STRUCTURE AND FUNCTION OF MAGNETOSOMES IN MAGNETOTACTIC BACTERIA

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Magnetotactic bacteria contain magnetosomes, which are mineral particles enclosed by membranes. The particles are ferrimagnetic magnetite, ferrimagnetic greigite, or greigite and non-magnetic pyrite. The particles constitute an elegant biomagnetic compass that orients the cell along the geomagnetic field lines as it swims. This paper discusses the structures of these particles and their possible formation mechanisms.

1.0 INTRODUCTION

A number of species of motile, aquatic bacteria are able to orient and navigate along geomagnetic lines.[1-3] This behavior, known as magnetotaxis, is based on the fact that each magnetotactic bacterium is a swimming, permanent magnetic dipole, that is, a motile bio-magnetic compass.[2] The permanent magnetic dipole moment of each magnetotactic cell is due to intracellular, membrane-bound, permanent single-magnetic-domain-sized inorganic particles known as magnetosomes, which are, in most cases, arranged in chains.[1,3,4,5] The biomineralization process, involving the composition, size, position, orientation, and even morphology of the particles, is highly controlled by the bacteria.[6] Moreover the magnetosome chain is a hierarchical structure that is a masterpiece of permanent magnet engineering.

The magnetosomes of most of the magnetotactic bacteria that have been studied to date contain particles of magnetite, Fe_3O_4. An example is shown in Figure 1. This organism, designated strain MV-4, is an unidentified magnetotactic bacterium from a marine marsh that has recently been isolated and grown in pure culture. It contains a chain of 16 uniformly sized and shaped magnetite particles, each about 60 nm along the chain axis. Magnetite was identified as the mineral form by electron diffraction measure-

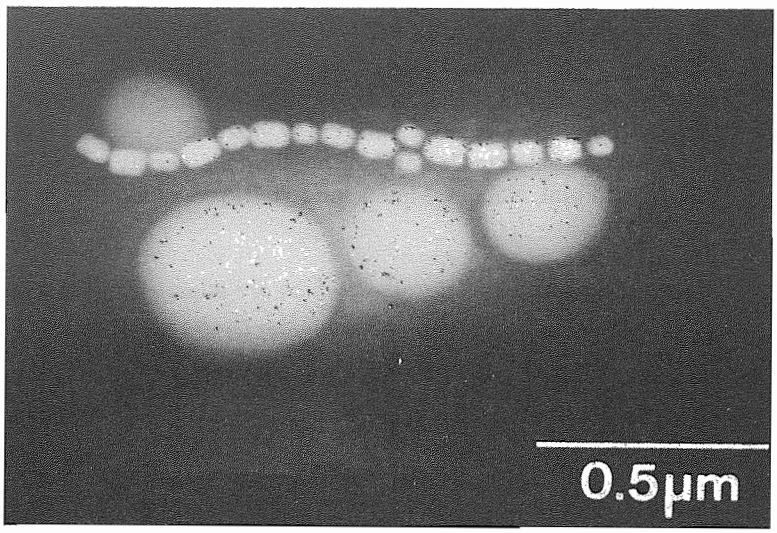

Figure 1. TEM/BF image of the marine, magnetotactic bacterium, strain MV-4. The chain of particles in the cell are the magnetite-containing magnetosomes. The globular structures contain polyphosphate or sulfur (see Figure 2).

ments on particles in whole cells.

In addition to the magnetosomes, cells of strain MV-4 contain other electron-dense structures, which also consist of partially inorganic materials. These are shown in Figure 2 where elemental maps of Fe, O, S, and P are shown for a cell of strain MV-4. These maps were produced in a scanning transmission electron microscope, fitted with a fluorescent X-ray detector. As the electron beam was rastered over the specimen, the instantaneous X-ray intensities of several selected elements were determined and recorded. It can be seen that Fe and O density correlate with the positions of the particles in the cell, which is consistent with their identification as magnetite. P and O map with the large, globular, electron-dense structures in the cell. These are presumably polyphosphate granules, which occur in many types of bacteria. Finally, two globular concentrations of S are also seen in the cell. These could be elemental sulfur globules which are obscured by the large polyphosphate granules in the electron micrograph. Thus the cell produced at least three spacially segregated inorganic products.

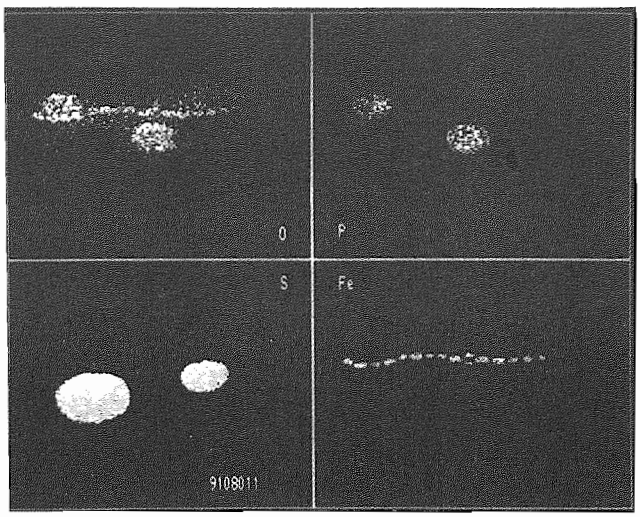

Figure 2. Elemental X-ray density maps of the organism shown in Figure 1: top left, 0; top right, P; bottom left, S; bottom right, Fe X-ray images.

The consistency of the magnetosome particle shape in strain MV-4 suggests a uniform particle morphology. This has recently been determined to be cubo-octahedral with a small elongation along the [211] direction.[7] In fact, narrow size distributions and species-specific morphologies are a characteristic feature of the magnetite biomineralization process in all magnetotactic bacteria.[6] These qualities are not characteristic of inorganically (chemically) produced magnetite, nor of extracellular magnetite produced by dissimilatory iron-reducing bacteria.[8] The latter type of indirect magnetite production results from chemical modifications of the extracellular environment by the organisms themselves. In this case the magnetite results from a chemical reaction between extracellular ferric oxyhydroxide and soluble ferrous ions which the cells secrete as they reduce ferric iron. The resulting magnetite particles have a wide size distribution and no organic component. This type of biomineralization has been termed "biologically-induced mineralization,"[9] in contrast to "biologically-controlled mineralization" in which the inorganic particles are produced within organic matrices.[9,10]

A number of idealized morphologies of bacterial magnetite in magnetotactic bacteria have been characterized[6] some of these are shown in Figure 3. The cubo-octahedral

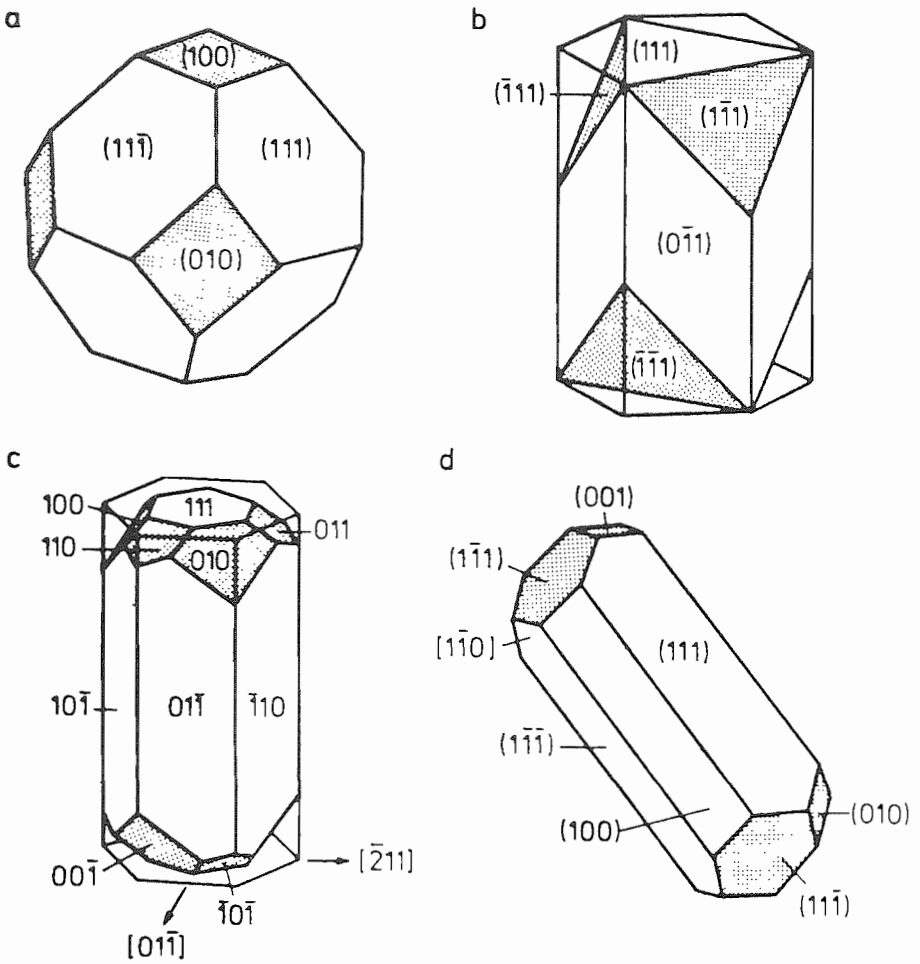

Figure 3. Idealized crystal morphologies of bacterial magnetite: (a) cubo-octahedron; (b) and (c) hexagonal prisms; (d) elongated cubo-octahedron (after Ref. 6).

morphology (Figure 3a) preserves the symmetry of the face-centered cubic, spinel crystal structure, and may be considered an equilibrium growth form. The other morphologies (Figures. 3b-d) all represent departures from the equilibrium form, presumably due to acceleration or deceleration of the growth of certain crystal faces,

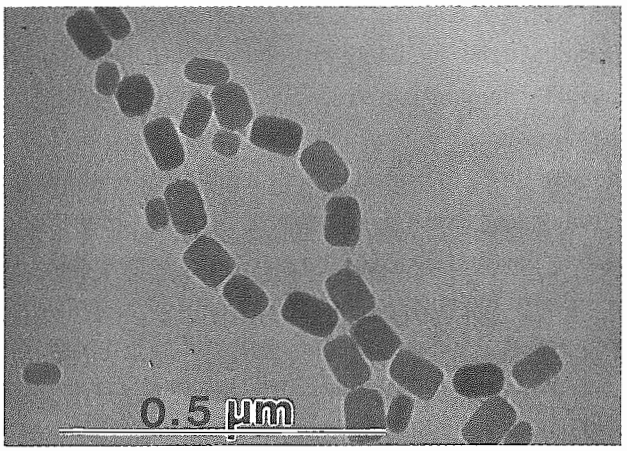

Figure 4. TEM/BF image of magnetite particles separated from cells of strain MV-1 showing the magnetosome membrane in this organism. Magnification: 100,000 x.

possibly resulting from the differential binding of macromolecules to those faces.

It is clear that magnetotactic bacteria exercise great control over the biomineralization process. How they do this is unclear but is currently under study. In several species of magnetotactic bacteria, the magnetite particles are enveloped in what appears to be a membrane vesicle. In *Aquaspirillum magnetotacticum*, the vesicle is a unit membrane (i.e., a lipid bilayer) consisting of phospholipids and numerous proteins, which is apparently not contiguous with the cytoplasmic membrane.[11] The magnetosome membrane is presumably the structural entity that anchors the mineral particle at a particular location in the cell, as well as the locus of control over the size and morphology of the particle. A magnetosome membrane has also recently been found in cells of the facultatively anaerobic, marine, magnetotactic bacterium, strain MV-1 (Figure 4). Clearly, more information about these membranes and their associated proteins in different magnetotactic bacteria would be highly desirable in elucidating the biomineralization process.

2.0 MAGNETIC STRUCTURE OF THE MAGNETOSOME CHAIN

The hierarchical structure of the magnetosome chain is significant when one considers its magnetic properties.[2] Firstly, consider the size of the individual particles. Large particles of any magnetic material, including magnetite, lower their magnetostatic energy by forming magnetic domains, thus reducing the remnant magnetic moment of the particle. Magnetic domains are regions of uniform magnetization and are separated from each other in the particle by transition regions known as domain walls. In the domain walls, the direction of magnetization changes smoothly from that of one domain to the other. The width of the domain walls in a particle is determined by the fundamental magnetic properties of the material including magnetic exchange and anisotropy energies, and is hence a constant for any magnetic material. Thus when the particle dimensions become comparable with the domain wall width, domains cannot form and the particle is forced to remain a single magnetic domain with uniform, maximum magnetization. For magnetite, this is 92 emu/g. Calculations by Butler and Banerjee[12] give 76 nm as the upper limit for the single-magnetic-domain-size range for equidimensional particles of magnetite. Because of shape anisotropy, the single-magnetic-domain volume increases with axial ratio for non-equidimensional particles. As a rule of thumb, magnetite particles with long dimensions of the order of 120 nm or less are single magnetic domains.

The thermal stability of the magnetization in single-magnetic-domain particles is determined by the particle volume. The magnetization is oriented along an energetically favorable direction in the particle known as an easy magnetic axis, which, for magnetite above the so-called Verwey transition at 118 K, is parallel to a <111> direction. There are several equivalent <111> directions in the lattice and thermal energy can spontaneously excite transitions of the magnetization over the intervening hard magnetic directions, or energy barriers due to magnetic anisotropy. This behavior, known as superparamagnetism, results in a time-averaged loss of remnant magnetization in an ensemble of particles. For single-magnetic-domain particles above a certain volume, the transition rate of the magnetization will be negligible and the particles will retain a permanent magnetization. For magnetite at 300 K, particles with dimensions greater than or equal to about 35 nm will be permanently magnetized. Thus, magnetite particles with long dimensions between about 35 and 120 nm are permanent, single magnetic

domains at ambient temperature. The magnetite particles produced by magnetotactic bacteria are typically within this size range. Thus the bacteria are not only producing magnetic, mineral particles, they are producing permanent, single-magnetic-domain-sized particles of that mineral.

When the particles are organized into chains, as they are in magnetotactic bacteria, the magnetic interactions between them cause their magnetic dipole moments to orient parallel to each other along the chain direction. The total magnetic dipole moment of the chain is thus the sum of the moments of the individual particles. By organizing the particles into chains, a bacterium is essentially constructing a permanent magnetic dipole which is sufficiently large to orient the cell in the geomagnetic field in water as it swims. That is, the magnetic energy of the dipole in the geomagnetic field is about a factor of 10 or greater than thermal energy at ambient temperature, which results in an 80% or better average projection of the moment on the field direction.

The cellular magnetic dipole can have two possible orientations with respect to the flagellum, which makes the organisms either North-seeking or South-seeking in the geomagnetic field. Organisms with the former and latter polarity predominate in the Northern and Southern hemispheres, respectively.[1-3] The advantage of magnetotaxis is presumably increased efficiency in finding and maintaining preferred position in redox and/or oxygen gradients.

We might note that permanent magnet manufacturers have been using biomimickry without realizing it. The basic strategy in the manufacture of permanent magnets from different materials, including alnico, samarium-cobalt, neodymium-iron-boron, and others, is to first produce permanent, single-magnetic-domain-sized particles of the material and then to press the particles together and sinter them to form the finished magnet. The magnet is then magnetized in an intense magnetic field. The bacteria have engineered a more elegant solution by forming chains of single magnetic domains which spontaneously produce the maximum magnetization.

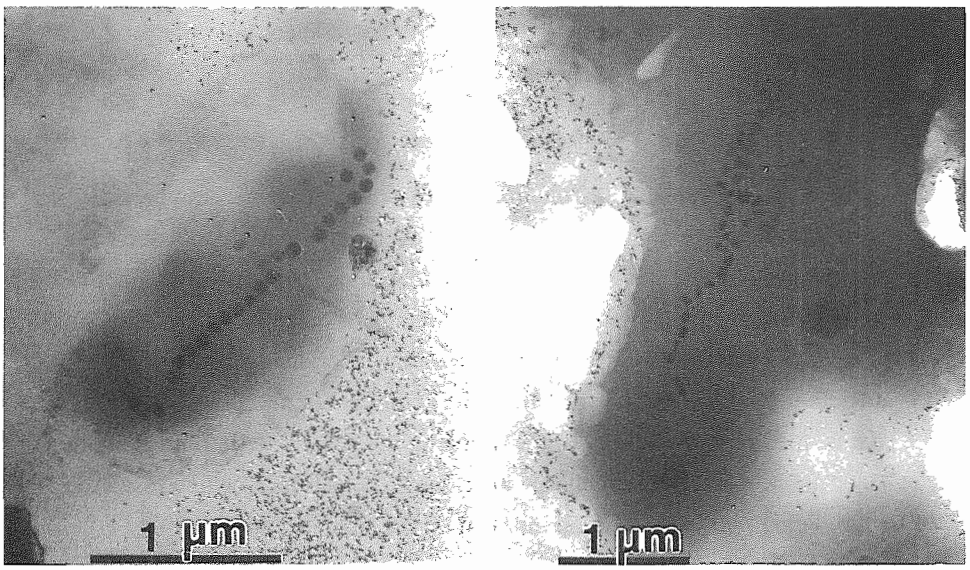

Figure 5. TEM/BF images of two magnetotactic rod-shaped bacteria collected from sulfidic aquatic habitats. Bars: 1 micron.

3.0 IRON SULFIDE PARTICLES IN MAGNETOTACTIC BACTERIA

Molecular oxygen is apparently required for cells of *A. magnetotacticum* to produce intracellular magnetite,[13] and this has led to the assumption that magnetite formation by magnetotactic bacteria is confined to surface sediments in aquatic habitats with microaerobic conditions.[14] However, morphologically diverse forms of magnetotactic bacteria are also common in reducing sediments and waters that contain high concentrations of hydrogen sulfide (H_2S) and probably no free oxygen.[15] Such conditions occur in coastal estuarine environments, salt marsh pools and certain shallow anaerobic basins.

Sulfide-rich sediments and water collected from both the west coast and east coast of the United States (Morro Bay, California and Woods Hole, Massachusetts, respectively) contained large numbers of rod-shaped magnetotactic bacteria. These were separated

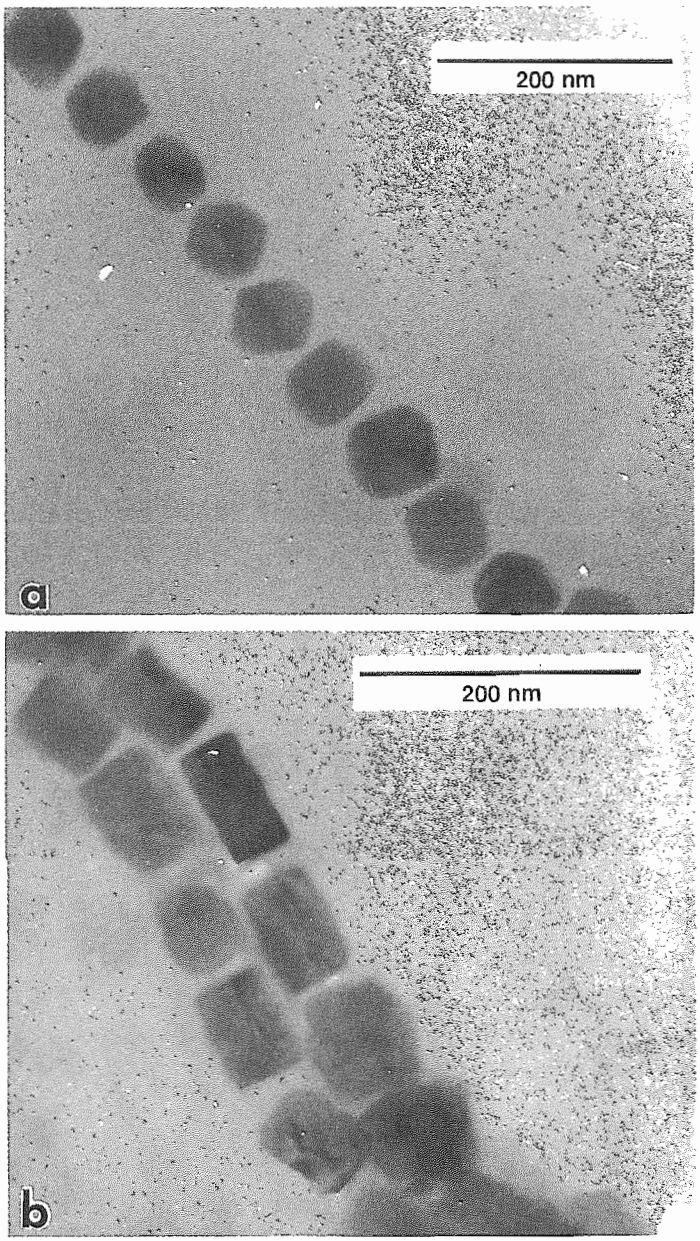

Figure 6. Magnified view of intracellular greigite, Fe_3S_4, particles in the cells shown in Figure 5, showing (a) cubo-octahedral and (b) rectangular prismatic morphologies.

magnetically and examined by electron microscopy. At least two types of rod-shaped organisms were found, a smaller one (ca. 2.5 x 1.3 mm) and a larger one (ca. 3 mm x 2 mm), containing cubo-octahedral and rectangular prismatic electron dense particles, respectively. These are shown in Figures 5 and 6. X-ray elemental mapping by scanning transmission electron microscopy revealed that the particles were composed of iron and sulfur, not iron and oxygen (Figure 7).[15] Identification of the iron-sulfur mineral phase by indexing isolated single crystal electron diffraction patterns revealed that both the smaller and larger rods contained particles of greigite, Fe_3S_4.[16,17] This mineral has a face-centered spinel structure and is isostructural with magnetite. Study of the particles by high resolution transmission electron microscopy revealed two distinct, idealized particle morphologies which are shown in Figure 8.[17] Thus greigite formation in these bacteria parallels magnetite formation in the microaerophilic bacteria described above, with narrow size distributions and species-specific morphologies, implying a high degree of control over the biomineralization process. Uncontrolled, biologically-induced mineralization of iron sulfide particles is known to occur in some dissimilatory sulfate-reducing bacteria.[18]

Greigite is ferrimagnetic at ambient temperature. Its saturation magnetization is approximately 30 emu/g, about one third that of magnetite. Thus it is less "efficient" than magnetite as a permanent magnet material. Nevertheless, it is perfectly adequate as the basis of the magnetotactic response in those bacteria which produce it. Many of the basic magnetic properties of griegite have not been determined so the single-magnetic domain size range is not known, but on the basis of the magnetization and Curie temperature we may infer that it is approximately the same as magnetite. Thus chains of magnetosomes containing greigite would function analogously to chains of magnetosomes containing magnetite.

Living in the same sulfidic habitats on the west and east coasts of the United States as the greigite-containing magnetotactic rods described above is an unusual multicellular, magnetotactic prokaryote (Figure 9) that is referred to casually as "the mulberry" because of its appearance in the light microscope.[19] A similar organism occurs in Brazil,[20] and probably in similar habitats in other parts of the world. The intact organism contains 7 to 20 individual cells, each of which has flagella on one side of the cell, and contains about 10 electron-dense particles arranged in chains Figure 10. There is

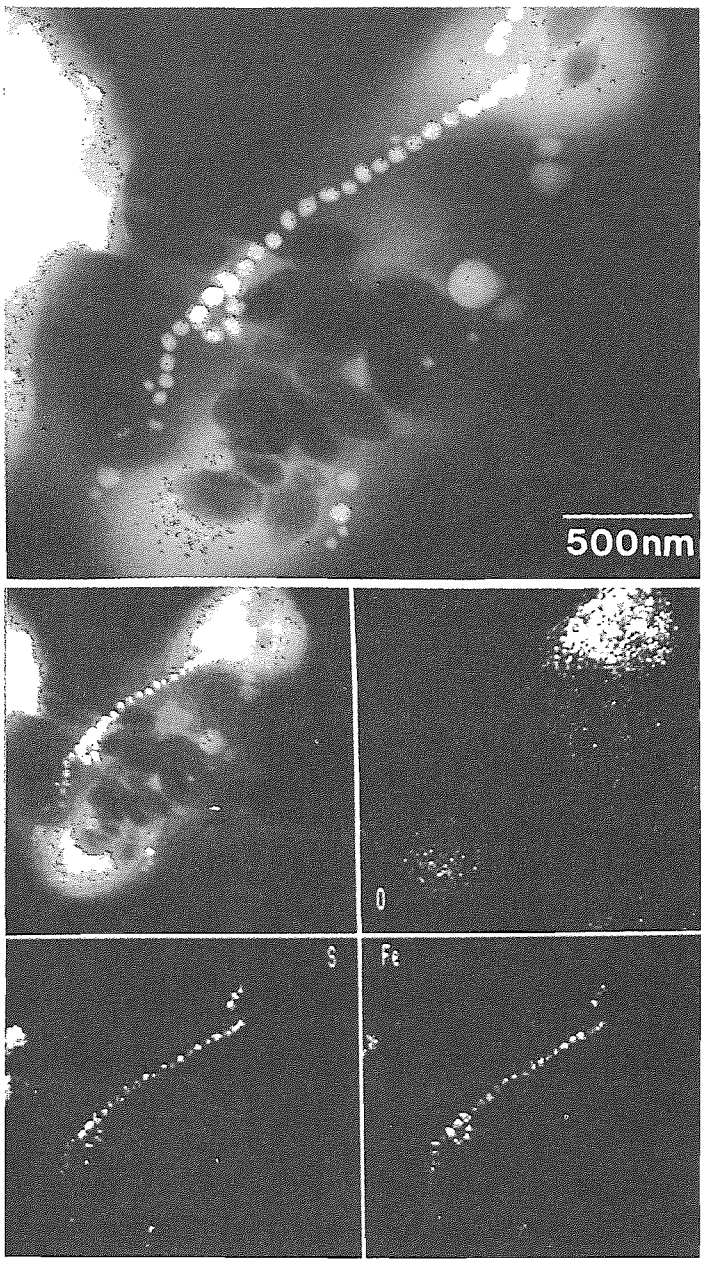

Figure 7. Elemental X-ray density maps for a magnetotactic rod-shaped bacterium with cubo-octahedral particles. Upper panel: STEM/BF image. Lower panel: upper left, STEM image; upper right, O; bottom left, S; bottom right, Fe. The correlation of Fe and S density with particle position shows that the particles contain Fe and S but not O. Similar results have been reported for cells containing rectangular prismatic particles.

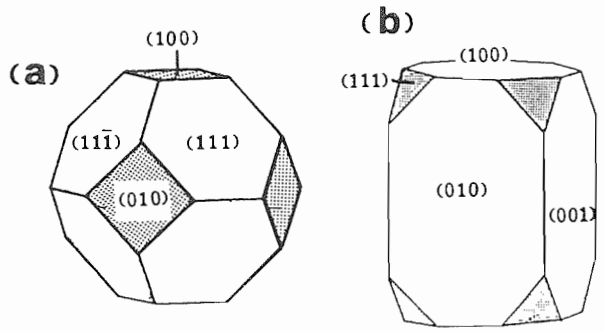

Figure 8. Idealized morphologies of greigite crystals formed in magnetotactic bacteria: (a) cubo-octahedron; (b) elongated, truncated cube. (After Ref. 17.)

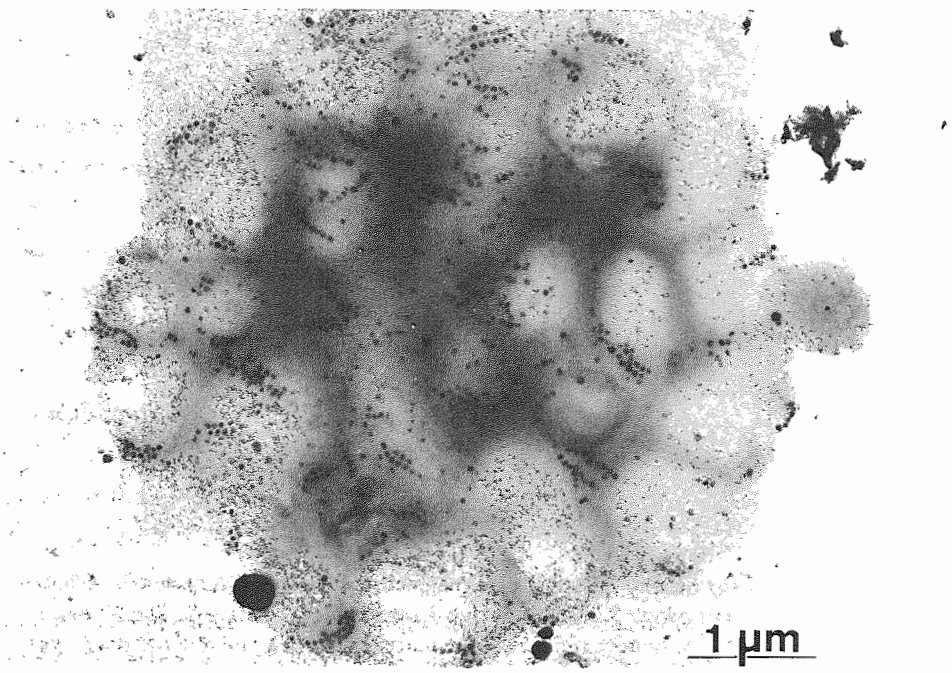

Figure 9. TEM/BF image of a magnetotactic, multicellular prokaryote ("mulberry") showing individual cells and chains of particle (Ref. 21).

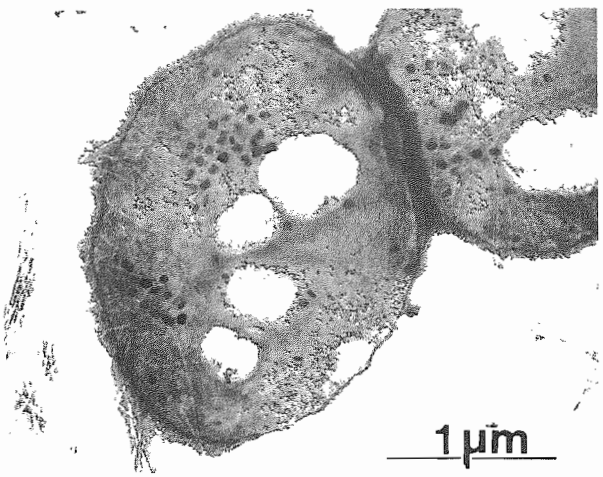

Figure 10. Electron micrograph of one of 20 constituent cells of the magnetotactic, multicellular prokaryote ("mulberry"). Each cell is flagellated and contains magnetosomes. The cell has been negatively stained (Ref. 19).

electron microscopical evidence that the magnetosome chains are oriented parallel to each other in the intact organism. The organism is motile and magnetotactic, but if it is disrupted, e.g., by osmotic shock, the individual cells are not motile, but are permanently magnetic.

As in the magnetotactic rods, elemental mapping shows that the electron-dense inclusions in this organism are iron sulfides (Figure 11).[21,22] However, electron diffraction data reveal that in addition to greigite, the cells contain nonmagnetic pyrite, FeS_2.[21] Since the morphologies of the particles in this organism are not uniform as in the magnetotactic rods, it is not possible to distinguish the particles of greigite and pyrite on the basis of simple examination of the transmission electron micrographs. Thus it is not known how the greigite and pyrite particles are distributed in the cells. However, the electron diffraction data suggest that pyrite is the predominant mineral. The role of non-magnetic pyrite in the cells is not known, but it clearly plays no role in magnetotaxis. Williams[23] has proposed that pyrite is involved in maintenance of iron and sulfide homeostasis in the organisms. Moreover, it has recently been found that copper

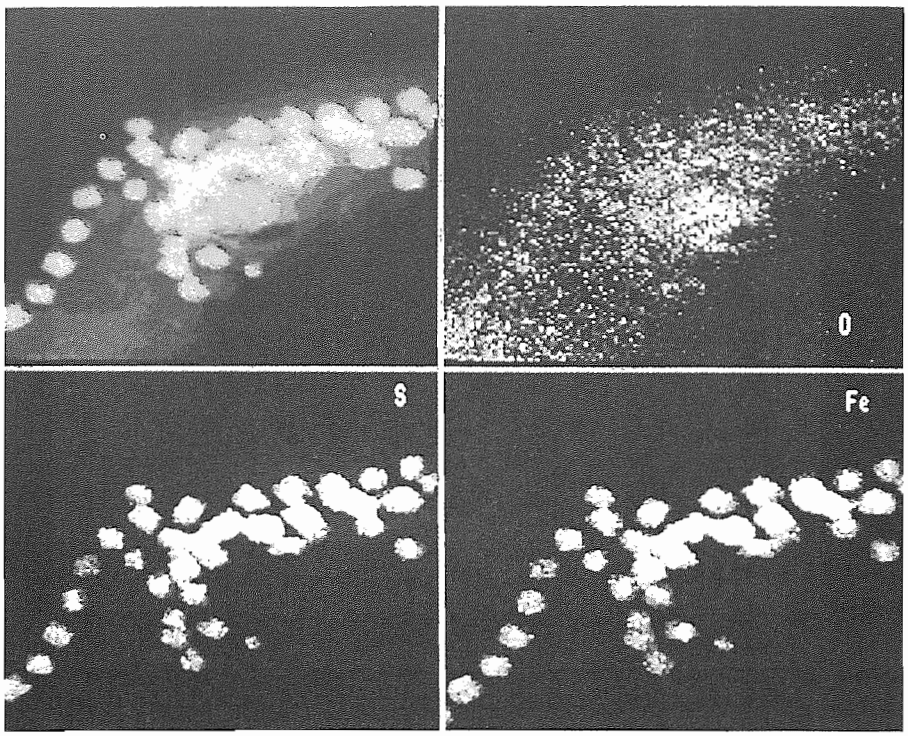

Figure 11. Elemental density maps for the particles in one of the constituent cells of the magnetotactic, multicellular prokaryote: upper left, transmission electron micrograph; upper right, O; bottom left, S; bottom right, Fe. Particles consist of greigite, Fe_3S_4, and pyrite, FeS_2 (Ref. 21). *(See also* ***Color Plate 5.****)*

can be incorporated into the greigite and/or pyrite particles,[24] suggesting a possible role in detoxification. Nevertheless, it is remarkable that these organisms are separately mineralizing two iron-sulfide minerals, probably in each of its constituent cells.

4.0 CONCLUSION

The rationale for biomimicking as a strategy for the design of novel materials and structures is that the materials and structures found in organisms have been refined and optimized in the course of evolution to have certain properties and to serve specific functions. Even if the functions are not completely understood, the properties can be determined and correlated with the structures. Thus strategies for the production of new ma-

terials and structures with similar properties can be determined. Much of the work in this area has focused on the mechanical properties of materials and structures in higher organisms, such as shell, bone, connective tissue, chitin, etc. The magnetotactic bacteria afford an example of optimization of magnetic properties in biomineralized magnetic particles by maintenance of control over particle size and morphology, and creation of a hierarchical structure by relative particle placement in the cell. It is remarkable that the same general scheme is utilized for two different magnetic minerals in different species of bacteria. It has recently been determined by phylogenetic analysis that magnetite-producing magnetotactic bacteria and the iron-sulfide-producing mulberry belong to distinct lineages of the proteobacteria in the domain bacteria.[25] This suggests that the biochemical basis of the biomineralization process could be different for the two mineral particles. In any case, elucidation of the details of bacterial biomineralization processes could yield clues to the production of ordered arrays of inorganic magnetic materials on a nanometer scale.

5.0 ACKNOWLEDGEMENTS

We gratefully acknowledge the participation of A. Garratt-Reed, B.R. Heywood, S. Mann, and N. Sparks in various aspects of this research. We also thank the Office of Naval Research for support.

6.0 REFERENCES

1. R. P. Blakemore, "Magnetotactic Bacteria," *Annu. Rev. Microbiol.*, **36**, 217-238 (1982).

2. R. B. Frankel, "Magnetic Guidance of Organisms," *Annu. Rev. Biophys. Bioeng.*, **13**, 85-103 (1984).

3. R. B. Frankel and R. P. Blakemore, "Magnetite and Magnetotaxis in Microorganisms," *Bioelectromagnetics*, **10**, 223-237 (1989).

4. D. A. Bazylinski, R. B. Frankel, and H. W. Jannasch, "Anaerobic Magnetite Produc-tion by a Marine Magnetotactic Microorganism," *Nature*, **333**, 518-519 (1988).

5. R. P. Blakemore, N. A. Blakemore, D. A. Bazylinski, and T. T. Moench, "The Magnetotactic Bacteria," in *Bergey's Manual of Systematic Bacteriology,* Vol. 3, M. P. Bryant, N. Pfennig, and H. T. Staley (eds.) (Williams and Wilkins, Baltimore, 1989) pp. 15-25.

6. S. Mann and R. B. Frankel, "Magnetite Biomineralization in Unicellular Microorganisms," in *Biomineralisation: Chemical and Biochemical Perspectives*, S. Mann, J. Webb, and R. J. P. Williams (eds.) (VCH, Weinheim, 1989) pp. 388-426.

7. F. Meldrum, S. Mann, B. R. Heywood, R. B. Frankel, and D. A. Bazylinski, "Electron Microscopy of Magnetosomes in Two Cultured Vibrioid Magnetotactic Bacteria," *Proc. Roy. Soc. B,* **251** 237-242 (1993).

8. N. H. C. Sparks, S. Mann, D. A. Bazylinski, D. R. Lovley, H. W. Jannasch, and R. B. Frankel, "Structure and Morphology of Anaerobically Produced Magnetite by a Marine Magnetotactic Bacterium and a Dissimilatory Iron-Reducing Bacterium," *Earth Planet. Sci. Lett.,* **98**, 14-22 (1990).

9. H. A. Lowenstam, "Minerals Formed by Organisms," *Science,* **211**, 1126-1131 (1981).

10. S. Mann, "On the Nature of Boundary Organised Biomineralization," *J. Inorg. Biochem.*, **28**, 363-370 (1986).

11. Y. A. Gorby, T. J. Beveridge, and R. P. Blakemore, "Characterization of the Bacterial Magnetosome Membrane," *J. Bacteriol.*, **170**, 834-841 (1988).

12. R. F. Butler and S. K. Banerjee, "Theoretical Single-Domain Grain Size Range in Magnetite and Titanomagnetite," *J. Geophys. Res.*, **80**, 4049-4058 (1975).

13. R. P. Blakemore, K. A. Short, D. A. Bazylinski, C. Rosenblatt, and R. B. Frankel, "Microaerobic Conditions are Required for Magnetite Formation within *Aquaspirillum magnetotacticum,*" *Geomicrobiol. J.*, **4**, 53-71 (1985).

14. D. R. Lovley, J. F. Stoltz, G. L. Nord, Jr., and E. J. P. Phillips, "Anaerobic Production of Magnetite by a Dissimilatory Iron-Reducing Microorganism," *Nature,* **330**, 252-254 (1987).

15. D. A. Bazylinski, R. B. Frankel, A. J. Garratt-Reed, and S. Mann, "Biomineralization of Iron Sulfides by Magnetotactic Bacteria from Sulfidic Environments," in *Iron Biominerals*, R. B. Frankel and R. P. Blakemore (eds.) (Plenum, New York, 1991) pp. 239-255.

16. B. R. Heywood, D. A. Bazylinski, A. Garratt-Reed, S. Mann, and R. B. Frankel, "Controlled Biosynthesis of Greigite (Fe_3S_4) in Magnetotactic Bacteria," *Naturwissenschaften*, **77**, 536-538 (1990).

17. B. R. Heywood, S. Mann, and R. B. Frankel, "Structure, Morphology, and Growth of Biogenic Greigite (Fe_3S_4)," in *Materials Syntheses Based on Biological Processes*, M. Alper, P. Calvert, R. Frankel, P. Rieke, and D. Tirrell (eds.) Proc. of MRS, Vol. 218 (Materials Research Society, Pittsburgh, 1991) pp. 93-108.

18. D. A. Bazylinski, "Bacterial Production of Iron Sulfides," in *Materials Syntheses Based on Biological Processes*, M. Alper, P. Calvert, R. Frankel, P. Rieke, and D. Tirrell (eds.) Proc. of MRS, Vol. 218 (Materials Research Society, Pittsburgh, 1991) pp. 81-91.

19. F. Rogers, R. P. Blakemore, N. A. Blakemore, R. B. Frankel, D. A. Bazylinski, D. Maratea, and C. Rogers, "Intercellular Structure in a Many-celled Magnetotactic Procaryote," *Arch. Microbiol.*, **154**, 18-22 (1990).

20. M. Farina, H. Lins de Barros, D. Motta de Esquivel, and J. Danon, "Ultrastructure of a Magnetotactic Microorganism," *Biol. Cell*, **48**, 85-88 (1983).

21. S. Mann, N. H. C. Sparks, R. B. Frankel, D. A. Bazylinski, and H. W. Jannasch, "Biomineralization of Ferrimagnetic Greigite (Fe_3S_4) and Iron Pyrite (FeS_2) in a Magnetotactic Bacterium," *Nature*, **343**, 258-260 (1990).

22. M. Farina, D. Motta de Esquivel, and H. G. P. Lins de Barros, "Magnetic Iron-Sulphur Crystals from a Magnetotactic Microorganism," *Nature*, **343**, 256-258 (1990).

23. R. J. P. Williams, "Biominerals and Homeostasis," in *Iron Biominerals*, R. B. Frankel and R. P. Blakemore (eds.) (Plenum, New York, 1991) pp. 7-19.

24. D. A. Bazylinski, A. J. Garratt-Reed, A. Abedi, and R. B. Frankel, "Copper Association with Iron Magnetosomes in a Magnetotactic Bacterium," *Arch. Microbiol.*, **60** 35-42 (1993).

25. E. F. DeLong, R. B. Frankel, and D. A. Bazylinski, "Multiple Evolutionary Origins of Magnetotaxis in Bacteria," *Science*, **259** 803-806 (1993).