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Biogenic Magnetite Nanoparticles

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ABSTRACT

Magnetite is widely used in various area including recording material, cancer treatment, magnetic resonance imaging, magnetic probes, detection of pathogens etc. The rocks and magnetofossils are the major source of magnetite. Magnetite may be synthesized chemically by various methods. But the magnetite obtained in nature and synthesized magnetite is not suitable for biomedical application due to its non-uniform shape which is hardly crystalline. Moreover magnetite obtained from ore and synthesized chemically may not be homogeneous in composition and in an agglomerated state. Biogenic magnetite is a step ahead of the magnetite synthesized otherwise in view of the above disadvantages of the latter. Biogenic magnetite nanoparticles are of single domain size, high chemical purity, crystallographic perfection, arranged in chain structure, unusual morphology, elongation, biocompatible, nontoxic, highly stable and disperse well in water owing to their natural lipid coating. Magnetite is synthesized by wide range of organisms including bacteria, chitons, honey bees, homing pigeons, dolphins, sharks, humans which helps to detect the earth's magnetic field. Biogenic magnetite nanoparticles can be isolated easily from magnetotactic bacteria.

Keywords: Biogenic magnetite; Nanoparticles; Magnetotactic bacteria; Magnetosomes; Biomineralization.

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INTRODUCTION

Magnetite is one of the naturally occurring iron oxide (Fe_3O_4) and is the only known biogenic material with ferromagnetic properties at room temperature. Of all the naturally occurring minerals on Earth, magnetite is known to be the best in view of magnetic property. Magnetite finds application in various fields including recording material, magnetic resonance imaging (MRI), environmental assessment, cell separation, DNA / RNA recovery, DNA discrimination within species, detection of single nucleotide polymorphisms related to human disease, receptor-binding assay for drug screening, hyperthermia, magnetic probes, constructing material for magnetic force microscopy cantilever. Immunoglobulin G bound magnetic nanoparticles are used to recognize bacteria including *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes* using pseudo immune interactions [1].

The chemical synthesis of Fe_3O_4 with various morphologies – nanowires [2], nanorods [3], films [4] and nanoparticles [5] by different methods were reported extensively. Synthetic magnetic nanoparticles have the following disadvantages that they are non-uniform, often not fully crystalline, may not be homogeneous in composition and in an agglomerated state, which imposes problems in processing [6].

Wide range of organisms has been reported to detect the earth's magnetic field with the help of localized superparamagnetic magnetite particles (biogenic magnetite). The presence of biogenic magnetite was first observed in marine molluscs [7]. The synthesis of biogenic magnetite inside the living organisms is genetically controlled. Biogenic magnetite crystals have unique features that distinguish them from geologically or synthetically produced crystals like single domain size, high chemical purity, crystallographic perfection, arranged in chain structure, unusual morphology and elongation.

MAGNETITE IN HIGHER ORGANISMS

Magnetotactic algae discovered in a lagoon in Brazil, identified as *Anisonema platysomum* (Euglenophyeeae) contain numerous tooth-shaped magnetite magnetosomes arranged as multiple, linear clusters of chains along the long axis of the cell [8]. In chitons (marine molluscs of the class Polyplacophora) the biomineralization process begins with an initial transport of metabolic iron to the posterior end of the radula sac where it is deposited as the mineral ferrihydrite within a preformed organic mesh of proteinaceous material [9]. Through an unknown process, this femhydrite is converted rapidly to magnetite, through a nontopotactic reaction, coupled with iron reduction and recrystallization [10]. Biomineralization of magnetite is also reported in diverse range of organisms including insects [11], honeybees [12], fish [13], birds [14] and even humans [15]. Eggs, larvae, and young pupae of the bees contain no measurable magnetic material, whereas the older pupae developed magnetic remanence within 2 days of the time they emerged as adults suggesting their biologic origin [16]. Isolation of biogenic magnetite from higher animals is very tedious and time-consuming.

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MAGNETITE IN BACTERIA

Magnetite nanoparticles along with the membrane constitute a unique structure called a magnetosome, which is only found in magnetotactic bacteria (MTB) and this feature distinguishes these bacteria from other prokaryotes. Several species of magnetite-producing MTB have been isolated from fresh, marine water habitats and cultivated in pure culture. Most magnetite-producing MTB are microaerophiles or anaerobic and present in oxic-anoxic zone at the sediment/water interface or just below [17]. MTB have a flagellum useful for their motility. MTB were phylogenetically and morphologically much more diverse with a variety of morphological types including cocci, rods, vibrios, spirilla and apparently multicellular forms [18] and can vary according to which hemisphere they are found as well as the type of ecosystem in which they live. Bacteria containing magnetosomes of magnetite (Fe_3O_4) are given in table 1.

Table 1: Magnetotactic bacteria producing biogenic magnetite

Magnetotactic Bacteria	Biogenic Magnetite (Particle size)	Ref.
Magnetospirillum magnetotacticum (*Aquaspirillum magnetotacticum*)	0-45 cubo-octahedral (42 nm)	[19]
Magnetospirillum gryphiswaldense MSR-1	60 magnetosomes (35–120 nm)	[20]
Magnetospirlillum magneticum AMB-1	Cuboidal (20-30nm)	[21]
Candidatus Magnetovibrio blakemorei MV-1	Anisometric	[22]
Candidatus Magnetococcus marinus MC-1	Psedudo-hexagonal prismatic	[23]
Candidatus Magnetococcus yuandaducum	truncated hexahedral prisms (30-115 nm)	[24]
Desulfovibrio magneticus sp.nov. (RS-1)	Six irregular bullet-shaped	[25]
Magnetobacterium bavaricum	1,000 projectile and hook (110 – 150 nm) magnetosomes in several straight chains	[26]
MHB-1	30 to 60 bullet-shaped magnetosomes in a single bundle, aligned in multiple chains	[27]
Candidatus Thermomagnetovibrio paiutensis (HSMV-1)	a single chain of 6-18 bullet-shaped magnetosomes	[28]
Itaipu-1	two chains of roughly square projections magnetosomes (length upto 250nm, and width/length ratios of ca.0.9)	[29]
Magnetospirillum strain WM-1	6-10 cuboidal magnetosomes (54 ± 12.3 nm × 43 ± 10.9 nm)	[30]
MWB-1	200-300 bullet-shaped magnetosomes (116 x 40nm) arranged in 4-7 bundles of magnetosome chains	[31]
Bilophococcus magnetotacticus	Hexagonal prism shaped (99.3 x 62.3 nm)	[32]

Magnetosomes are characterized by narrow size distributions, species-specific crystal habits of various combinations of the isomeric forms (111), (110) and (100). Each MTB contains 10-20 magnetosomes each containing a magnetite nanoparticle. These magnetosomes are aligned in a chain-like fashion, imparting a magnetic dipole to the bacterial cell and allows the cells to sense the Earth's geomagnetic field [20]. The magnetic crystal's morphology, size and type vary from species to species but are very much conserved within the same bacterial species or genus. The three most common magnetic crystal morphologies are elongated prismatic, roughly cuboidal, and tooth-shaped [33]. However, magnetite crystals produced by

chemical methods have low crystallinity and broad size distributions. Biomineralisation in MTB provides uniform magnetite crystals of high chemical purity with an average diameter of 50-110nm [34, 35].

Synthesis of Biomagnetite in Bacteria

Iron exists in oxidized ferric iron, Fe^{3+} in aerobic environment and reduced ferrous iron Fe^{2+} state in anaerobic environments. Magnetosomes production was influenced by changes in the bacteria's natural habitat, which is directly controlled by climate. The formation of different crystal shapes under the same experimental conditions in a closed system has been observed only in magnetotactic bacteria [36]. Magnetic nanoparticles are synthesized by a specific set of proteins that are present in membrane-bound organelles called magnetosomes. The process of synthesis of magnetite magnetosomes by Magnetotactic bacteria is still unclear. It is believed several different steps are involved in this biomineralization process. These steps include iron uptake by the bacteria, magnetosome vesicle formation within the bacteria, iron transfer into the magnetosome vesicle, and protein-mediated Fe_3O_4 or Fe_3S_4 biomineralization within the magnetosomes [37, 38].

Fe^{2+} is very soluble at neutral pH and is quickly oxidized to Fe^{3+} (insoluble at neutral pH) under the oxygenated conditions. In order to utilize solid-phase Fe^{3+}, some MTB are believed to synthesize iron-binding biomolecules called a siderophores [38, 39]. The Fe^{3+}-sideophore complex enters the cell and then Fe^{3+} is cleaved from the siderophore. Once inside the cell, proteins reduce the Fe (III), converting the iron to Fe^{2+} which is then taken up by the magnetosomes. Pre-existance of empty and partially filled vesicles prior to the biomineralization of the mineral phase in iron-starved cells of *M. magnetotacticum* and *M. gryphiswaldense* is reported [20, 40]. *M. magneticum* AMB-1 use MagA protein to transport and accumulate Fe^{2+} within the subcellular vesicle in an energy dependent manner [41]. MamB and MamM proteins within the magnetosome membrane of *M. gryphiswaldense* have been shown to function in the transport of iron into the magnetosomes. Once inside the magnetosome, four Mms-proteins (membrane proteins) in *M.magneticum* AMB-1: Mms5, Mms6, Mms7 (homolog MamD in *M. gryphiswaldense*), and Mms13 (homolog MamC in *M.gryphiswaldense*) are believed to control the nucleation and growth of magnetite or greigite within the magnetosome vesicles [42]. All four proteins contain a hydrophobic N-terminus that allows the four proteins to become integrated within the magnetosome lipid bilayer membrane. The proteins also contain a hydrophilic C-terminus which has strong affinity for metal ions. The magnetite crystals formed in vitro in the presence of Mms6 are similar to those produced by intact cells while the magnetite crystals produced without Mms6 in vitro showed no homogeneity in shape and size. The hydroxyl group at the C-terminus of the Mms6 protein might function as a template for Fe_3O_4 crystal formation, and control the shape of the crystals.

EXTRACELLULAR FORMATION OF MAGNETITE

Under some restricted conditions anaerobic organisms may catalyze the extra cellular formation of magnetite. Formation of magnetite by Fe (III)-reducing mesophilic bacterium,

Geobacter metallireducens, GS-15 [43] and thermophilic bacterium, *Thermoanerobacter ethanolicus*, TOR-39 [44] represents biologically facilitated mineralization in which the particles are extracellularly formed as a byproduct of microbial Fe (III) respiration. Magnetite formation by *Shewanella* (NV-1 and W3-7-1) was slower than those of *Thermoanerobacter* (TOR-39) [45]. Other Fe(III)-reducing bacteria also form magnetite in culture [46-48]. Extracellularly formed crystals are clearly epicellular and are not aligned in chains [49]. Bharde et al. (2006) synthesized nanoparticulate magnetite at room temperature extracellularly by challenging the fungi*, Fusarium oxysporum* and *Verticillium* sp., with mixtures of ferric and ferrous salts [50]. The role of bacteria in the formation of extracellular magnetite remains poorly understood. Several factors particularly pH, ionic strength, lattice geometry, polarity, stereochemistry, and topography may act in concert to control nucleation and growth of crystals [51]. These magnetite grains have poor crystallinity, nonuniform shapes, grain sizes ranging from 10-50 nm and superparamagnetic. Extracellular synthesis method generates 5,000 times more magnetite than a magnetotactic bacterium. The amount of magnetite produced is primarily limited by the amount of Fe(III) present in the culture that is available for reduction by cells [52]. The biochemical and molecular processes involved in electron transfer and iron reductions that are essential for extracellular precipitation of Fe (II)-containing phases such as magnetite are also reported [53]. From the above discussion it is clear that the extracellular synthesis of magnetic nanoparticles posses most of the disadvantages similar to the chemical synthesis of nanoparticles. However, biogenic magnetite nanoparticles are better as compared to the chemically synthesized ones owing to the eco-friendly aspect of the former which does not involve the use of harsh parameters and toxic chemicals [54].

CONCLUSIONS AND PERSPECTIVES

Since the discovery of biogenic magnetite particles nearly 50 years ago, considerable progress has not been made in bulk synthesis. Moreover, completely utilizing them as tools in various bio-applications is still in its infancy. The greatest hurdle is the isolation and cultivation of MTB for bulk synthesis. Further, there are many attributes of biogenic magnetite nanoparticles that make them a potential alternative to synthetic magnetic nanoparticles. Biogenic magnetite nanoparticles are biocompatible, nontoxic, highly stable and disperse well in water owing to their natural lipid coating, whereas the synthetic particles need to be rendered water soluble, which is usually nontrivial and can be extremely difficult for those with sizes beyond the superparamagnetic range. Their innate high chemical purity, species-specific shape, narrow size range promises their use in wide range of biomedical applications such as magnetic separation, drug delivery etc. The innate lipid layer on the biogenic magnetite particles is an excellent platform for immobilization of biomolecules through a variety of bionconjugation techniques. Although most of these studies are still at the proof-of-concept level, the biogenic magnetite nanoparticles have already been proved to be better than the synthetic magnetite nanoparticles in some cases.

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