Magnetic microbes: bacterial magnetite biomineralization

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ABSTRACT

Magnetotactic bacteria are a diverse group of prokaryotes with the ability to orient and migrate along the magnetic field lines in search for a preferred oxygen concentration in chemically stratified water columns and sediments. These microorganisms produce magnetosomes, the intracellular nanometer-sized magnetic crystals surrounded by a phospholipid bilayer membrane, typically organized in chains. Magnetosomes have nearly perfect crystal structures with narrow size distribution and species-specific morphologies, leading to well-defined magnetic properties. As a result, the magnetite biomineralization in these organisms is of fundamental interest to diverse disciplines, from biotechnology to astrobiology. This article highlights recent advances in the understanding of the bacterial magnetite biomineralization.

Keywords:

Magnetite biomineralization, magnetotactic bacteria; magnetosome magnetite nanocrystal

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1. Introduction

Biomineralization is a process by which living organisms convert chemical elements obtained from the local environment into minerals, producing functional organic-inorganic biocomposites. In biologically induced mineralization, the inorganic phase is formed by adventitious precipitation. In biologically controlled mineralization, proteins and other

biological macromolecules are thought to play an important role in controlling the size and shape of the forming biomineral¹. The products of biomineralization, such as silica shells of diatoms or skeleton in animals, provide a number of benefits to the organism and often exhibit superior physical and chemical properties as compared to their synthetic counterparts.

Iron is the fourth most common chemical element on our planet, as reflected by the abundance of iron-based minerals². Metallic iron, found in meteorites and in the Earth's inner and outer core, is not very common on the planetary surface, as it tends to oxidize to produce a variety of oxides and oxyhydroxides². Due to the presence of iron's unpaired electrons, these compounds are magnetic, with magnetite being the most magnetic of all naturally occurring minerals on Earth. Present in many rocks, small magnetite crystals typically carry the dominant magnetic signature, preserving the record of the geomagnetic history of our planet. This is of great importance to paleogeomagnetism, magnetohydrodynamics, and other scientific fields.

Iron cycling on Earth is an extremely complex biogeochemical process involving both abiotic and biotic components, and magnetite is also common among biogenic materials. It is found as biochemical precipitate in four out of the five kingdoms of living organisms (Monera, Proctista, Animalia, and Plantae), however has not yet been recorded in fungi. Millions of creatures, from fishes and sea turtles, to birds and numerous insects, migrate to spawn and reproduce, often against adverse conditions. Some navigate vast distance to the exactly same place, year after year, while others do this only once and then die. The mechanisms of magnetosensing in these animals, viewed as a trait of complex and highly evolved living systems, continue to fascinate and puzzle researchers^{3,4}. But there are other, small and previously thought to be simple organisms responding to the Earth's magnetic field, the subject of this review.

2. Magnetotactic bacteria

The smallest known organisms that respond to magnetic fields are magnetotactic bacteria. The term "*magnetotactic bacteria*" represents a diverse group of fastidious microorganisms with the cellular morphologies ranging from coccoid (ovoid), vibroid (curved rod-shaped) and spirilloid (helical), to multicellular organisms^{3,5-7}, as exemplified in **Figure 1**. These are heterogeneous group of Gram-negative bacteria sharing the ability to orient in magnetic field. These microorganisms, found in freshwater and marine sediments around the world, produce chains of unique intracellular structures called *magnetosomes*, comprised of magnetic magnetite (Fe_3O_4) or greigite (Fe_3S_4) nanometer-sized crystals enveloped in a 2-3 nm thick phospholipid bilayer membrane⁵. Although these microorganisms were discovered more than four decades ago and extensively studied, the exact biomineralization mechanisms of how

the synthesis of these mineral crystals remain poorly understood, in part because these microbes are notoriously difficult to handle^{5,8-12}. Based on the biomineral formed, magnetotactic bacteria can be divided into two general types: the obligate microaerophiles, facultative anaerobes, or obligate anaerobes iron-oxide types that mineralize crystals of magnetite (Fe_3O_4), and the obligate anaerobes iron-sulfide types that mineralize crystals of greigite (Fe_3S_4)¹³. Recently, a greigite-producing magnetotactic bacterium, strain BW-1, was shown to biomineralize greigite and magnetite depending on culture conditions¹⁴. Common traits of the magnetotactic bacteria as a group include motility by means of flagella, negative tactic and often growth response to atmospheric concentrations of oxygen, respiratory form of metabolism in pure culture, and formation of magnetosomes. The latter is governed by specific gene clusters.

Iron can account for over 2 percent of the total weight in these bacterial cells, making them highly magnetic¹⁵⁻¹⁸. Regardless of the type of the biomineral, the magnetosome crystals are in the single magnetic domain size range (35-120 nm), which have the highest possible magnetic moment per unit volume. In most magnetotactic bacteria, the arrangement of magnetosome crystals in chain along the long axis of the cell yields maximal magnetic dipole moment to the cell. This arrangement was long believed to be due to magnetic interactions between individual magnetic magnetosomes within the chain. However, cytoskeletal elements have now been shown to play a major role in magnetosome chain formation, thereby anchoring the chain within the cell^{12,19,20}.

3. Ecology, diversity, genetics, and cell biology

Magnetosome chains act as miniature compass needles, torquing the bacteria into alignment with the Earth's geomagnetic field lines. This passive alignment allows the cell to efficiently migrate and seek the boundary in liquid known as oxic-anoxic interface, which is characterized by opposing gradients of oxygen from the surface and reduced compounds (usually, sulfur) in water columns or sediments⁵. This phenomenon is known as magnetotaxis, or magnetically assisted aerotaxis. Use of the magnetosome chain, arguably, reduces the random three-dimensional bacterial movement to a one-dimensional search for an optimal oxygen concentration, likely providing an advantage over other non-magnetotactic microorganisms.

Before the advent of molecular phylogenetics, the considerable morphological diversity of magnetotactic bacteria was apparent from light microscopic observations of environmental

samples of water and sediment. The phylogenetic diversity of magnetotactic bacteria, based on their 16S rRNA gene sequences, is relatively extensive. Most known cultured and uncultured magnetotactic bacteria belong to the *Alpha*-, *Gamma*-, and *Deltaproteobacteria* classes of the *Proteobacteria* phylum, however several uncultured species are affiliated with the *Nitrospirae* phylum, and one, strain SKK-01, was assigned to the candidate division OP3, part of the *Planctomycetes-Verrucomicrobia-Chlamydiae* (PVC) bacterial superphylum^{10,21-27}.

In magnetite-producing magnetotactic bacteria, proteins present in the magnetosome membrane control biomineralization of the magnetite crystal. These proteins are encoded by the magnetosome genes present as clusters within the genomes of all examined magnetotactic bacteria so far^{21,22,24-26}. The functions of several of these genes and their associated proteins in magnetosome synthesis and construction of the magnetosome chain have been elucidated through bioinformatics and experimental evidence²⁶. These clusters are in relatively close proximity to each other within the genomes, and are surrounded or interrupted by certain types of genomic structures. The latter suggests that in some microorganisms, genes that encode for magnetosome membrane proteins, as well as genes involved in the construction of the magnetite magnetosome chain, are organized within a genomic island that might be transmitted to other different bacteria through horizontal gene transfer. A greigite-producing magnetotactic bacterium, *Candidatus Desulfamplus magnetomotoris* strain BW-1, was shown to biomineralize greigite and magnetite depending on culture conditions. Based on a phylogenetic comparison of BW-1 and similar uncultured greigite- and/or magnetite-producing magnetotactic bacteria from freshwater to hypersaline habitats, these microorganisms represent a previously unknown group of sulfate-reducing bacteria in the *Deltaproteobacteria*. Genomic analysis of BW-1 revealed the presence of two different magnetosome gene clusters, suggesting that one may be responsible for greigite biomineralization and the other for magnetite¹⁴. The distribution of magnetosome genes in different phylogenetic groups of magnetotactic bacteria can be attributed to the horizontal gene transfer between these organisms, although there is no strong direct proof of this process²³. Recent successes in determining a large number of magnetosome gene sequences of cultured magnetotactic bacteria, some of which have been only recently isolated, as well as characterization of many uncultured organisms using culture-independent techniques, allowed researchers to determine phylogenies based on mam protein amino acid sequences, providing a new insight into the evolutionary origin of magnetotaxis and magnetosome biomineralization in magnetotactic bacteria.

From comparative genomic analyses, mam (magnetosome membrane) genes responsible for magnetosome biomineralization, are organized as clusters in the genomes of

magnetotactic bacteria, in some as a magnetosome genomic island (**Figure 2**)^{23,26}. A set of genes within the *mamAB* operon, recently shown to be crucial for magnetite biomineralization, is common among all magnetotactic bacteria. Ten genes (*mamABEIKLMOPQ*) are conserved in all magnetite-producers, whereas only nine of these, excluding *mamL*, seem to be conserved in the two greigite-producers²⁶.

Recent reports showed that a phylogenetic tree based on magnetosome protein amino acid sequences from a number of magnetotactic bacteria was congruent with the tree based on the 16S rRNA gene sequences of the organisms, suggesting that evolution and divergence of these proteins and the 16S rRNA gene occurred similarly. Some of the *mam* genes involved in the production of magnetite magnetosomes were also present in greigite-producing magnetotactic bacteria. On the other hand, while the minimal set of *mam* genes necessary for magnetosome formation in *Magnetospirillum* was shown to be conserved in magnetite-producing magnetotactic *Deltaproteobacteria*, the number of genes associated with magnetotaxis in these microorganisms appears to be larger than previously thought²⁴. A comparative genomic analysis of magnetotactic *Deltaproteobacteria* revealed that in addition to *mam* genes, there is a conserved set of genes, designated *mad* genes, specific to the magnetotactic *Deltaproteobacteria*, some also being present in *Candidatus Magnetobacterium bavaricum* of the *Nitrospirae* phylum, but absent in the magnetotactic *Alphaproteobacteria*²⁴.

Taken together, these findings suggest that the biomineralization of magnetite and greigite by these diverse microorganisms did not have evolved independently, and that the ability to biomineralize magnetosomes and the possession of the *mam* genes was acquired by these microorganisms from a common ancestor of all proteobacteria^{23,28}. Novel magnetotactic bacteria are isolated from sediment and water collected around the globe, as evidenced by a growing number of reports. Each finding expands the knowledge of phylogenetic diversity and physiology of these remarkable microorganisms, revealing their potential in nitrogen, iron, carbon and sulfur cycling in natural environments.

4. Living magnets: magnetosomes

Fabrication of monodisperse magnetic nanocrystals at ambient temperature is a relatively straightforward process; however, synthetic magnetite, in general, exhibits wide distribution of sizes and inferior crystallinity, having negative impact on its magnetic properties¹⁰. And while the inorganic production of structurally perfect magnetite nanocrystals with narrow size distribution is challenging, magnetotactic bacteria produce magnetite nanocrystals with uniform shapes and sizes at physiological conditions.

Magnetite biomineralization by the magnetotactic bacteria is a complex process involving the bacterial uptake of iron, nucleation and growth, interaction with biopolymers and proteins, and emergence of the resultant magnetic biogenic crystal^{5,6,10,29-31}. Formation of nanometer-sized magnetite crystals within the magnetosome organelles of magnetotactic bacteria can serve as a model for elucidating the molecular mechanisms of the biomineralization process. Understanding the magnetite biomineralization in these organisms in terms of crystal formation, as well as the involvement of biological macromolecules and cellular processes, is of fundamental interest to scientists, because similar principles can be used to develop synthetic nanomaterials with desired morphological and structural control. If the intricate pathways used by bacteria to build complex intracellular structures can be revealed, the findings could be ultimately applied to rational design of synthetic nanomagnetics for specific applications. Owing to the magnetosome mineral phase's purity, well-ordered crystal structure, narrow size distribution and consistent species-specific morphologies, and resulting well-defined magnetic properties, magnetite biomineralization in magnetotactic bacteria is a topic of great interest in a variety of fields, from nanotechnology to astrobiology^{10,15,27,31-35}.

While all magnetotactic bacteria produce magnetosomes, the shapes of these magnetic nanocrystals differ from one type of bacteria to another, as schematically shown in **Figure 3**. The remarkably consistent species-specific morphologies of magnetosome magnetite nanocrystals, leading to their well-defined magnetic properties, are indicative of highly controlled biological mineralization^{5,33,36-38}.

Magnetosome magnetite provides an attractive model for probing magnetic phenomena at the nanometer scale, as these nanocrystals are chemically pure, highly uniform and free of undesired particle agglomeration, which often accompanies the conventional magnetite synthesis. For example, a magnetic signature of the Verwey transition can be clearly seen in magnetotactic bacteria that contain almost perfect crystals of magnetite, thus disproving the literature claims that the Verwey transition temperature is suppressed in nanoparticles³¹. Instead, the lack of the Verwey transition observable by the bulk magnetometry is related to the superparamagnetic nature of these small nanocrystals³¹. Magnetization data, acquired with various forms of bacterial and synthetic magnetite, shown in **Figure 4**, illustrates such a case and establishes that the Verwey transition is not sensitive to the size of the magnetite crystal as long as one can speak of chemically-pure bulk magnetite with a well-defined crystal structure. In simple terms, all the tested bacterial magnetosome magnetite samples exhibit the Verwey transition above 100 K, and its magnetic signature appears to be due to the presence of extended crystalline structure³¹.

The membrane encasing each magnetic nanocrystal contains a number of proteins not present in other parts of the cell. Among those, several acidic proteins associated with the magnetic crystal are presumed to influence formation of the biomineral³⁹⁻⁴¹. These proteins are likely involved in transporting the iron compounds taken by the cell out of solutions and concentrating them inside the membrane, although the exact role of each protein in this process remains largely unknown. By understanding the functions of individual proteins, it might be possible to elucidate the manner in which the cell exerts control over the shape and size of magnetite crystals^{5,39-49}. However, despite the vast research, little is known about the biological mechanisms controlling the biomineralization of magnetosome nanocrystals. Equally limited is the knowledge of the nucleation, growth and maturation of the formed magnetosome crystal. Probing the early stages of magnetite magnetosome biomineralization by utilizing the spatially-resolved electron microscopy findings and those produced by the synchrotron analyses sometimes yields inconclusive, or even contradictory information^{29,30,46,50-53}. It becomes increasingly clear, that the variety of processes involved in magnetosome formation, ranging from the bacterial uptake of iron to nucleation and growth of nanoscale magnetite, necessitates the use of various analytical approaches, and their implementation provides new insights on the complex processes of magnetosome magnetite biomineralization.

In the a recent work, Siponen and co-authors described the structure of the magnetosome-associated protein MamP, reportedly built on a unique arrangement of a self-plugged PDZ domain fused to two magnetochrome domains, thus defining a new class of c-type cytochrome found exclusively in magnetotactic bacteria. Based on mutational analysis, enzyme kinetics, co-crystallization with ferrous iron and an *in vitro* MamP-assisted magnetite production assay, MamP has been established as an iron oxidase contributing to the formation of ferric ferrihydrite, which could be required for magnetite crystal growth *in vivo*. These results shed light on some of the molecular mechanisms of iron management taking place inside the magnetosome and highlight the role of magnetochrome in iron biomineralization⁵⁴.

Recent Komeili *et al.*, while performing a genetic and biochemical study of proteins proposed to function in iron mineralization, showed that the redox sites of c-type cytochromes of the *Magnetospirillum magneticum* strain AMB-1 magnetosome island, MamP and MamT, appeared to be essential to their physiological function. The ablation of one or both heme motifs led to a loss of function, pointing to their importance in ability to carry out redox chemistry *in vivo*⁵⁵. The developed method to heterologously express fully heme-loaded MamP from AMB-1 for *in vitro* biochemical studies, showed that its ferric-ferrous redox couple is set at an unusual potential (-89 ± 11 mV) compared with other related cytochromes involved in iron oxidation or reduction, however it remains able to oxidize Fe(II) to Fe(III) and mineralize iron to

produce mixed-valent iron oxides. The results of the *in vitro* mineralization experiments are indicative of Mms mineral-templating peptides from AMB-1 modulation of MamP iron redox chemistry⁵⁵.

Recent comprehensive transmission electron microscopy (TEM) study of magnetic and structural properties on bullet-shaped magnetites produced by the uncultured strain MYR-1 belonging to the Nitrospirae phylum, revealed a multiple-step crystal growth of MYR-1 magnetite. Initial isotropic growth yielding ~40 nm cubooctahedral particles was followed by subsequent anisotropic growth and a systematic final elongation along [001] direction²⁷. During the crystal growth, one major {111} face was shown to be well developed and preserved at the larger basal end of the crystal, while the basal {111} face appeared to be terminated by a tetrahedral-octahedral-mixed iron surface. Dimensional advantages for binding protein could be indicative of protein-templated magnetite crystallization. These findings offer new insights for understanding magnetite biomineralization within the Nitrospirae phylum and²⁷.

Bacterial magnetite is highly relevant for numerous commercial and biomedical applications; however the potential use of magnetosomes in nanotechnology is limited by the difficult, fastidious nature of cultivating magnetotactic bacteria. Some redundancy in the protein architecture of magnetosomes raises the possibility that a more basic set of magnetosome genes might be sufficient for iron biomineralization and compartmentalization within a lipid vesicle. If the magnetosome island could be transferred into less fastidious foreign recipient, the host organisms would possibly gain the ability to biomineralize highly ordered magnetic nanostructures. This task has long been hindered by the structural and genetic complexity of the magnetosome organelle and insufficient knowledge of the involved biosynthetic functions. Recently, the expression of a minimal set of genes from the magnetotactic bacterium *Magnetospirillum gryphiswaldense* resulted in groundbreaking magnetosome biosynthesis within the photosynthetic model organism *Rhodospirillum rubrum*¹¹. This represents a giant step toward sustainable tailored production of magnetic nanocrystals in suitable microorganisms¹¹.

Finally, high-resolution TEM and cryo-TEM provide vital information about the organization of the cellular structures and the development of the magnetosomes, however, because of the high vacuum requirement, conventional TEM analysis can only be used to image air dried or vitrified bacteria devoid of their natural environment^{12,56}. This technique is restricted to the static mode and does not permit following the evolution of a single biomineralized nanocrystal. Therefore, alternative techniques are devised to visualize time-resolved magnetite biomineralization in live magnetotactic bacteria. Recently, a correlative scanning TEM (STEM)

and fluorescence microscopy technique was devised for imaging viable cells of *Magnetospirillum magneticum* strain AMB-1 in liquid using an *in situ* fluid cell TEM holder⁵⁷. Cells were visualized in a fluid cell with HAADF-STEM, followed by correlative fluorescence imaging to verify their cell wall membrane integrity. Based on the post-STEM fluorescence imaging, the bacterial cell wall membrane did not sustain damage during STEM analysis at low electron dose conditions (**Figure 5**), although the effects of the electron beam and confinement in the fluid cell on viability of bacteria are yet to be carefully investigated.

Correlative STEM and fluorescence imaging of magnetotactic bacteria is a first step in directly observing biomineralization of magnetite nanocrystals *in vivo*⁵⁷. Once developed, this method will enable dynamic visualization of magnetosome formation in individual cells.

5. Iron *et al.*: expanding the menu

Ferric iron has a poor solubility near neutral pH, and iron acquisition at these conditions poses problems for many microorganisms^{2,58}. Magnetotactic bacteria, however, have evolved high-affinity iron sequestering agents to accumulate the necessary amounts of iron from their surroundings, and cultured strains of magnetotactic bacteria are typically grown in the medium with iron added as citrate or quinate at the millimolar concentrations. It is worth noting, that even though the both natural aqueous environment and a nutrient growth medium routinely contain ions of other metals, for example calcium, aluminum and magnesium, the biomineralized magnetosome magnetite nanocrystals remain chemically pure. In fact, the chemical purity of magnetosome magnetite is considered to be a truly biogenic fingerprint, and for a longest time biomineralization of “doped” magnetite was considered impractical. However, the possibility to culture magnetotactic bacteria to produce doped magnetite nanoparticles would have significant implications for applications. Magnetite is relatively soft magnetic material, and its magnetic stability can be enhanced by doping, for example with cobalt^{10,59}.

Recently, researchers have exploited the knowledge and current understanding of magnetotactic bacteria to synthesize more complex magnetic nanoparticles *in vivo*, opening up the possibility of using these bacteria as reactors for magnetic nanomaterials with tailored magnetic response. Staniland and co-authors reported on controlled cobalt doping of magnetite magnetosomes *in vivo* in an attempt to tune magnetic properties of magnetosomes⁶⁰. This complex topic has generated numerous discussions amongst the scientific communities around the world and led to several follow-up reports on the incorporation of zinc and nickel or manganese into the magnetosome magnetite under various conditions^{61,62}. It remains unclear,

whether such doping actually happens *in vivo* during growth in a controlled process, or rather by a diffusion of the dopant after the cell's death: it is well known that cobalt is generally toxic for microorganisms, and cells would likely have difficulty surviving in the presence of cobalt at the concentrations described in Staniland *et al.*^{2,58,60,63}. In another study, cells of *Magnetospirillum gryphiswaldense* strain MSR-1 were grown in the presence of manganese, ruthenium, zinc and vanadium. The Verwey transition served as a unique marker to probe the incorporation of the dopant within the magnetosome: manganese incorporation into the magnetite nanocrystals was signaled by a suppression of the Verwey transition, as well as by changes in crystalline structure and chemical composition of magnetosome magnetite. Notably, unlike the case of vanadium and ruthenium, manganese became incorporated in the crystal structure of magnetosome magnetite produced by some magnetotactic bacteria grown in the presence of either MnCl_2 or MnSO_4 ⁶⁴.

The outcome of the magnetosome doping experiments is indicative of dopant ions interfering with iron at the metabolic level: when the foreign ion is offered to the growing cell in large quantities, it likely competes with iron for non-specific metal transport sites in the cell, accumulates in the cell, and either expelled by specific membrane proteins, or sequestered within the cells, the latter leading to biomineralization of doped magnetite. It is worth noting, however, that although doping of magnetosome magnetite is feasible, as shown by differences in the magnetic signal with respect to the non-doped control specimen in several cases, the ability to control it remains highly debatable^{10,60,63-65}. Moreover, if magnetite biomineralization were to be tweaked by environmental conditions, this would imply that this process is not strictly genetically controlled - this would contradict the vast indications of the strict biological control over this process.

Regardless of the feasibility, magnetotactic bacteria grown in such a way would provide an ideal system to investigate impurities introduced into structurally perfect, uniform magnetite nanometer-size crystals, to affect their magnetic properties. This permits a targeted, effective tailoring of the magnetic response, which, in turn, will lead to new applications of these, already widely used, materials.

6. Summary

In this review, magnetotactic bacteria and magnetosome magnetite nanocrystals they produce, were surveyed focusing on recent advances in understanding bacterial magnetosome formation. Magnetite biomineralization by the magnetotactic bacteria is one of the oldest and simplest types of biologically controlled mineralization, and it proceeds under strict biological control over their chemical composition, size, crystal habits, and placement within the cell.

While many aspects of this remarkable process remain unclear, with the discovery and isolation of new strains and the development of new techniques and synthetic biology, there has been much progress in understanding magnetosome formation. Recent discoveries open new possibilities in rational design and sustainable production of magnetic particles with the properties highly relevant numerous commercial and biomedical applications.

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Figure 5