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Inter-phylum structural conservation of the magnetosome-associated TPR-containing protein, MamA

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ABSTRACT

Magnetotactic bacteria enclose the magnetosome, a unique prokaryotic sub-cellular organelle that allows the biomineralization of magnetic nano-crystals. Membrane-coated magnetosomes are arranged into a linear chain that permits magnetotactic bacteria to navigate geomagnetic fields. Magnetosome assembly and biomineralization are controlled by conserved magnetosome-associated proteins, including MamA, a tetra-trico-peptide repeat (TPR)-containing protein that was shown to coat the magnetosome membrane. In this study, two MamA structures from *Candidatus Magnetobacterium bavaricum* (Mbav) were determined via X-ray crystallography. These structures confirm that Mbav MamA folds as a sequential TPR protein and shares a high degree of structural similarity with homologous MamA proteins from *Magnetospirillum* species. Furthermore, the two TPR-containing domains of MamA are separated by an interphylum-conserved region containing a flexible hinge that is involved in ligand binding and recognition. Finally, substantial differences were found in the local stabilization of the MamA N-terminal domain as a result of the loss of an evolutionary conserved salt bridge.

1. Introduction

Magnetotactic bacteria (MTB) comprise a group of aquatic Gram-negative micro-organisms that contain a unique prokaryotic sub-cellular organelle termed the magnetosome (Komeili, 2012). The magnetosome allows for the biomineralization of iron oxides or iron sulfides, namely magnetite or greigite, respectively. Each magnetic nanocrystal is enclosed by a phospholipid-based membrane derived from the bacterial inner membrane. Several magnetosomes are arranged into linear chains along actin-like filaments, promoting the formation of a single and stable magnetic dipole. This magnetic dipole allows the MTB to navigate geomagnetic fields more efficiently while seeking the growth-favoring conditions of the micro-oxic transition zone (Faivre and Schüler, 2008; Bazylinski and Frankel, 2004).

Magnetotatctic bacteria are a diverse phylogenetic group, with representatives being found in α, γ, and δ-proteobacteria subdivisions, as well as in the phylum, *Nitrospira* (Jogler et al., 2009). Recently, two studies uncovered new MTB species. The first such species is the δ-proteobacteria BW-1 that is able to biomineralize both greigite and magnetite, depending upon culture conditions (Sakaguchi et al., 1993; Kawaguchi et al., 1995; Lefèvre et al., 2011). The second novel species reported is the uncultivated *Candidatus Magnetobacterium bavaricum* (Mbav) strain, belonging to the *Nitrospira* (Jogler et al., 2010a,b). In comparison to other MTB that usually contain up to 50 magnetosomes per cell, Mbav, with its non-typical large size (3–10 μm) and distinct cell biology, can contain up to 1,000 magnetosomes in a single cell. Mbav contains bullet-shape magnetosomes that are aligned in multiple bundles of parallel chains. Although magnetosome size, number and shape vary dramatically between MTB species, it was shown that magnetosome formation and biomineralization processes are genetically controlled, highly conserved and governed by a unique set of genes which encode for a set of soluble and integral membrane proteins. Most magnetosome-associated genes are located in a single genomic island, implying that the trait of magnetotaxis was horizontally transferred between the different phyla and species (Nakazawa et al., 2009; Abreu et al., 2008; Jogler et al., 2009, 2010b; Lefèvre et al., 2011; Komeili, 2012). While the genomic island of the α-proteobacterial *Magnetospirillum* species

Abbreviations: Mbav, *Candidatus Magnetobacterium bavaricum*; TPR, tetra-trico-peptide repeat; MTB, magnetotactic bacteria; AMB-1, *Magnetospirillum magneticum*; MSR-1, *Magnetospirillum gryphiswaldense*; RMSD, root mean square deviation; NTD, N-terminal domain; CTD, C-terminal domain; 2MUC, two monomers unit cell (PDB code: 3VTX); 4MUC, four monomers unit cell (PDB code: 3VTY); H11, helix 11; P11, peptide 11 analogous to H11.

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contains the *mamAB*, *mamCD*, *mms6* and *mamXY* operons, the most essential genes, controlling magnetosomal membrane biogenesis, magnetosome alignment and iron transport, are located in the *mamAB* operon, common to all known MTB (Murat et al., 2010; Lohsse et al., 2011). In the BW-1 strain, two genomic islands were found, with one predicted to contain genes encoding for magnetite biomineralization and the other containing genes predicted to code for greigite formation (Lefèvre et al., 2011). A similar putative genomic island containing conserved magnetosome-related genes associated with the biomineralization of magnetite was recently discovered in Mbav (Jogler et al., 2010b).

One of the most abundant and conserved magnetosome-associated proteins is MamA (also known as Mms24 (Okamura et al., 2000) and Mam22 (Okuda and Fukumori, 2001; Okuda et al., 1996)). Deletion of *mamA* in *Magnetospirillum* species has no effect on membrane invagination, however, iron accumulation throughout the magnetosome chain is altered in such mutants, as most of the invaginations do not contain iron oxide crystals (Komeili et al., 2004). In these species, MamA was shown to have a dynamic sub-cellular localization pattern during bacterial growth (Komeili et al., 2004), localizing to the magnetosomal chain matrix and dissociating from this matrix upon treatment with alkaline buffer (pH 11.0) (Taoka et al., 2006; Yamamoto et al., 2010). Other studies revealed that MamA exists in homo-oligomeric complexes both *in vivo* and *in vitro* (Yamamoto et al., 2010; Zeytuni et al., 2011).

Structural and biochemical studies of MamA from *Magnetospirillum* species have shown that a 41-residue deletion mutant (MamAΔ41) folds into a structure containing five sequential tetra-trico-peptide repeat (TPR) motifs, with the additional putative TPR motif at the N-terminus (amino acids 1–40) being responsible for the cellular localization of the protein, as well as for the formation of MamA homo-oligomers (Zeytuni et al., 2011). TPR is a structural motif that contains a degenerate primary consensus sequence of 34 amino acids. Although these 34 amino acids define the TPR motif, there are no fully invariable positions but rather a preference for small and large amino acids in specific positions. A single TPR motif adopts a helix-turn-helix fold, while adjacent TPR units packed in parallel usually create an overall super-helix structure. This super-helix forms a pair of concave and convex curved surfaces which permit the binding of diverse ligands, usually via the concave TPR surface (D'Andrea and Regan, 2003; Zeytuni and Zarivach, 2012). TPR proteins or TPR domains within multi-domain proteins can be found in a wide range of organisms, where they promote protein–protein interactions and protein complex formation. Solved MamAΔ41 structures had revealed some unique structural features, in comparison to other TPR-containing proteins, including three protein–protein interactions sites at the protein concave and convex surfaces. The putative MamA TPR motif (residues 1–40) is presumed to bind at the concave surface. In addition, MamAΔ41 contains two distinct TPR domains that undergo conformational changes upon peptide binding (Zeytuni et al., 2011); other TPR-containing proteins do not exhibit such elasticity (Zeytuni and Zarivach, 2012).

In general, magnetosome formation is thought to be conserved across distinct phyla, as MTB all share a similar genomic island containing the same genes. While the genome of several MTB species from proteobacterial phyla have been sequenced (Matsunaga et al., 2005) the partial genome sequence of Mbav serves as the sole representative of an MTB from the *Nitrospira*. Given that MamA is one of the most conserved magnetosome-associated proteins (~23% amino acids sequence identity and ~38 similarity between MamA from AMB-1 or MSR-1 to Mbav), it represents a candidate of choice for comparative and comprehensive interphylal structural studies. In this article, we present two crystallographic structures of MamA from Mbav and discuss structural conservation and differences of MamA proteins from distantly-related MTB species.

2. Results

2.1. MamAΔ41 folds as a TPR-containing protein

To obtain structural and biochemical information, recombinant MamAΔ41Mbav was over-expressed in *Escherichia coli* cells. MamAΔ41Mbav was found to be soluble and stable as a monomer in solution, similar to MamAΔ41 from *M. magneticum* AMB-1 (Supplementary Fig. S1). Crystallization trials using a sitting drop vapor diffusion methodology resulted in the appearance of two crystal forms which diffracted to a resolution of 1.75 and 2.0 Å, respectively (see Table 1 for data collection and refinement statistics). Phase information for both crystal forms was obtained by the molecular replacement technique. After manual rounds of rebuilding and refinement, both crystals yielded high quality structures (Table 1) with almost full coverage of the protein sequence (Supplementary Table S1). Upon analyzing the crystal packing, we noticed that although lattice parameters indicated that the two crystal forms crystallized in a primitive orthorhombic space group, each crystal form displayed different cell dimensions and packing properties (Table 1). The asymmetric unit of the 4MUC (PDB code: 3VTY) crystal form contains four MamAΔ41Mbav monomers (chains A–D), whereas only two monomers are found in the 2MUC crystal form (PDB code: 3VTX) (chains A and B) (Fig 1A and B).

The overall structure of the MamAΔ41Mbav monomer contains 10 anti-parallel α-helices and turn motifs folded as five TPR motifs, namely TPR1 (H1 and H2), TPR2 (H3 and H4), TPR3 (H5 and H6), TPR4 (H7 and H8) and TPR5 (H9 and H10), similar to previously determined MamA structures (Fig. 1C). These TPR motifs give rise to a structure displaying concave and convex surfaces. An addi-

Table 1
Data collection and refinement statistics.

PDB code	3VTX	3VTY
Aberration	2MUC	4MUC
Protein	MamAΔ41	MamAΔ41
Data collection	ID14-4–ESRF	ID14-1–ESRF
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	77.457, 77.548, 77.963	51.701, 101.315, 139.553
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	1.745	2.003
*R*sym or *R*merge	6.5 (56.2)	9.6 (53.4)
$I/\sigma I$	39.33 (3.36)	21.33 (3.53)
Completeness (%)	98.3 (99.9)	99.7 (100)
Redundancy	5.3	10.4
Wavelength (Å)	0.939	0.933
Refinement		
Resolution (Å)	1.745	2.003
No. reflections	47365	49963
*R*work/*R*free	16.18/20.04	16.25/21.93
No. atoms		
Protein	2938	5619
Ligand/ion	12	6
Water	376	604
B-factors		
Protein	27.74	20.46
Ligand/ion	46.67	20.277
Water	39.21	28.17
R.m.s. deviations		
Bond lengths (Å)	0.0273	0.0244
Bond angles (°)	2.0236	1.7345

Values in parentheses are for the highest resolution shell. Data was collected at 100 K for all crystals.