protozoa, in contrast to other eukaryotic microorganisms, such as alga and fungi, etc. Other advantages could be: (i) most of the free living protozoa have short life, (ii) easy to culture, (iii) the maintenance of culture is inexpensive and thus NPs production could be very cost effective, (iv) most widespread in water bodies all over the world and (v) the motile nature, which could be capitalized for easy separation of protozoa from NPs containing medium (Mortimer et al. 2016). Contrary to this, the production of NPs by the pathogenic protozoa could be an uphill task for a nanotechnologist, because of their limited technical skills for parasite handling, cumbersome culture maintenance, which needs costly reagents and equipments, thus the cost effectiveness for the production of NPs will be compromised. First and foremost, use of the parasitic forms may be against the scientific aptitude, if better alternatives are available.

The only disadvantage could be that the protozoa are very much sensitive and the extreme temperature, physical or chemical treatments may disrupt the plasma membrane of the organisms.

8.3.2 Plausible Mechanism(s) for the Synthesis of Biocompatible Nanoparticles (NPs) by Protozoa

Nothing has been investigated on the mechanistic pathways for synthesis of NPs in protozoa. The physiology and biochemistry of free living nonpathogenic protozoa suggest that they may use both intracellular and extracellular modes for synthesis of NPs. Comprehensively, the protoza can use different innate processes for NPs' synthesis, such as (a) heavy metal detoxification by metal binding proteins, (b) antioxidant enzymes as metal reducers, (c) sequestration of heavy metals by negatively charged plasma membrane and/or by many other reducing molecules of the cell, such as enzymes, proteins and sugars. Before discussing these mechanisms for the biosynthesis of NPs, it is important to know certain basic physiological and biochemical aspects of these innate processes in protozoa.

The protozoa are equipped with the detoxification system, which neutralizes the noxious activity of heavy metals. Gutiérrez et al. reviewed that the ciliate protozoa are evolved both intracellular and extracellular mechanisms for heavy metal detoxification by metallothionein (MTs) and other reducing macromolecules (Gutiérrez et al. 2011). The metal detoxification in ciliate protozoa may occur by four different methods: 1. The intracellular quelling of heavy metal stress by the chelators and/or antioxidant enzymes. 2. A resistant mechanism, which employed adsorption of positively charged heavy metal ions on the negatively charged outer surface of cell membrane (extracellular detoxification). 3. The third mechanism is biotransformation in which the biomethylation and biovolatilization occur, which was first reported in *Tetrahymena thermophila*. 4. The fourth mechanism is the active transport of the metal ion out of the cells, which is evident by the presence of 485 putative

genes encoding membrane transporter in *T. thermophila*. The most important mechanism is the intracellular sequestration of heavy metals by MTs and accumulation in vacuoles, which actively releases these nontoxic insoluble metals outside of the organisms. The metal bioaccumulation has been reported in various ciliate protozoa (Gutiérrez et al. 2011). The ciliate protozoa's MTs family is divided into subfamily 7a or CdMTs or subfamily 7b or CuMTs, depending upon metal induction pattern and Cys residue clustering. Moreover, the protozoa, e.g. *Euglen*a, also have the ability to accumulate heavy metals, such as Cd2+, Cu2+, Zn2+, Pb2+, Tc7+, and Cr6+ (RodrÍguez-Zavala et al. 2007; Rehman 2011). This accumulation is facilitated by the formation of a complex with the cysteine, GSH, and chelatins, which are thought to be the components of antioxidative strategy of *Euglena* against heavy metal toxicity (Mendoza-Cozatl et al. 2002; Jasso-Chávez et al. 2010). Thus, these chelators, such as enzymes [e.g. glutathione or (GSH)], proteins (e.g., thioredoxin), inner and outer surface of plasma membrane could act as a reducing and stabilizing sites, which may biotransform heavy metal ions into the nanoparticles, under appropriate conditions.

Interestingly, the protozoa are also well equipped with the antioxidant machinery. If the heavy metal exposure is too high not to get saturated by the metal chelators, then the over production of reactive oxygen species (ROS) may damage the organism. Under this circumstance, the antioxidant system plays vital role in protection of protozoa, by scavenging of ROS. Moreover, the reducing capacity of antioxidant enzymes may also promote the transformation of toxic metal ions into nontoxic insoluble metals. The antioxidant enzymes, such as glutathione peroxidases (GPXs), which use GSH as an electron donor (Overbaugh and Fall 1985), NADPH dependent thioredoxin (Trx) reductase (Yoshida et al. 2016), ascorbate peroxidase (APX) (Shigeoka 2002) act as reducing agents for ROS in protozoa, and thus protect the organisms from heavy metal toxicity.

The chelation or sequestration of metal ions is indispensable for the nanoparticle formation. As we know that the protozoa have MTs, different antioxidant enzymes and other proteins, as a metal ion reducers and stabilizers. These biomolecules can reduce the heavy metal ions by electron donation, under suitable conditions. Thus, the metal ions can biotransform into insoluble metals that may act as a NP precursors. Subsequently, these NP precursors will nucleate and grow into NPs, which could be stabilized by biomolecules that contain amine bonds, C=O, N=O, C=N, COOH as functional groups NPs. The role of metal chelators in the formation of NPs is supported by an observation, where various phytochelators with reducing property are used for the synthesis of nanoparticles (Husen and Siddiqi 2014). The main enzymatic chelators in microorganisms are MTs, which are cysteine rich, low molecular weight proteins. The MTs can bind both physiological (like, zinc, selenium, copper) and xenobiotic heavy metals (like, silver, cadmium, mecury, arsenic). This metal chelating property of MTs has been used for the large scale production of silver nanoparticles by the engineered bacteria containing MT gene insert from *Candida* sp. (Yuan et al. 2019). Conclusively, at least four probable mechanisms for the production of NPs by the free living nonpathogenic protozoa, could be deduced.

1. Intracellular Synthesis of Nanoparticles in Protozoa: The three possible mechanisms for synthesis of NPs in free living nonpathogenic protozoa are individually discussed below. These categorizations have made for the easy understanding of these phenomena. However, these mechanisms may operate simultaneously, if the conditions are uncontrolled. Nonetheless, the genetic modification in protozoa, just like bacteria (Yuan et al. 2019), may give preference to one process over the others, for the synthesis of desired NPs.
   (a) Synthesis of NPs by the Protozoa using Metallothioneins (MTs):
   The MTs are cysteine rich low molecular weight metal-binding proteins. The main function of MTs is to regulate the metabolism of essential metals (zinc, copper and selenium) within the cell. The cysteine residues of MTs are involved in the sequestration of free radicles and heavy metals into insoluble metals. The MTs have Cys-Xaa-Cys clusters, which can act as functional group for the reduction of metal ions and their stabilization and accumulation within the cells (Yuan et al. 2019). The high metal binding affinity and metal reducing properties can make MTs a biofactory for the synthesis of metal NPs. Interestingly, the protozoa also contain MTs, which act as heavy metal chelators during detoxification process (Gutiérrez et al. 2011). The reducing and stabilizing properties of MTs have already been employed for the synthesis of NPs in other microorganisms (Yuan et al. 2019). Therefore, MTs from protozoa can also reduce metal ions and thus, can transform them into nontoxic NPs. Heavy metal ions exposure to protozoa causes massive influx of heavy metal ions inside the organisms (RodrÍguez-Zavala et al. 2007; Rehman 2011). These ions activate the heavy metal response elements present on metallothionein gene promoter and cause overexpression of MTs. These MTs bind with the metal ions, eventually reduce and stabilize them into nontoxic metal NPs. Finally, these NPs will be accumulated in vacuoles and effluxed out of the cells via vacuolar exocytosis or through ATP dependent transporters (Fig. 8.3a).
   (b) Synthesis of NPs by Protozoa using reducing Enzymes from Anti-Oxidant Machinery:
   The protozoa have efficient antioxidant system for scavenging reactive oxygen species. This antioxidant system has many enzymes, viz. GPXs, Trx reductase, APX, etc., which neutralize toxic free redicals, generated in response to the heavy metal ions. These molecules can reduce the metal ions into the NP precursors. These NP precursors will nucleate and form NPs, which will be actively transported out of the cell (Fig. 8.3b).
   (c) Inner surface of the Plasma membrane as a Biotemplate for the synthesis of NPs:
   This mode of NPs formation may be similar to the other microorganisms. The only difference between them is in cell covering. The protozoan is enclosed with plasma membrane, while other microorganisms have cell wall (see section and Fig. 8.2). The cell membrane of protozoa is composed of lipoprotein embedded in lipid bilayer. The layer facing the cytoplasm is negatively charged. This negatively charged surface can donate the electrons to metal

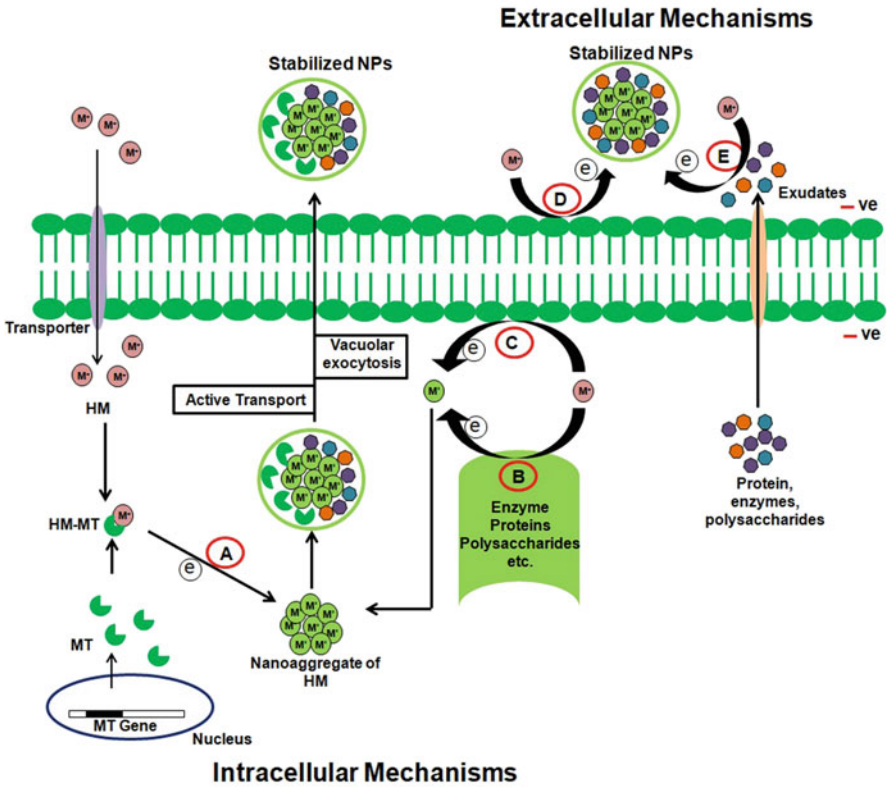

Fig. 8.3 Plausible mechanisms of nanoparticle's synthesis by protozoa. (**a**) Metallothioneins (MTs) induced NPs' synthesis, (**b**) enzyme mediated nanoparticles (NPs) synthesis, (**c**) inner surface of the cell as a biotemplate for NPs synthesis, (**d**) extracellular synthesis of NPs, (**e**) Induction of NPs' formation by reducing biomolecules present in cellular exudate. *HMs*: heavy metal ions, M^+: metal ion, M°: nanoparticle precursor, *−ve*: negatively charged surface

ions and thus, can form NP precursors, which will nucleate and finally produce NPs. (Fig. 8.3c).

2. Extracellular Synthesis of NPs by Protozoa:
   The outer surface of the protozoa consist of glycolipid with sugar group extended outwards. These proteins, extending outwards, may act in similar manner as discussed in method, 1c. But, the formation of NPs at the surface of a protozoan could be a resistant mechanism, which may reduce heavy metal ions by negatively charged outer surface or by cellular exudates. These reduced ios can form nontoxic NPs outside the cell (Fig. 8.3d, e).

8.4 Conclusion

The biocompatible nanoparticles are used in various biomedical and therapeutic research to avoid toxicity of chemically synthesized NPs. The reduction of metal ions into NP precursors is imperative for the formation of nanoparticles. Fortunately, different microorganisms, viz. protozoa, bacteria, fungi, alga etc. can provide suitable biotemplates for such reduction processes in cell interior as well as exterior. Nevertheless, the formation of nontoxic nanoaggregates by these microorganisms is just a surrogate of defense machinery against heavy metal toxicity. Considering the biocompatibility, the protozoa have edge over other microorganisms, owing to their remarkable resemblance with animals. However, inadequate data on protozoa mediated synthesis of NPs and on its applications, limit us to draw any final conclusion. But, the indistinct nature and wide spread presence of protozoa throughout the world may prove a boon for the synthesis of human friendly NPs.

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