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## Precipitation of $\text{Fe}_3\text{O}_4$ in Magnetotactic Bacteria [and Discussion]

R. B. Frankel, R. P. Blakemore and A. L. Mackay

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Precipitation of  $\text{Fe}_3\text{O}_4$  in magnetotactic bacteriaBY R. B. FRANKEL<sup>1</sup> AND R. P. BLAKEMORE<sup>2</sup><sup>1</sup> *Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, U.S.A.*<sup>2</sup> *Department of Microbiology, University of New Hampshire, Durham, New Hampshire 03824, U.S.A.*

The magnetotactic bacterium *A. magnetotacticum* contains ferrous ions, a low density hydrous ferric oxide, a high density hydrous ferric oxide (ferrihydrite) and  $\text{Fe}_3\text{O}_4$ , which is precipitated by partial reduction of the ferrihydrite precursor.

## MAGNETOTACTIC BACTERIA

Magnetotactic bacteria are various species of aquatic micro-organisms that orient and swim along magnetic field lines (Blakemore 1975, 1982; Blakemore & Frankel 1981; Moench & Konetzka 1978). All magnetotactic cells examined to date by electron microscopy contain iron-rich, electron-opaque particles (Balkwill *et al.* 1980; Towe & Moench 1981). In several species of magnetotactic bacteria, and possibly all, the particles consist of magnetite,  $\text{Fe}_3\text{O}_4$  (Frankel *et al.* 1979). Cuboidal, rectangular parallelepiped, and arrowhead shaped particles occur in different species with typical dimensions of 400–1200 Å†. These are within the single-magnetic-domain size range for  $\text{Fe}_3\text{O}_4$ . In most species the particles are arranged in chains, which impart a magnetic moment to the cell, parallel to the axis of motility. The moment is sufficiently large that the bacterium is oriented in the geomagnetic field at ambient temperature as it swims, i.e. the chain of  $\text{Fe}_3\text{O}_4$  particles functions as a biomagnetic compass (Frankel & Blakemore 1980). By this means the organism propels itself along the geomagnetic field lines. The direction of migration depends on the orientation of the biomagnetic compass. Those with the north-seeking pole forward migrate north along the field lines. Those with the south-seeking pole forward migrate south. It has been found that north-seeking bacteria predominate in the Northern Hemisphere while south-seeking bacteria predominate in the Southern Hemisphere (Blakemore *et al.* 1980; Kirschvink 1980). The vertical component of the inclined geomagnetic field selects the predominant polarity in each hemisphere by presumably favouring those cells whose polarity causes them to be directed downward towards the sediments and away from the toxic effects of the oxygen rich surface waters. At the geomagnetic equator where the vertical component is zero both polarities coexist; presumably, horizontally directed motion is equally beneficial to both polarities in reducing harmful upward migration (Frankel *et al.* 1981).

In the freshwater magnetotactic spirillum, *Aquaspirillum magnetotacticum*, iron composes 2% or more of the cellular dry mass (Blakemore *et al.* 1979). Electron microscopy studies of this organism show that the  $\text{Fe}_3\text{O}_4$  particles are cuboidal, 40–50 nm wide, and are arranged in a chain that longitudinally traverses the cell (figure 1). The particles are enveloped by

† 1 Å =  $10^{-10}$  m.

[ 157 ]

electron-transparent and electron-dense layers; a particle and its enveloping membrane has been termed a magnetosome (Balkwill *et al.* 1980).

Since *A. magnetotacticum* is cultured in a chemically defined medium in which iron is available as soluble ferric quinate (Blakemore *et al.* 1979), the presence of intracellular  $\text{Fe}_3\text{O}_4$  implies a process of bacterial precipitation of this mineral, with control of particle size, number and location in the cell.

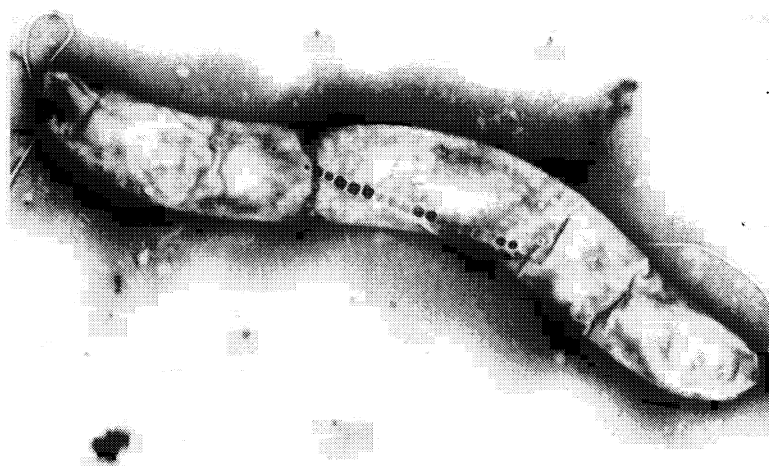


FIGURE 1. Electron micrograph of *A. magnetotacticum*. The cell is approximately 3  $\mu\text{m}$  long. Electron-opaque spots in the middle of the cell are the magnetosomes.

To elucidate the  $\text{Fe}_3\text{O}_4$  biomineralization process, cells and cell fractions, some isotopically enriched in  $^{57}\text{Fe}$ , have been studied by Mössbauer spectroscopy (Frankel *et al.* 1983). Cells of a non-magnetotactic variant that accumulated iron but did not make  $\text{Fe}_3\text{O}_4$  and of a cloned, non-magnetotactic strain that accumulated less iron, were also studied. The results suggest that  $\text{Fe}_3\text{O}_4$  is precipitated by reduction of a hydrous ferric oxide precursor.

#### MÖSSBAUER SPECTROSCOPY OF MAGNETOTACTIC BACTERIA

Mössbauer spectra of wet packed cells enriched in  $^{57}\text{Fe}$  at 200 K and at 80 K are shown in figures 2 and 3, respectively. The 200 K spectrum can be analysed as a superposition of spectra corresponding to  $\text{Fe}_3\text{O}_4$  (spectra  $A_1$  and  $A_2$ ), a broadened quadrupole doublet with parameters characteristic of ferric iron (spectrum B), and a weak quadrupole doublet with parameters corresponding to ferrous iron (spectrum C) (table 1). Spectra  $A_1$  and  $A_2$  correspond to  $\text{Fe}^{3+}$  in tetrahedral sites and to  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  in octahedral sites in  $\text{Fe}_3\text{O}_4$ , respectively (Hargrove & Kundig 1970).

Spectrum B was also observed in lyophilized cells and has isomer shift and quadrupole splitting parameters similar to iron in ferritin and in the mineral ferrihydrite, indicative of ferric iron with oxygen coordination. The relative intensity of B to  $A_1 + A_2$  was somewhat variable from sample to sample, depending on growth conditions. At 80 K, spectra  $A_1$  and  $A_2$  correspond to  $\text{Fe}_3\text{O}_4$  below the Verwey transition and the parameters of spectrum B and the relative intensity of B to  $A_1 + A_2$  are relatively unchanged compared to the spectrum at 250 K. Between

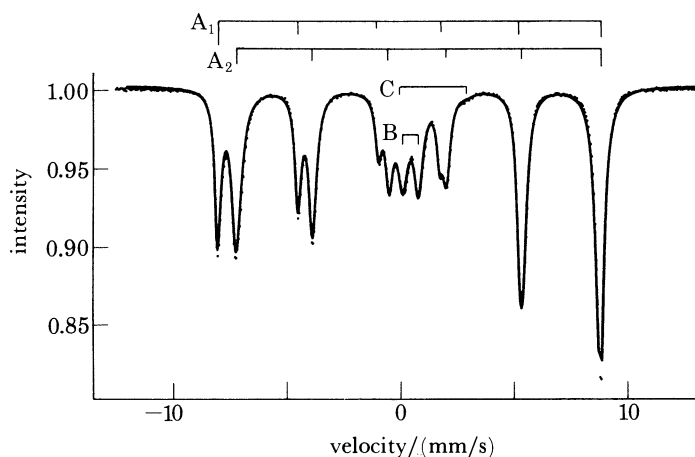
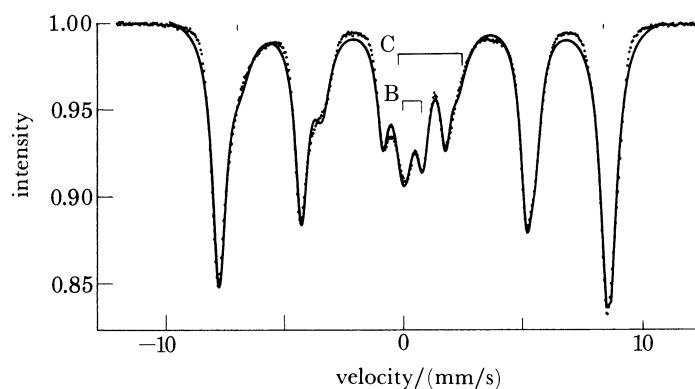
FIGURE 2. Mössbauer spectrum of *A. magnetotacticum* wet, packed cells at 200 K.FIGURE 3. Mössbauer spectrum of *A. magnetotacticum* wet, packed cells at 80 K.

TABLE 1. MÖSSBAUER PARAMETERS AT 80 K

material	$\delta$ /(mm/s) <sup>(a)</sup>	$\Delta E_Q$ /(mm/s) <sup>(b)</sup>
<i>A. magnetotacticum</i> spectrum B	$0.47 \pm 0.03$	$0.65 \pm 0.05$
<i>A. magnetotacticum</i> spectrum C	1.32	3.17
<i>A. magnetotacticum</i> non-magnetic cells	0.47	0.68
<i>A. magnetotacticum</i> cloned, non-magnetic cells	0.51	0.65
ferritin <sup>(c)</sup>	0.47	0.73
<i>E. coli</i> <sup>(d)</sup> storage material	0.50	0.66
ferrihydrate <sup>(e)</sup>	0.47	0.74
amorphous ferric gel <sup>(f)</sup>	0.47	0.81

(a) Isomer shift relative to iron metal at room temperature. (b) Quadrupole splitting. (c) Blaise *et al.* (1965). (d) Bauminger *et al.* (1980). (e) Murad & Schwertmann (1980). (f) Coey & Readman (1973).

80 and 4.2 K, however, the intensity of B decreased with decreasing temperature so that at 4.2 K only a residual doublet remained. A similar temperature dependence for spectrum B was also obtained in lyophilized cells.

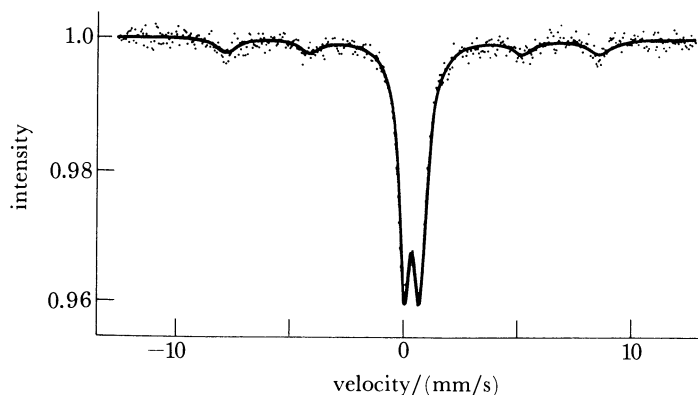


FIGURE 4. Mössbauer spectrum of non-magnetotactic cells at 80 K. Some residual  $\text{Fe}_3\text{O}_4$  is present in the sample.

The isomer shift and quadrupole splitting parameters of spectrum C correspond to high spin ferrous iron in coordination with oxygen or nitrogen. This spectrum was not observed with lyophilized cells, possibly as a result of oxidation during sample preparation. Wet, packed cells kept unfrozen under anaerobic conditions contained increased amounts of material responsible for spectrum C and correspondingly less material with spectral characteristics B. Thawing and aeration of these frozen cells resulted in increases in B spectral lines and concomitant decreases in C spectral lines. This indicates that the iron atoms responsible for spectrum C came from reduction of the iron atoms giving spectrum B. Unlike that of spectrum B, the intensity of spectrum C did not decrease between 80 and 4.2 K.

The decrease in the intensity of spectrum B between 80 and 4.2 K can be explained as the onset of magnetic hyperfine interactions at low temperature, resulting in a concomitant decrease in the intensity of the central absorption doublet. This phenomenon has been observed with Mössbauer spectroscopy of ferritin (Blaise *et al.* 1965). However, in the present case, the magnetic hyperfine lines were obscured by the magnetite spectral lines ( $A_1$  and  $A_2$ ). To further resolve the nature of the materials responsible for spectrum B, we studied the temperature dependent Mössbauer spectra of non-magnetotactic cells, which lacked the interfering magnetite.

For  $T \geq 80$  K, the spectrum of lyophilized non-magnetotactic cells (figure 4) consisted primarily of the quadrupole doublet characteristic of ferric iron, denoted by spectrum B in figures 2 and 3. In addition, a very low intensity spectrum due to  $\text{Fe}_3\text{O}_4$  (spectral lines  $A_1 + A_2$  in figures 2 and 3) was observed. These latter spectral lines might have been due to a small fraction of magnetotactic cells in the sample or trace amounts of magnetite possibly present in the non-magnetotactic cells. Below 80 K, the intensity of the quadrupole doublet decreased with decreasing temperature while the intensity of a six line spectrum flanking the doublet increased. At 4.2 K the spectrum (figure 5) consisted primarily of the six broadened magnetic hyperfine lines, with a small residual doublet in the centre. Spectral lines  $A_1 + A_2$  were obscured by the six line spectrum. Application of a longitudinal magnetic field of  $4.78 \text{ MA m}^{-1}$

produced broadening of the six line spectrum, but with no appreciable shifts in the line positions or decreases in any line intensities.

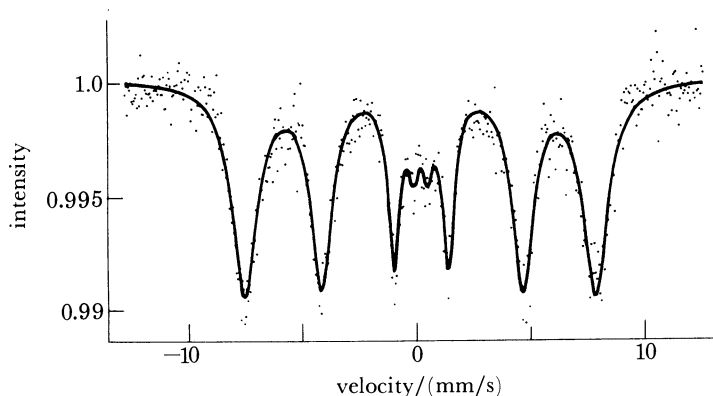


FIGURE 5. Mössbauer spectrum of non-magnetotactic cells at 4.2 K.

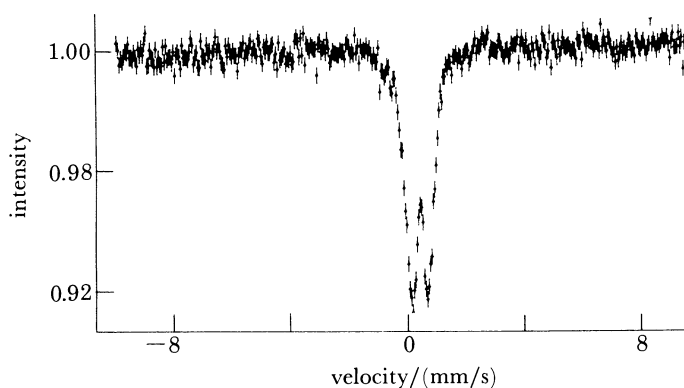


FIGURE 6. Mössbauer spectrum of cloned, non-magnetotactic strain of *A. magnetotacticum* at 4.2 K.

These spectral characteristics are indicative of small particles of hydrous ferric oxide with antiferromagnetic exchange interactions similar to those of the ferric iron within ferritin micelles and in ferrihydrite. By comparison with ferritin, the experimental results indicate that hydrous ferric oxide particles in the non-magnetotactic cells are of the order of 100 Å in diameter, or less. Unlike ferritin or ferrihydrite, however, there was a residual quadrupole doublet in the 4.2 K spectrum of magnetotactic and non-magnetotactic cells. The intensity of this residual doublet varied from sample to sample, but its presence suggests another high spin ferric material with high temperature spectral characteristics similar to those of ferrihydrite, but with iron atoms less densely packed so that magnetic exchange interactions between them are weaker and the spectrum is not magnetically split at 4.2 K. This latter material was also observed in a cloned, non-magnetotactic strain of *A. magnetotacticum* that accumulates less iron.

The Mössbauer spectrum of wet, packed cells of the cloned non-magnetotactic strain consisted of a quadrupole absorption doublet for  $T \geq 4.2$  K (figure 6). The spectral parameters obtained at 80 K were similar to those of spectrum B in magnetotactic cells (table 1), which indicates the presence of a high spin ferric iron material. Application of an external  $4.78 \text{ MA m}^{-1}$  magnetic field at 4.2 K results in spectra with a broad distribution of hyperfine

fields. These spectral characteristics indicate the presence of high spin  $\text{Fe}^{3+}$  in a hydrous oxide with magnetic exchange interactions below 4 K, that is, where the iron atoms are less densely packed than in ferrihydrite. This material has similar spectral characteristics to the iron storage material in *E. coli* (Bauminger *et al.* 1980).

When these wet, packed cells were held above 275 K in an anaerobic environment, a ferrous spectrum similar to spectrum C appeared, in addition to the ferric iron doublet. This indicates that the hydrous ferric oxide in cells of this strain can be reduced to ferrous iron as with cells of the other strain.

In summary, cells of *A. magnetotacticum* contain ferrous ions, a low density hydrous ferric oxide, a high density hydrous ferric oxide (ferrihydrite) and  $\text{Fe}_3\text{O}_4$ . Additional experiments with cell fractions show that ferrihydrite in the magnetotactic cells is associated with the magnetosomes (Frankel *et al.* 1983).

#### DISCUSSION

On the basis of our results we have proposed that *A. magnetotacticum* precipitates  $\text{Fe}_3\text{O}_4$  in the sequence:  $\text{Fe}^{3+}$  quinate  $\rightarrow \text{Fe}^{2+} \rightarrow$  low density hydrous ferric oxide  $\rightarrow$  ferrihydrite  $\rightarrow \text{Fe}_3\text{O}_4$  (Frankel *et al.* 1983). In non-magnetotactic cells the process stops with ferrihydrite. In cells of the cloned, non-magnetotactic strain the process stops with low density hydrous ferric oxide.

In the proposed process, iron enters the cell as  $\text{Fe}^{3+}$  chelated by quinic acid. Reduction to  $\text{Fe}^{2+}$  releases iron from the chelator.  $\text{Fe}^{2+}$  is re-oxidized and accumulated as the low density hydrous iron oxide. By analogy with the deposition of iron in the micellar cores of the protein ferritin (Clegg *et al.* 1980), this oxidation step might involve molecular oxygen, which is required for  $\text{Fe}_3\text{O}_4$  precipitation in *A. magnetotacticum* (D. Bazylinski and R. P. Blakemore, personal communication 1983). Dehydration of the low density hydrous ferric oxide results in ferrihydrite. Finally, partial reduction of ferrihydrite and further dehydration yields  $\text{Fe}_3\text{O}_4$ .

$\text{Fe}_3\text{O}_4$  is thermodynamically stable with respect to haematite and ferrihydrite at low  $E_{\text{H}}$  and high pH (Garrels & Christ 1965). However, rapid transformation of ferrihydrite to magnetite appears to involve more than simple reduction and dehydration. While the degree of crystallinity of ferrihydrite can vary, in crystalline samples it has a structure related to haematite, with hexagonal close-packed oxygen atoms and  $\text{Fe}^{3+}$  octahedrally coordinated sites.  $\text{Fe}_3\text{O}_4$  has a cubic, inverse spinel structure with  $\text{Fe}^{3+}$  in octahedral and tetrahedral sites, and  $\text{Fe}^{2+}$  in octahedral sites. This, plus the fact that the precipitation process requires spatial segregation of regions of differing  $E_{\text{H}}$  and possibly pH, suggests that the process is organic matrix mediated (Lowenstam 1981). So the magnetosome envelope is probably an integral element in the precipitation process, functioning as a locus for enzymatic activities including control of  $E_{\text{H}}$  and pH, as well as a structural element.

Reduction of a ferrihydrite precursor to  $\text{Fe}_3\text{O}_4$  occurs in the marine chiton, a mollusc of the genus *Polyplacophora* (Towe & Lowenstam 1981). In this organism the radular teeth undergo a sequential mineralization process that results in a surface coating of  $\text{Fe}_3\text{O}_4$ . Iron is transported to the superior epithelial cells of the radula as ferritin. Then iron is transferred to a preformed organic matrix on the tooth surface as a ferrihydrite. Finally, the ferrihydrite is reduced to  $\text{Fe}_3\text{O}_4$ . So the  $\text{Fe}_3\text{O}_4$  precipitation process in chitons appears to be similar to that in the magnetotactic bacteria.

Lowenstam (1981) and Webb (1983) have observed that biogenic iron oxides and oxyhydroxides are present in each of the five kingdoms of the biological world, with ferrihydrite

the third most extensively formed mineral of biological origin and magnetite the fourth. Magnetotactic bacteria present an excellent opportunity for elucidating the  $\text{Fe}_3\text{O}_4$  precipitation process in detail.

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## Discussion

A. L. MACKAY (*Department of Crystallography, Birkbeck College, London, U.K.*). It is known that in anaerobic conditions, magnetite can be produced topotactically by the decomposition of the



so called green-rust material, which has a hexagonal unit cell and is a layer structure of sjögrenite–pyroaurite type, so that dehydration gives crystallites of magnetite that are hexagonal in form. Are the crystals of magnetite in magnetotactic bacteria of this form rather than equi-axed, as might be expected from the crystal structure? If so then this would be a pointer to the process of formation involved.

R. B. FRANKEL. Particles with a variety of forms occur in various species.



FIGURE 1. Electron micrograph of *A. magnetotacticum*. The cell is approximately 3  $\mu\text{m}$  long. Electron-opaque spots in the middle of the cell are the magnetosomes.