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Cryo-electron tomography of the magnetotactic vibrio *Magnetovibrio blakemorei*: Insights into the biomineralization of prismatic magnetosomes

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ABSTRACT

We examined the structure and biomineralization of prismatic magnetosomes in the magnetotactic marine vibrio *Magnetovibrio blakemorei* strain MV-1 and a non-magnetotactic mutant derived from it, using a combination of cryo-electron tomography and freeze-fracture. The vesicles enveloping the *Magnetovibrio* magnetosomes were elongated and detached from the cell membrane. Magnetosome crystal formation appeared to be initiated at a nucleation site on the membrane inner surface. Interestingly, while scattered filaments were observed in the surrounding cytoplasm, their association with the magnetosome chains could not be unequivocally established. Our data suggest fundamental differences between prismatic and octahedral magnetosomes in their mechanisms of nucleation and crystal growth as well as in their structural relationships with the cytoplasm and plasma membrane.

1. Introduction

Magnetotactic bacteria comprise a diverse group of motile, Gram-negative prokaryotes capable of producing intracellular, membrane-bounded, nano-sized magnetic crystals called magnetosomes (Bazylinski and Frankel, 2004). Each magnetosome consists of a magnetite (Fe_3O_4) or greigite (Fe_3S_4) crystal enveloped by a phospholipid bilayer membrane (Gorby et al., 1988). Specific proteins are expressed by the cell in the magnetosome membrane and modulate the biomineralization of the magnetic crystals within the magnetosome membrane vesicle (Komeili, 2007). As a result of the controlled biomineralization process, magnetosomes contain magnetite and greigite crystals with different morphologies that are dependent on the magnetotactic bacterial species (Bazylinski and Frankel, 2004). For magnetite magnetosomes, crystal habits include cuboctahedral, elongated prismatic and elongated-anisotropic (bullet-shaped crystals). Magnetite magnetosome formation in the *Alphaproteobacteria* has been extensively characterized in the freshwater magnetotactic spirilla belonging to the genus *Magnetospirillum* (Greene and Komeili, 2012). In *Magnetospirillum gryphiswaldense* MSR-1 magnetosomes consist of cuboctahedral magnetite crystals that are enveloped individually by vesicles of the magnetosome membrane associated with a set of specific proteins (Gorby et al., 1988; Katzmann et al., 2010; Schüler, 2004; Scheffel et al., 2006). In *Magnetospirillum magneticum* AMB-1 the magnetosome membrane originates by invagination from the cytoplasmic membrane and remains attached to the cell membrane (Greene and Komeili, 2012; Komeili et al., 2006).

In the genomes of many magnetotactic bacteria, magnetosome genes are located in clusters (Komeili, 2007). These genes encode for proteins that are responsible for controlling the size and morphology of magnetite crystals in magnetotactic bacteria, as well as magnetosome chain organization (Schüler, 2004). In the genus *Magnetospirillum*, these genes comprise the *mamAB*, *mamGFDC*, *mamXY* and *mms6* operons (Jogler and Schüler, 2009) that reside in a genomic region known as the magnetosome island (MAI) (Schüler, 2004). Comparative genomic analysis shows that the gene content and organization in the MAI differ among cultured magnetite-producing magnetotactic bacteria. These differences are thought to be responsible for diversity of magnetosome crystal morphology, size and magnetosome organization within the cell (Jogler et al., 2009a). In uncultured magnetotactic bacteria, *mam* genes have also been detected in both magnetite-producing

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magnetotactic bacteria belonging to the *Alphaproteobacteria* (Jogler et al., 2009b) and the *Nitrospirae* phylum (Jogler et al. 2011) as well as in greigite-producing magnetotactic bacteria belonging to *Deltaproteobacteria* (Abreu et al., 2011; Lefèvre et al., 2011) suggesting a monophyletic origin for the trait of magnetotaxis.

In freshwater magnetotactic spirilla, the magnetosome vesicles enveloping the magnetite crystals are distributed along a filamentous structural element, formed by the protein MamK (Komeili et al., 2006). MamK, encoded by the *mamK* gene of the *mamAB* cluster in the MAI of several magnetotactic bacteria, is a homolog of the bacterial actin-like protein involved in the determination of cell shape called MreB (Komeili et al., 2006; Jogler et al., 2009a,b). The magnetosome chain is organized by a filamentous network which runs parallel to the cell membrane and extends to the cell poles (Komeili et al., 2006; Scheffel et al., 2006; Pradel et al., 2006). This filamentous network is composed of MamK filaments of about 200–250 nm in length (Komeili, 2007). In *Magnetospirillum magneticum* strain AMB-1, the MamK assemblage is highly dynamic and the protein nucleates at multiple sites resulting in the formation of a new filament or the association with a preexistent one (Pradel et al., 2006). More recently, Katzmann et al. (2011) showed that the function of the magnetosome cytoskeleton elements is also involved in positioning and segregation of the magnetosome chain. Using cryoelectron tomography and analysis of mutant cells, they also demonstrate that septation in *Magnetospirillum gryphiswaldense* MSR-1 proceeds asymmetrically from one lateral edge of the cell and MamK filaments probably mediate the recruitment of magnetosome chains to the midcell before cell division. Magnetosomes are also organized along a cytoskeletal filamentous structure in the uncultured *Candidatus Magnetobacterium bavaricum* although a *mamK* gene was not found during partial genome sequencing of this organism (Jogler et al., 2011). The filamentous structure observed in this magnetotactic bacterium might be formed from MamK or a MamK-like protein encoded for a gene elsewhere in the genome.

MamJ, another protein that seems to be essential for magnetosome chain organization in *Magnetospirillum* species (Scheffel et al., 2006), is encoded by the *mamJ* gene also present in the *mamAB* cluster (Grünberg et al. 2001). Transmission electron microscopy of *Magnetospirillum gryphiswaldense* mutants lacking a functional *mamJ* gene showed that mature magnetosomes were arranged in clusters within the cell, whereas empty and partially filled vesicles containing immature magnetosomes were scattered throughout the cytoplasm. The filamentous structure, consisting of MamK filaments, was morphologically identical in mutant and wild-type cells. The phenotype generated in the absence of a functional MamJ protein suggests its function involves linking magnetosomes and magnetosome vesicles to the MamK filamentous structure (Scheffel et al., 2006). In *Magnetospirillum gryphiswaldense* MSR-1, it has been recently demonstrated that MamJ and protein LimJ regulate the dynamics behavior of MamK filaments *in vivo* (Draper et al., 2011).

The precise determination of the position of individual magnetosomes and of magnetosome chains and their relation to cytoskeleton elements is pivotal for the understanding of the complex structure underlying magnetosome biomineralization and magnetosome chain formation, as well as magnetotactic behavior of the organism. Cryo-electron tomography (cryo-ET) is the most promising methodological approach (Milne and Subramaniam, 2009) that has revealed the three-dimensional (3D) ultrastructure of the intact magnetotactic bacterial cell in a near-native, frozen hydrated state (Komeili et al., 2006). Most cryo-ET studies on magnetotactic bacteria have focused on *Magnetospirillum* species (Draper et al., 2011; Katzmann et al., 2010, 2011; Komeili et al., 2006; Scheffel et al., 2006.), which biomineralize cuboctahedral crystals of magnetite, and thus little is known regarding the cellular biology of magnetotactic species that produce elongated prismatic magnetosomes.

Here, we applied cryo-ET to characterize cytoplasmic structures of *Magnetovibrio blakemorei* (Bazylinski et al., 2012), a marine magnetotactic vibrio which produces elongated prismatic crystals of magnetite. In particular, we determined physical characteristics of magnetosome chain positioning and magnetosome nucleation, as well as established the presence of cytoskeleton elements in this organism.

2. Materials and methods

2.1. Cell culture

Cells of *Magnetovibrio blakemorei* strain MV-1 were grown at 28 °C in heterotrophic medium in sealed flasks purged first with nitrogen and then nitrous oxide (Dean and Bazylinski, 1999). A spontaneous non-magnetotactic mutant of *Magnetovibrio blakemorei* (strain NMV-1), unable to produce magnetosomes (Dubbels et al., 2004), was grown similarly and also examined in this study.

2.2. Electron microscopy

For cryo-ET, frozen vitreous cells were prepared by pipetting drops of cell culture onto Quantifoil 300-mesh copper EM grids. The grids were then blotted with filter paper and immediately frozen in liquid propane at −175 °C using a plunge freezing machine (Leica model KF80, Allendale, NJ). Frozen specimen grids were cryo-transferred into the microscope using a Gatan 626 cryoholder and cryo transfer stage (Pleasanton, CA). Electron tomography was performed using a Tecnai TF30 transmission electron microscope (FEI Company, Hillsboro, OR) equipped with a Shottky field emission gun and operated at 300 kV. Tomographic tilt series were acquired by tilting the samples from at least -54° to $+54^{\circ}$ at 2° intervals. The defocus value at each image was −10 μm. The total electron dose was 10,000 e nm^{-2}. Images at each angular increment were recorded by a 2048 × 2048 pixel Ultrascan cooled CCD detector (Gatan) after zero-loss filtering by a Tridiem post-column imaging filter (Gatan). Tomograms were reconstructed using the IMOD software package (University of Colorado, Boulder, CO) and surface-rendered in Amira (Visage Imaging, Germany). Most tomograms were post-processed in IMOD by anisotropic diffusion filtering to improve signal-to-noise ratio.

For electron energy-loss spectroscopy, cells were freeze-dried inside the microscope and analyzed with the Tridiem post-column imaging filter and Ultrascan CCD detector. Electron energy loss spectra were obtained with dispersions of 0.2 and 0.3 eV/channel using a 40 μm objective aperture. Data acquired were analyzed with the Digital Micrograph software package.

2.3. Freeze fracture

For freeze-fracture experiments, cells were centrifuged at 4 °C and washed in phosphate buffered saline and directly placed on gelatin cushions without chemical fixation. The samples were immediately frozen by impact against a liquid-nitrogen cooled sapphire block of a Life Cell CF-100 quick-freezing machine (Research and Manufacturing Co, Tucson, AR). Frozen specimens were then transferred to a Balzers freeze-fracture machine (Balzers/Leica, Allendale, NJ) and fractured at −150 °C. After fracturing, the samples were shadowed with platinum at 45 °C and carbon at 90 °C. The organic material was cleaned from the replicas by hypochlorite bleach for 1 h. Replicas were then washed in distilled water, collected on 400 mesh nickel grids, and examined with a