JOURNAL OF BACTERIOLOGY, Feb. 1988, p. 834–841
0021-9193/88/020834-08$02.00/0

Vol. 170, No. 2

Characterization of the Bacterial Magnetosome Membrane

YURI A. GORBY,[1] TERRY J. BEVERIDGE,[2] AND RICHARD P. BLAKEMORE[1]*

Department of Microbiology, Spaulding Life Sciences Building, University of New Hampshire, Durham, New Hampshire 03824,[1] and Department of Microbiology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1[2]

Received 28 August 1987/Accepted 4 November 1987

Intact magnetosomes of *Aquaspirillum magnetotacticum* were purified from broken cells by a magnetic separation technique. Electron microscopic and chemical analyses revealed the magnetite to be enclosed by a lipid bilayer admixed with proteins. Lipids were recovered in fractions expected to contain (i) neutral lipids and free fatty acids, (ii) glycolipids and sulfolipids, and (iii) phospholipids (in a weight ratio of 1:4:6). Phospholipids included phosphatidylserine and phosphatidylethanolamine. Two of the numerous proteins detected in the magnetosome membrane were not found in other cell membranes or soluble fractions.

The permanent-magnetic character of magnetotactic bacteria (3) and algae (27) results from a conspicuous intracellular structure characterizing the group, the magnetosome (1). Those magnetosomes which have been studied are enveloped single crystals of the iron oxide magnetite, commonly arranged in one or more linear arrays within the cytoplasm (7, 14, 17, 28). Magnetite crystal morphology may vary among species. In some bacterial species, the crystals are truncated hexagonal prisms as revealed by crystal lattice imaging (17); in others they are bullet shaped (3). Those within the axenically cultivable species *Aquaspirillum magnetotacticum* (16) are truncated octahedrons (14) which lie in a single helical line along the cell axis and adjacent to the cytoplasmic membrane. The structure and composition of the magnetosome envelope has not been widely studied, although trilaminate membrane structures have occasionally been observed surrounding magnetosomes of thin-sectioned magnetotactic bacteria collected directly from mud (2). Balkwill et al. (1) considered the possibility that magnetite particles of *A. magnetotacticum* were each surrounded by a lipid bilayer. However, because of the high electron density of the magnetite core, it was not possible to discern the electron-opaque inner leaflet expected of a closely apposed lipid bilayer in these stained preparations. We applied magnetic separation methods to disrupted cells as a unique and effective means of purifying magnetosomes for chemical and structural analyses, and from these magnetosomes we obtained definitive proof of an attendant bilayer envelope.

MATERIALS AND METHODS

Media and culture conditions. *A. magnetotacticum* was grown in 15-liter batch cultures as previously described (4). The chemically defined mineral medium contained 4 mM $NaNO_3$ and lacked organic forms of nitrogen. Iron, at a final concentration of 20 μM, chelated with an equimolar concentration of quinic acid, was added to autoclaved and cooled medium. To study iron limitation, cells were transferred at least three times in medium from which iron compounds were omitted. The total trace iron concentration in media to which no iron was intentionally added was less than 1 μM, as determined with ferrozine (26).

Cells concentrated by filtration were centrifuged at 5,000 × *g* for 10 min at 4°C. They were suspended and washed three times in buffer A, consisting of 10 mM *N*-2-hydroxyethylpiperizine-*N'*-2-ethanesulfonic acid buffer (pH 7.4) containing 10 μg of the protease inhibitor phenylmethylsulfonyl fluoride per ml.

Magnetosome purification. Approximately 10^{12} cells suspended in 30 ml of buffer A were disrupted by three passes through a French pressure cell at 18,000 lb/in^2. DNase (50 μg/ml), RNase (100 μg/ml), and $MgCl_2$ (10 μM) were added to the disrupted cells and incubated for 60 min at 23°C. Disrupted cells in a centrifuge tube were placed in the gap of a large (2 kG) radar magnet. The black magnetic fraction accumulated within 10 min at the sides of the tube nearest the magnet. The nonmagnetic fluid fraction was removed by aspiration, and the magnetic phase was suspended in 100 times its volume of buffer A. This procedure was repeated at least 10 times. The partially purified magnetosome fraction was suspended in 100 times its volume of buffer A containing 1 M NaCl. The salt was added to remove adventitious electrostatically associated proteins. Purified magnetosomes were washed at least 10 more times with buffer A.

Fractionation of nonmagnetic subcellular components. The nonmagnetic cell fraction was separated into outer membrane, inner membrane, and soluble fractions by methods described by Schnaitman (24). Nonmagnetic cellular debris (approximately 30 ml obtained from 10^{12} cells) was centrifuged at 500 × *g* for 15 min at 4°C to remove unbroken cells. The supernatant fluid was centrifuged (200,000 × *g*; 1 h; 4°C) to remove membranes, and the supernatant fluid from this high-speed centrifugation, considered to contain soluble proteins, was stored on ice. The brown pellet, containing outer and inner membranes, was suspended in 30 ml of buffer A containing 2% (vol/vol) Triton X-100 and 10 mM $MgCl_2$. The solubilized cytoplasmic membrane proteins were precipitated with cold 95% ethanol overnight at 0°C and collected by centrifugation (500 × *g*; 15 min; 4°C). Fractionation was evaluated by assaying specific activity of succinic dehydrogenase, an inner membrane enzyme (6), and by measuring the quantity of 2-keto-3-deoxyoctonate, a constituent of outer membrane lipopolysaccharide (20).

Freeze-etching. Cells of *A. magnetotacticum* MS-1 and the nonmagnetic mutant strain NM-1A were flash frozen in Freon 22 kept at liquid nitrogen temperature. Frozen preparations were fractured and etched for 10 s, and platinum-carbon replicas were made at −100°C in a Balzers BA 360M freeze-etching apparatus. Magnetically purified magnetosomes were similarly prepared. Replicas were examined at

* Corresponding author.

60 kV with a Philips EM300 or EM400 electron microscope equipped with a goniometer stage under standard operating conditions.

Thin sections. Cells grown with or without 20 μM iron were fixed for 1 h with glutaraldehyde (5% [vol/vol]), followed by washing and secondary fixation for 30 min with osmium tetroxide (1% [vol/vol]) in 50 mM cacodylate buffer (pH 6.8) containing 10 mM $MgCl_2$. Samples were dehydrated in ethanol, followed by propylene oxide, and embedded in Epon 812 or Epon 812-Araldite. Thin sections obtained with an LKB 8800 Ultratome III ultramicrotome were stained with 5% uranyl acetate and 0.4% lead citrate (22) and viewed with either a Hitachi H600 or a Philips EM400 STEM at 80 kV in the transmission electron microscopy mode.

Lipid analysis. Lipids were extracted from purified magnetosomes with chloroform-methanol as described by Bligh and Dyer (5) and purified by the Sephadex bead (Pharmacia Fine Chemicals, Piscataway, N.J.) method of Wurthier (29). The purified total lipids were separated into three fractions by an acid-treated Florisil (Sigma Chemical Co., St. Louis, Mo.) column (9). The fractions were expected to contain (i) neutral lipids and free fatty acids, (ii) glycolipids, sulfolipids, and possibly phosphatides, and (iii) phospholipids. Each lipid fraction was dried and weighed.

The dry phospholipid fraction was dissolved in 0.3 ml of chloroform-methanol (1:1 [vol/vol]), spotted onto a glass thin-layer chromatography plate [20 by 20 cm] of silica gel, and chromatographed in chloroform-methanol-water (65:25:4 [vol/vol]). Subsequently, the plate was air dried, rotated 90°, and chromatographed in the second dimension with chloroform–methanol–7 N ammonium hydroxide (60:35:5 [vol/vol]). The developed plate was completely air dried. Lipids were stained with iodine vapors. Each spot was scraped from the thin-layer chromatography plate and transferred to a Pasteur pipette plugged with glass wool. Phospholipids were eluted from the pipettes with 2 ml of chloroform-methanol (1:1 [vol/vol]), followed by 2 ml of absolute methanol. Each sample was collected in a 5-ml glass ampoule and evaporated to dryness under a stream of nitrogen. The residues were each dissolved in 2 ml of 1 N HCl and, after sealing of the ampoule, heated to 100°C for 4 h. Cooled ampoules were opened, and 2 ml of redistilled hexane was added. The mixture was vigorously shaken and allowed to separate, and the aqueous phase was removed and lyophilized. The residues were dissolved in 0.1 ml of distilled water and spotted onto Whatman no. 1 chromatography paper. The chromatogram was developed with redistilled phenol-absolute ethanol-glacial acetic acid (50:5:6 [vol/vol]), air dried, and sprayed with ninhydrin reagent, which stains serine and ethanolamine. A duplicate chromatogram was sprayed with Dragendorf reagent (9), which stains choline and dimethylethanolamine. The color and R_f value of each unknown sample were compared to those of lipid standards.

Gel electrophoresis. The protein concentration of each subcellular fraction was determined by the method of Lowry et al. (13). Magnetite, liberated from organic material during the Lowry assay, was removed by centrifugation before spectrophotometric analysis at A_{760}. Proteins (5 μg) from each subcellular fraction were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis through a 4% stacking gel and a 12% separating gel as described by Laemmli (10).

Proteins from partially purified magnetosomes and nonmagnetic fractions from cells of strain MS-1 were separated by two-dimensional gel electrophoresis. Samples (30 μg of protein each) were separated in a pH gradient ranging from 3.5 to 10.0 with tube gels as described by O'Farrell (19). At 16 h, the constant voltage (400 V) was increased to 800 V for an additional hour. Each tube gel was fixed with 1% agarose to the top of a sodium dodecyl sulfate-polyacrylamide gel (10 to 20% linear gradient of acrylamide). Proteins were separated in the second dimension of constant current (15 mA) for 9 h. The gels were stained with silver as described by Oakley et al. (18).

RESULTS

Magnetosome purification. Magnetosomes within *A. magnetotacticum* cells were always arranged in a linear array in the manner described by Balkwill et al. (1). They appeared to be enveloped and were separated from one another by a distance of about 9.0 nm. The interparticle spacing decreased to about 6.8 nm in crude preparations of magnetosomes, although particles remained attached end to end and were still enveloped (Fig. 1A). After NaCl treatment and extensive washing, magnetosomes appeared free of contaminating cellular components. However, each particle remained enveloped and separated from adjacent particles by a distance of 5.0 nm (Fig. 1B). Purified magnetosomes did not exhibit succinic dehydrogenase activity or contain 2-keto-3-deoxyoctonic acid. Sodium dodecyl sulfate detergent or Triton X-100 treatment, which dissolves lipid bilayers, removed the enveloping material, destroyed the linear arrangement of the electron-dense particles, and allowed them to clump with virtually no interparticle spacing (Fig. 1C).

Freeze-etching. In frozen and etched preparations of magnetic cells of strain MS-1 (Fig. 2A), intact magnetosomes appeared as convex protrusions (M⌣M). Cup-shaped depressions with raised rims (M⌣M) were interpreted to be regions through which the fracture plane had passed with removal of magnetite cores. The raised edges making up the rim were attributed to a magnetosome envelope differing in composition and structure from adjacent cytoplasm. A number of representative fracture surfaces associated with magnetosomes within strain MS-1 cells are shown in Fig. 2B. The results are those expected if a lipid bilayer were present around each magnetite crystal. Some fractures appeared to expose the external surfaces of intact magnetosomes (M⌢M). In other cases, the magnetite crystal appeared to have been extracted (as revealed by characteristic raised rims), and the internal surface of the magnetosome-enveloping layer (M⌣M) was evident. Occasionally, the fracture appeared to have penetrated the magnetosome envelope without removal of the mineral core, thereby exposing a surface which was either the magnetite core or the external face of the internal leaflet of the magnetosome envelope (M⌢MF). The latter would be possible only if the boundary were a lipid bilayer which fractured internally along its hydrophobic region.

Frozen and etched magnetosomes, isolated from cells, displayed these same structural features. When the alignment of magnetosomes to the fracture angle was correct, the particles appeared to be arranged in linear arrays (Fig. 3). Replicas of magnetosomes treated with detergent to remove enveloping layers (data not shown) did not exhibit fractures characteristic of intact magnetosomes.

Frozen and etched cells of a nonmagnetic mutant strain lacked features associated with magnetite cores or associated enveloping layers (Fig. 4), although fracture surfaces associated with the inner and outer membranes appeared similar to those of other gram-negative bacteria. Fracture surfaces associated with external wall layers, such as capsules or surface arrays, were not observed with either the magnetic or the nonmagnetic strain.

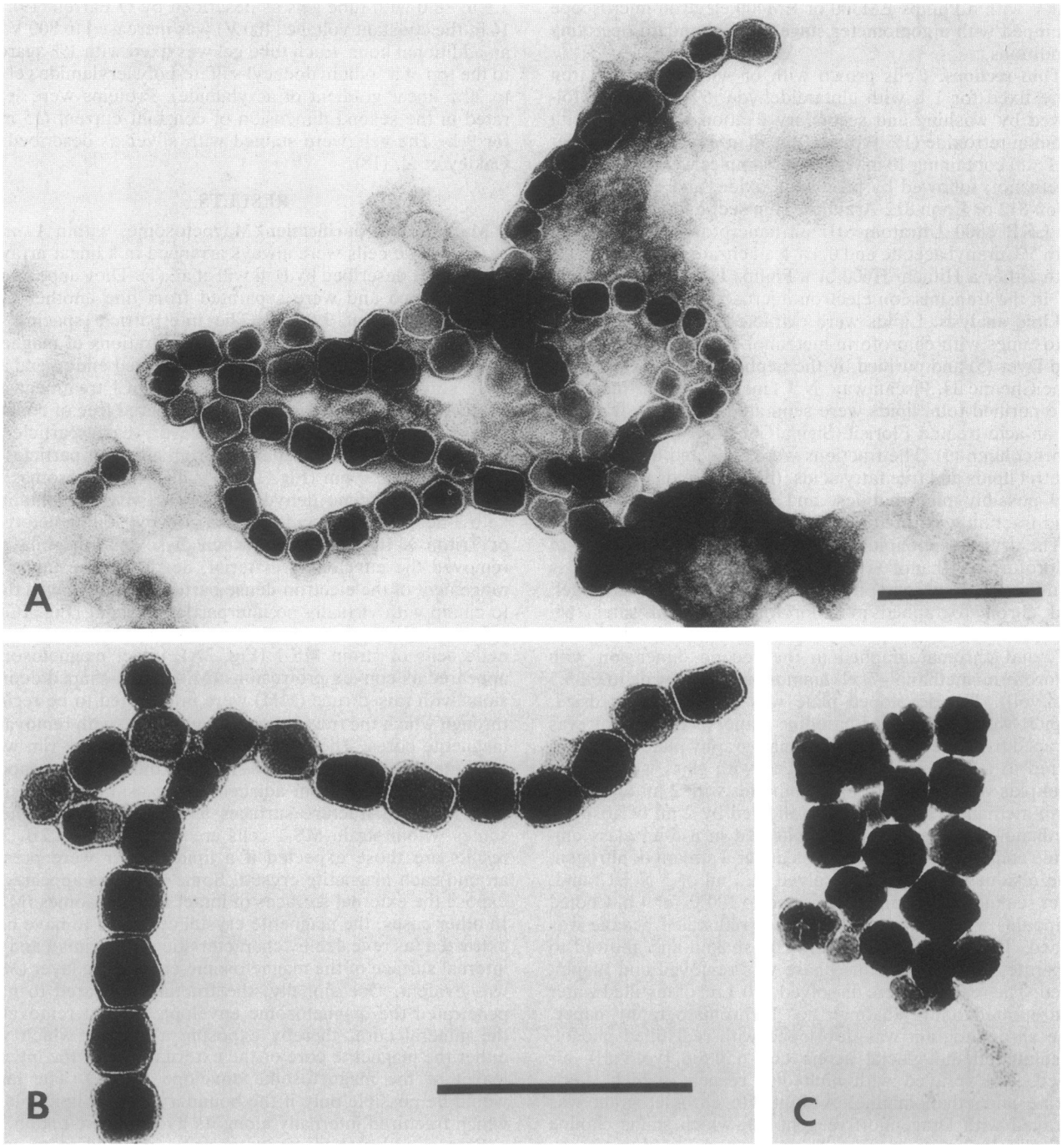

FIG. 1. Electron microscopic evaluation of magnetosome purity. (A) Magnetosomes liberated from cells after three passages through a French pressure cell. Particles, separated by a distance of 6.8 nm, remain in chains. Note the contaminating cellular debris. (B) Purified magnetosomes after treatment with 1 M NaCl and extensive washing. Interparticle spacing has decreased to 5.0 nm, yet magnetosomes remain in chains. Note the covering around each particle and the lack of contaminating cellular debris. (C) Magnetosomes after treatment with 10% sodium dodecyl sulfate. Enveloping material has been removed, and particles are randomly oriented. Bar, 250 nm.

Thin sections. Magnetosomes within cells cultured in medium containing 20 μM iron (Fig. 5) appeared in thin sections as electron-dense crystalline iron cores, each enveloped by a 1.7-nm-thick, electron-transparent layer and a 2.0-nm electron-dense layer. These results were comparable to those of Balkwill et al. (1). Stereo views of our thin sections (not shown) offered additional evidence of the bilayer nature of this envelope and suggested that it was not merely an electron phase artifact.

Magnetic cells cultured with no added iron contained some typical magnetosomes (Fig. 6A). In addition, however, numerous 40-nm-diameter membranous vesicles were present. These vesicles lacked electron-dense cores and were adjacent to one another along the long axis of the cell

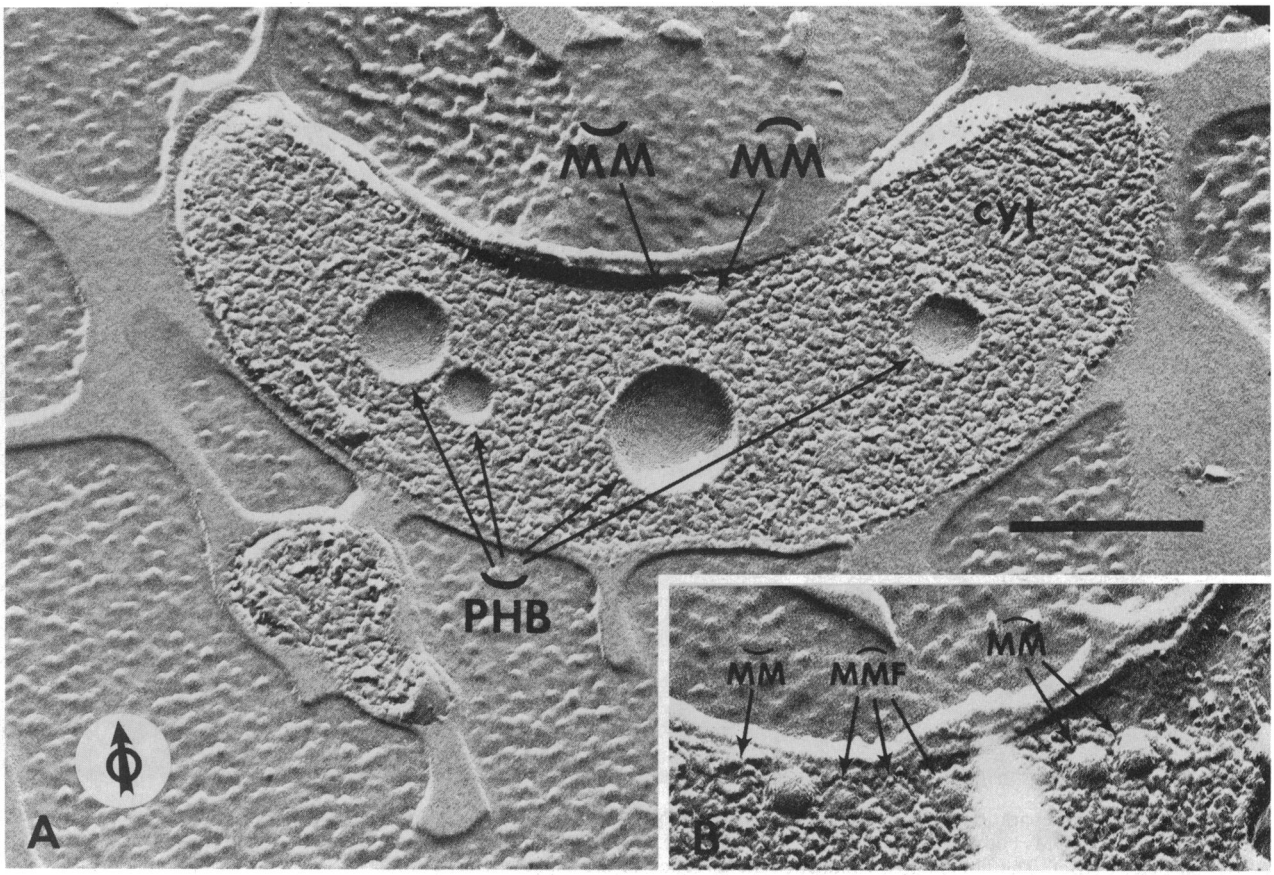

FIG. 2. Freeze-etch preparations of magnetic cells of strain MS-1. (A) An intact magnetosome appears convex, with the magnetosome membrane surface ($\widehat{MM}$) exposed, whereas a magnetosome from which the iron core has been removed by the fracture appears concave, revealing the inside of the membrane ($\overset{\frown}{MM}$). Poly-β-hydroxybutyrate, PHB; cytoplasm, cyt. (B) The fracture has penetrated the magnetosome membrane and exposed what appears as either the face of the magnetite particles or the convex fracture of the inner leaflet of the magnetosome membrane ($M\widehat{MF}$). The circled arrow indicates the direction of the shadow. Bar, 250 nm.

in the position normally occupied by intact magnetosomes within cells cultured with iron. Each "empty" membrane vesicle consisted of two 1.7-nm-thick, electron-dense layers separated by a 2.2-nm-thick electron-transparent layer as characteristic of a lipid bilayer unit membrane (23). Sometimes these vesicles were not filled with crystalline magnetite. Instead (as determined from energy-dispersive X-ray analysis and selected-area electron diffraction), they contained amorphous iron (Fig. 6B), which was presumably a derivative of polyferric hydroxide.

Lipid analysis. Magnetically separated and washed magnetosomes from 25 g of wet-packed cells yielded 25 mg of purified lipids and 150 mg of purified magnetite. Lipids associated with this magnetosome fraction included (i) neutral lipids and free fatty acids making up 8% of total lipids by weight, (ii) a fraction expected to contain glycolipids, sulfolipids, and phosphatides making up 30% of total lipids by weight, and (iii) phospholipids making up 62% of total lipids by weight. The neutral and free fatty acids were not identified, nor were the components of the glycolipid fraction. The phospholipids included phosphatidylethanolamine and phosphatidylserine, as determined by thin-layer chromatography.

Gel electrophoresis. Proteins of the cell outer and inner membranes and of the soluble cell fraction were compared with those associated with the purified magnetosome fraction (Fig. 7). The outer membrane protein profile of this

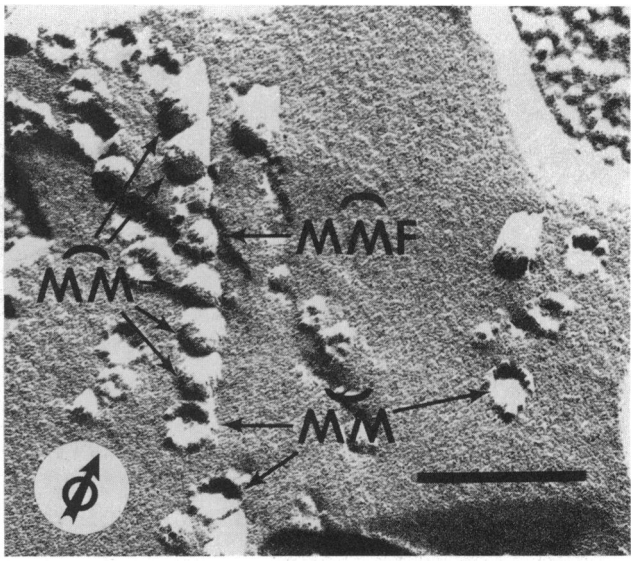

FIG. 3. Freeze-etch preparation of purified magnetosomes. Note the chain formation of characteristic fracture surfaces. Abbreviations: outer surface of the magnetosome membrane, $\widehat{MM}$; inside surface of the magnetosome membrane, $\overset{\frown}{MM}$; either the face of the magnetite particle or the convex fracture of the inner leaflet of the magnetosome membrane, $M\widehat{MF}$. The circled arrow indicates the direction of the shadow. Bar, 100 nm.

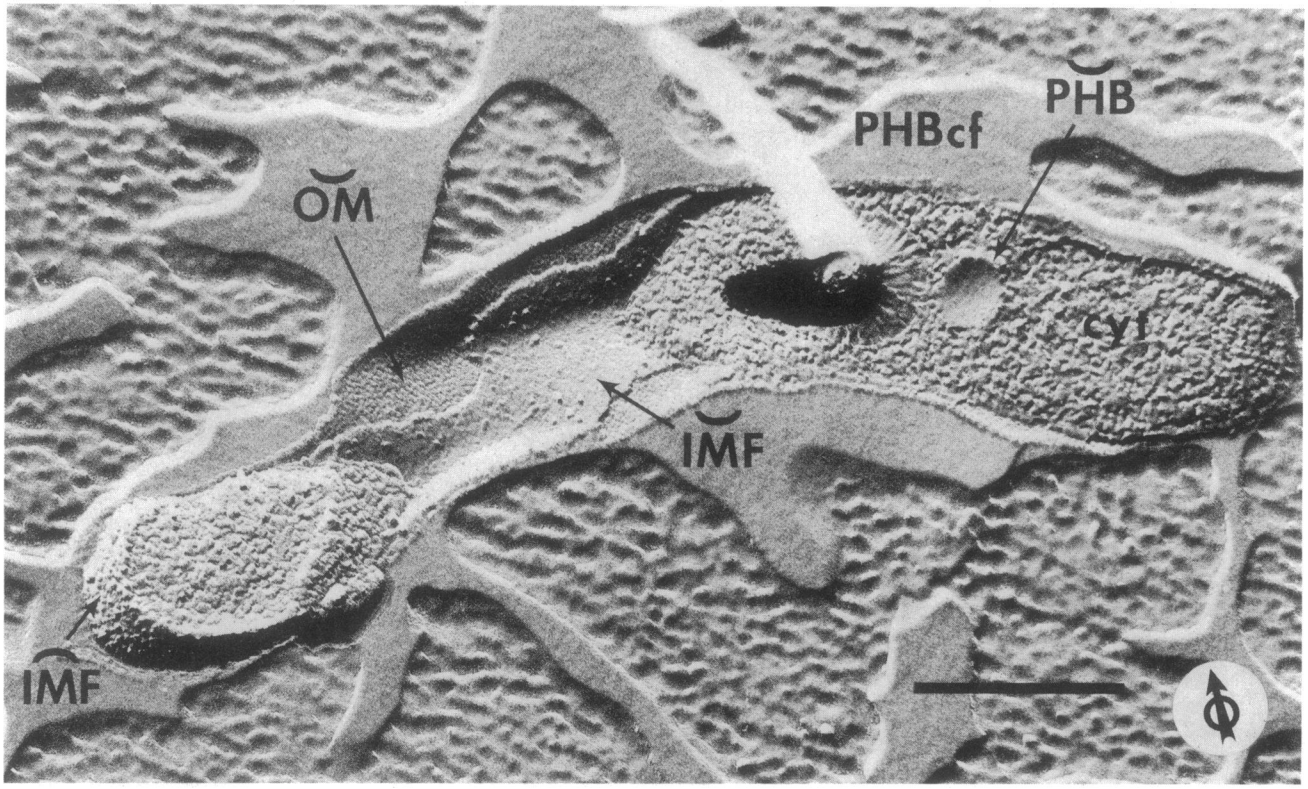

FIG. 4. Freeze-etch preparation of nonmagnetic mutant strain NM-1A. Note the lack of magnetic particles. Abbreviations: inner surface of the outer membrane, OM; convex fracture of the inner leaflet of the inner membrane, IMF; poly-β-hydroxybutyrate, PHB; cross-fracture of a PHB particle, PHBcf. The circled arrow indicates the direction of the shadow. Bar, 250 nm.

organism was similar to that described by Paoletti and Blakemore (21). Several proteins of identical molecular masses were shared between the magnetosome membrane and either the outer or inner membrane, but the intensities of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis bands differed, reflecting concentration differences. Two

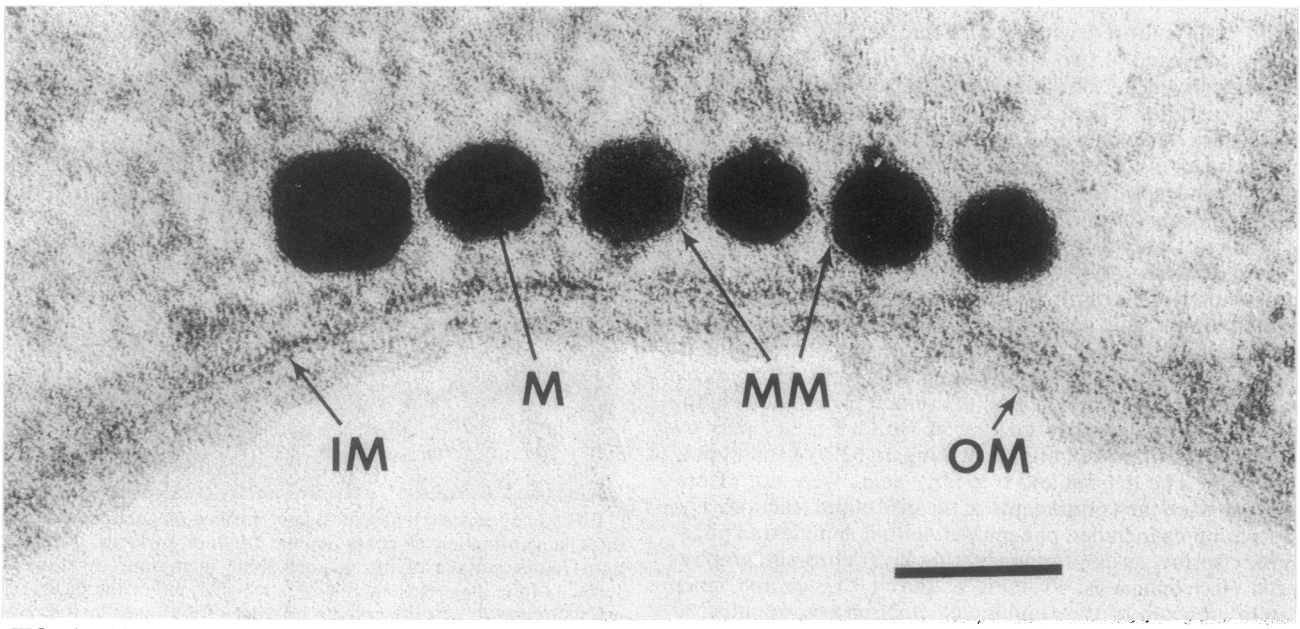

FIG. 5. Thin section of a magnetic cell. Abbreviations: outer membrane, OM; inner membrane, IM; magnetosome membrane, MM; magnetite, M. Bar, 100 nm.

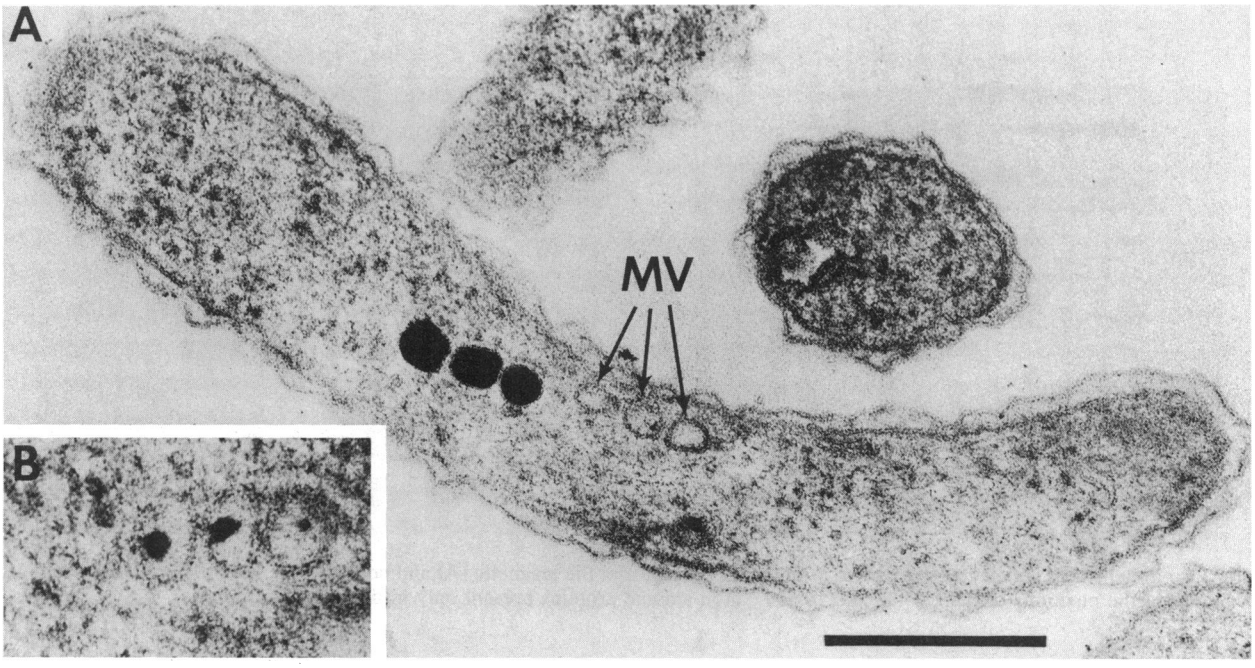

FIG. 6. Thin sections of cells cultured under iron limitation. (A) Note the trilaminate structure of the membranous vesicles (MV), which lie along the same axis as complete magnetosomes. Bar, 250 nm. (B) Note the small electron-dense deposits of amorphous iron within the membranous vesicles.

proteins, with apparent molecular masses of 15,000 and 33,000 daltons, appeared to be restricted to the magnetosome membrane fraction.

Characteristic magnetosome protein profiles obtained after two-dimensional gel electrophoresis are shown in Fig. 8. Two abundant anodically migrating proteins, with apparent molecular masses of 15,500 and 16,500 daltons, were present only in the magnetosome fraction. In concert, the one- and two-dimensional gels showed the magnetosome membrane to be distinct from the other outer and inner cell membranes.

DISCUSSION

Previous studies (1, 2) provided suggestive evidence for a lipid bilayer envelope surrounding the bacterial magnetosome. However, conclusive evidence has been lacking because of the difficulty in interpreting thin sections and the absence of data on purified magnetosomes. We extended previous cytological studies and used a magnetic separation method to recover intact magnetosomes from cellular debris. Our data, obtained by freeze-etching and thin sectioning of both cells and magnetically extracted magnetosomes, indicate the presence of a trilaminate membrane surrounding each magnetite core. This membranous envelope was absent from purified magnetosomes treated with detergent to remove lipids and proteins. Trilaminate membrane vesicles with dimensional and spatial characteristics of magnetosomes, but devoid of magnetite cores, were present in wild-type magnetic cells grown without iron. Amorphous iron was occasionally present in small quantity within these vesicles. Magnetosomes, vesicles with amorphous iron, or empty vesicles were not present within cells of the nonmagnetic mutant strain NM-1A. It was apparent, therefore, that these membranes were an integral part of magneto-

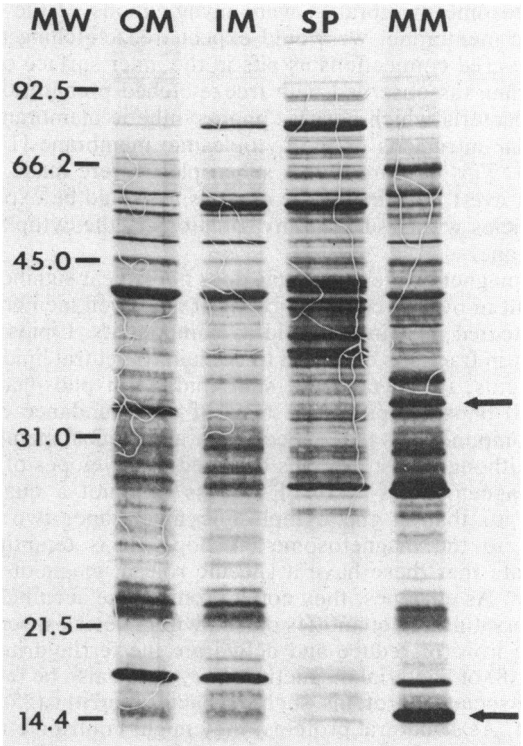

FIG. 7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cell fractions of strain MS-1. Lanes: Outer membrane proteins, OM; inner membrane proteins, IM; magnetosome membrane proteins, MM; soluble proteins, SP; molecular weight standards, MW. Arrows indicate the positions of the 15,000- and 33,000-dalton magnetosome membrane proteins.

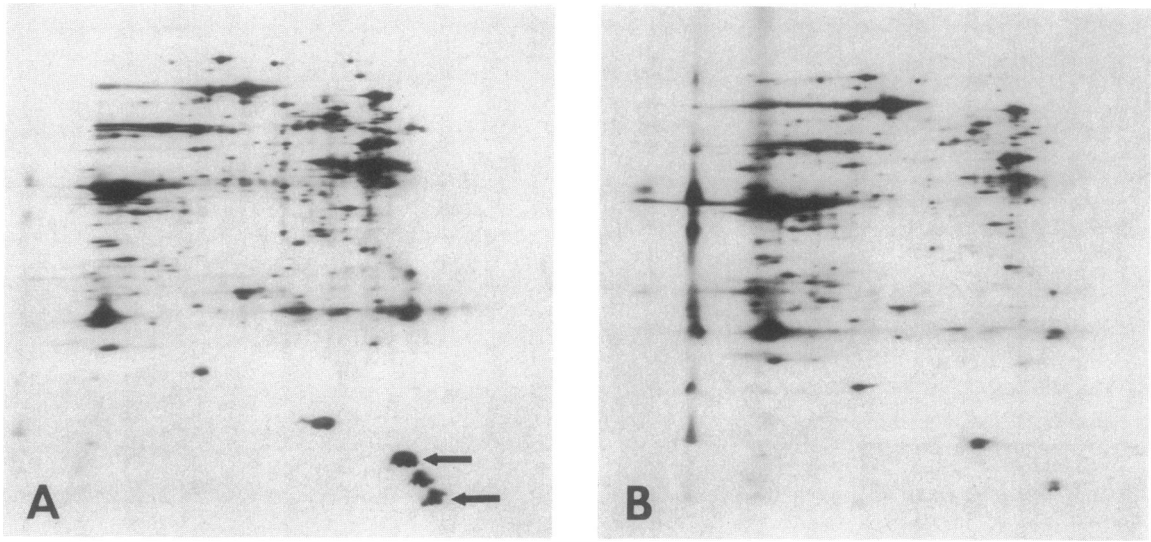

FIG. 8. Two-dimensional gel electrophoresis of proteins recovered from the magnetic (A) and nonmagnetic (B) fractions from strain MS-1. Arrows indicate the positions of the 15,500- and 16,500-dalton anionic proteins present only in the magnetic fraction.

somes, and we consider them to be magnetosome boundary membranes.

Magnetosome membranes do not appear to be contiguous with the cytoplasmic membrane. We have never observed connections between the two membranes in numerous thin sections, including stereo views, of magnetic cells. If the magnetosome membranes were invaginations of the cytoplasmic membrane, we would expect freeze-etching to reveal severed connections as pits in the inner surface of this membrane (as observed with freeze-etched preparations of cyanobacteria which possess photosynthetic membranes as vesicular intrusions of the cytoplasmic membrane [11]); it did not. Furthermore, when spheroplasts were made, they did not evert their magnetite crystals as would be expected of particles within surficial invaginations of the cytoplasmic membrane.

The magnetosome membrane does not appear significantly different in overall composition from other cell membranes. We detected protein and lipid as components. Lipids were present in fractions expected to contain (i) neutral lipids and fatty acids, (ii) glycolipids, sulfolipids, and phosphatides, and (iii) phospholipids. The ratio of the abundance of the lipid components is that expected for a biological membrane (23). Although most proteins detected in envelopes of purified magnetosomes were of a mass (but not a quantity) similar to that of the cytoplasmic membrane, two were unique to the magnetosome envelope. It is tempting to speculate that these have a specific role in magnetite production. As enzymes, they could promote the accumulation of supersaturating quantities of iron within vesicles, serve to oxidize iron, or reduce and dehydrate the ferrihydrite precursor (8) of bacterial magnetite. They could also be ferrihydrite-associated proteins such as bacterioferritin (25) apoprotein. As structural proteins, they might contribute to the compartmentalization deemed essential for "organic matrix-mediated" (12) biomineralization. The use of artificial membranous vesicles to study iron biomineralization, as recently initiated by Mann et al. (15), would undoubtedly be advanced by purification and inclusion of these magnetosome-specific proteins.

ACKNOWLEDGMENTS

We thank N. Blakemore, R. Frankel, T. Laue, and F. Rodgers for helpful advice and discussions. J. Cherry and B. McGrew provided help with electrophoresis.

A portion of this research was performed by the Natural Sciences and Engineering Council of Canada Guelph Regional STEM Facility in the Department of Microbiology, College of Biological Science, University of Guelph, and was supported by Natural Sciences and Engineering Council and Medical Research Council operating grants to T.J.B. Research conducted in the laboratory of R.P.B. was supported by Office of Naval Research contract N00014-85-K-0502 and National Science Foundation grant DMB-85-15540. Y.A.G. gratefully acknowledges partial support from University of New Hampshire Central University Research Fund award S396.

LITERATURE CITED

1. **Balkwill, D. L., D. Maratea, and R. P. Blakemore.** 1980. Ultrastructure of a magnetotactic spirillum. J. Bacteriol. **141:** 1399–1408.
2. **Blakemore, R. P.** 1975. Magnetotactic bacteria. Science **190:** 377–379.
3. **Blakemore, R. P.** 1982. Magnetotactic bacteria. Annu. Rev. Microbiol. **36:**217–238.
4. **Blakemore, R. P., D. Maratea, and R. S. Wolfe.** 1979. Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium. J. Bacteriol. **140:**720–729.
5. **Bligh, E. G., and W. S. Dyer.** 1951. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. **37:**911–917.
6. **Dobrogosz, W. J.** 1981. Enzymatic activity, p. 365–392. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
7. **Frankel, R. B., R. P. Blakemore, and R. S. Wolfe.** 1979. Magnetite in freshwater bacteria. Science **203:**1355–1356.
8. **Frankel, R. B., G. C. Papaefthymiou, R. P. Blakemore, and W. O'Brien.** 1983. Fe_3O_4 precipitation in magnetotactic bacteria. Biochim. Biophys. Acta **763:**147–159.
9. **Hanson, R. S., and J. A. Phillips.** 1981. Chemical composition, p. 328–364. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American

Society for Microbiology, Washington, D.C.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Lommen, M. A. J., and J. Takemoto.** 1978. Comparison, by freeze-fracture electron microscopy, of chromatophores, spheroplast-derived membrane vesicles, and whole cells of *Rhodopseudomonas sphaeroides*. J. Bacteriol. **136**:730–741.
12. **Lowenstam, H. A.** 1981. Minerals formed by organisms. Science **211**:1126–1131.
13. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
14. **Mann, S., R. B. Frankel, and R. P. Blakemore.** 1984. Structure, morphology and crystal growth of bacterial magnetite. Nature (London) **310**:405–407.
15. **Mann, S., J. P. Hannington, and R. J. P. Williams.** 1986. Phospholipid vesicles as a model system for biomineralization. Nature (London) **324**:565–567.
16. **Maratea, D., and R. P. Blakemore.** 1981. *Aquaspirillum magnetotacticum* sp. nov., a magnetic spirillum. Int. J. Syst. Bacteriol. **31**:452–455.
17. **Matsuda, T., J. Endo, N. Osakabe, and A. Tonomura.** 1983. Morphology and structure of biogenic magnetite particles. Nature (London) **302**:411–412.
18. **Oakley, B. R., D. R. Kisch, and N. R. Morris.** 1980. A simplified stain for detecting proteins in polyacrylamide gels. Anal. Biochem. **105**:361–363.
19. **O'Farrell, P. H.** 1975. High resolution two dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
20. **Osborn, M. J., and H. C. P. Wu.** 1980. Proteins of the outer membrane of gram-negative bacteria. Annu. Rev. Microbiol. **34**: 369–422.
21. **Paoletti, L. C., and R. P. Blakemore.** 1986. Hydroxamate production by *Aquaspirillum magnetotacticum*. J. Bacteriol. **167**:73–76.
22. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**:208–212.
23. **Rogers, H. J.** 1983. Bacterial cell structure, p. 28–53. American Society for Microbiology, Washington, D.C.
24. **Schnaitman, C.** 1981. Cell fractionation, p. 52–61. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
25. **Stiefel, E. I., and G. D. Watt.** 1979. *Azotobacter* cytochrome $b_{557.5}$ is a bacterioferritin. Nature (London) **279**:81–83.
26. **Stookey, L. L.** 1970. Ferrozine—a new spectrophotometric reagent for iron. Anal. Chem. **42**:779–781.
27. **Torres De Araujo, F. F., M. A. Pires, R. B. Frankel, and C. E. M. Bicudo.** 1986. Magnetite and magnetotaxis in algae. Biophys. J. **50**:375–378.
28. **Towe, K. M., and T. T. Moench.** 1981. Electron-optical characterization of bacterial magnetite. Earth Planet. Sci. Lett. **52**:213–220.
29. **Wurthier, R. E.** 1966. Purification of lipids from nonlipid contaminants on Sephadex bead columns. J. Lipid Res. **7**:558–561.