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A protein-protein interaction in magnetosomes: TPR protein MamA interacts with an Mms6 protein

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ABSTRACT

Magnetosomes are membrane-enveloped bacterial organelles containing nano-sized magnetic particles, and function as a cellular magnetic sensor, which assist the cells to navigate and swim along the geomagnetic field. Localized with each magnetosome is a suite of proteins involved in the synthesis, maintenance and functionalization of the organelle, however the detailed molecular organization of the proteins in magnetosomes is unresolved. MamA is one of the most abundant magnetosome-associated proteins and is anchored to the magnetosome vesicles through protein-protein interactions, but the identity of the protein that interacts with MamA is undetermined. In this study, we found that MamA binds to a magnetosome membrane protein Mms6. Two different molecular masses of Mms6, 14.5-kDa and 6.0-kDa, were associated with the magnetosomes. Using affinity chromatography, we identified that the 14.5-kDa Mms6 interacts with MamA, and the interaction was further confirmed by pull-down, immunoprecipitation and size-exclusion chromatography assays. Prior to this, Mms6 was assumed to be strictly involved with biomineralizing magnetite; however, these results suggest that Mms6 has an additional responsibility, binding to MamA.

1. Introduction

In 1975, magnetotactic bacteria (MTB) were first discovered by Blakemore [1,2]. These bacteria contain unique organelles called magnetosomes that biomineralize magnetic minerals using specific proteins that are only associated with the organelle [3]. In 1996, the first magnetosome associated protein was isolated, sequenced and found to consist of tetratricopeptide repeat (TPR) motifs which are known to mediate protein-protein interactions [4]. Since 1996, this protein has been designated MamA (Mam22), and researchers in the field of biochemistry and genetics have studied it, and recently the structures of four different MamA proteins from four different MTB have been resolved [5–7]. However, even using cutting edge techniques, researchers have merely confirmed the idea that MamA interacts with other magnetosome-associated protein(s), but the function of MamA still remains enigmatic. We have made a major discovery towards identifying the binding partner of MamA, which gives a significant clue to its function.

MamA is conserved in all known MTB [8], and even though it is a soluble cytoplasmic protein [9], it localizes in the magnetosome matrix, a proteinaceous layer surrounding magnetosome vesicles of *Magnetospirillum* species [10,11]. The entire primary structure of MamA consists of five TPR motifs and one putative TPR motif [9]. These motifs consist of a helix-turn-helix fold, which has been known to promote protein-protein interactions [12]. Proteins with TPR motifs are important to cells which use them in a wide variety of ways such as protein transport, protein folding, transcription and splicing, and cell cycle control [13].

Two different functions for MamA have been proposed. Based on studies of a *mamA* deletion mutant, MamA appears to activate or prime preformed magnetosomes for biomineralization [14]. A different study used the atomic force microscopy (AFM) to observe chains of magnetosomes with and without MamA and proposed that MamA is anchored to the magnetosome membrane and may stabilize the magnetosome chain [11]. According to the MamA crystal structures, the five TPR motifs form a superhelix structure which has at least three putative protein binding sites, and one of the sites specifically binds to one of the magnetosome-associated proteins [5–7], However, the question remains as to which magnetosome-associated protein(s) interacts with MamA.

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In this study, we used MamA affinity chromatography to screen the proteins from the magnetosomes of *Magnetospirillum magneticum* AMB-1 which bind to MamA. We found that Mms6, a magnetosome membrane-bound protein, binds to MamA. We further confirmed this binding using immuno-precipitation, pull-down and size-exclusion chromatography experiments. In addition to this, we established that two different types of Mms6 exist in the magnetosome membrane, a 14.5-kDa and 6.0-kDa version. Until now, Mms6 was thought to be exclusively involved in biomineralization, however these new results imply an additional function of Mms6 within magnetosomes and provide a clue to answer the question of how MamA binds to magnetosomes in *M. magneticum* AMB-1.

2. Materials and methods

2.1. Microorganisms and cultures

Bacterial strains and plasmids are listed in Table S1. *M. magneticum* AMB-1 (ATCC 700,264) was cultured as described [15]. *Escherichia coli* strains were cultivated in LB broth [16] at 37 °C, unless specified otherwise. When necessary, the antibiotics kanamycin (20 μg/ml) or ampicillin (100 μg/ml) were added to the *E. coli* cultures.

2.2. Expression and purification of MamA and Mms6 proteins

The MamA expression vector was generated as previously described [9]. Primer sequences are shown in Table S2. For C-terminal His-tagged full-length Mms6 expression, the plasmid pET29b-mms6$^{1-133}$ was constructed by cloning the entire PCR-amplified *mms6* gene (*mms6*$^{1-133}$; accession number: AB096081) fragment into the *Nde*I/*Kpn*I sites of pET-29b (Merck-Millipore) using a primer set (mms6–1f and mms6–1r). The pET29b-mms6$^{1-133}$ was also used as the template to create the expression plasmid of Mms6$^{75-133}$-His. For protein expression, *E. coli* strain BL21(DE3) containing these recombinant plasmids were grown at 30 °C until an $A_{600\ nm}$ of ~0.6, and then induced by 1 mM (final concentration) of isopropyl-β-D-thiogalactopyranoside (IPTG) for 7 h. The cells were then harvested by centrifuging at 8000 × *g* for 15 min.

The purification of recombinant MamA in *E. coli* was performed by Ni-affinity chromatography as previously described [9]. For purifying the recombinant Mms6$^{1-133}$-His and Mms6$^{75-133}$-His, cells were suspended in 10 mM Tris–HCl (pH 8.0) and disrupted using sonication (80 W for 15 min). The lysate was centrifuged at 8000 × *g* for 15 min to remove the cell debris, and then the supernatant was ultra-centrifuged at 100,000 × *g* for 1 h to separate the membrane and soluble protein fractions. The membrane fraction was suspended in 10 mM Tris–HCl (pH 8.0) containing 2% CHAPS and 200 mM NaCl, and then incubated at 4 °C for 2 h to solubilize the membrane proteins. The solubilized fraction was harvested by ultracentrifugation (100,000 × *g* for 1 h) and the supernatant was subjected to a Ni-NTA resin (QIAGEN) column. The proteins bound to the column were eluted with 50 mM NaH_2PO_4 containing 250 mM imidazole, 0.2% CHAPS and 300 mM NaCl (pH 8.0). The eluted protein fraction was dialyzed against 10 mM Tris–HCl (pH 8.0) containing 0.2% CHAPS.

2.3. MamA-affinity chromatography

Preparation of His-MamA affinity resin was performed as previously described [11]. Bovine serum albumin (BSA) was also immobilized to the CNBr-activated Sepharose resin and used as a control column. Magnetosomes (770 mg wet weight) were purified from *M. magneticum* AMB-1 cells (125 *g* wet weight collected from 600 L of medium) by magnetic separation as previously described [10]. The purified magnetosomes were treated by an alkaline buffer, 0.1 M CAPS-NaOH (pH 11.0), to remove MamA specifically from the magnetosomes [10,11] (Fig. S1). The proteins associated with the MamA-eliminated magnetosomes were solubilized with 10 mM Tris–HCl (pH 8.0) containing 2% sucrose monocaprate at 4 °C for 16 h. This suspension was centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was dialyzed against the equilibration buffer (10 mM Tris–HCl (pH 8.0) containing 0.2% sucrose monocaprate), the protein solution (11 ml, 0.32 mg proteins/ml) was subjected to the His-MamA-column (1 × 10 cm^2) and BSA-column (1 × 10 cm^2) with a flow rate of 1 ml/h. After that, the columns were washed with 25 ml of equilibration buffer and the bound proteins were eluted with 0.1 M CAPS-NaOH buffer (pH 11.0) containing 0.2% sucrose monocaprate. The eluted protein fractions were concentrated approximately two hundred times by ultrafiltration. The concentrated samples were applied to SDS-PAGE, and the protein bands were analyzed by mass spectrometry and identified.

2.4. Physical and chemical measurements

SDS-PAGE was performed using the method of Laemmli [17] and tricine-SDS-PAGE was performed as previous described [18]. His-tagged protein bands were visualized using InVision His-Tag In-Gel Stain (Thermo Fisher Scientific). The protein concentration was determined using a BCA Protein Assay Kit (Thermo Fisher Scientific). The in-gel protein identification was performed as previously described using the 4800Plus MALDI-TOF/TOF Analyzer (Applied Bioscience, Carlsbad, CA) and the results were analyzed using Protein PILOT software [19].

2.5. Immunoblotting analyses

Anti-Mms6$^{1-133}$ polyclonal rabbit antibodies were raised against the purified recombinant Mms6$^{1-133}$-His. Immunoreactivity of anti-Mms6$^{1-133}$ and anti-MamA [10] antibodies was detected at dilutions of 1:50,000 for each. Goat anti-Rabbit IgG conjugated to horseradish peroxidase (GE Healthcare Bioscience) was diluted 1:10,000 using the Pierce Western Blotting Substrate Plus (Thermo Fisher Scientific). The chemifluoresence data were collected using a Luminescent Image Analyzer, LAS 3000 (Fujifilm) and the band intensities were quantified using Multi Gauge software v. 2.2 (Fujifilm). The protein weights of the 14.5-kDa Mms6 and 6.0-kDa Mms6 from the purified magnetosomes were calculated according to the relative intensities for equal weights of these two protein bands in the immunoblot. The relative intensities for the 14.5-kDa Mms6 (Mms6$^{1-133}$) and 6.0-kDa Mms6 (Mms6$^{75-133}$) protein bands were calculated from the immunoblotting profiles of the two purified proteins, 0.1 μg Mms6$^{1-133}$-His and 0.9 μg Mms6$^{75-133}$-His, against anti-Mms6$^{1-133}$ polyclonal antibodies (Fig. S2).

2.6. Immunoprecipitation assay

The immunoprecipitation assay was performed as described [20] with some modification. A 200 μl mixture containing 2 μM His-MamA and 1 μM Mms6$^{1-133}$-His was incubated at 28 °C for 1 h. After incubation, 2 μl of anti-Mms6$^{1-133}$-antibody, *anti*-MamA antibody or normal serum were added to the mixture and incubated for 1 h. A slurry of protein A-Sepharose resin (GE Healthcare Bioscience) was added, and the proteins that co-precipitated with the protein A-Sepharose resin were analyzed by SDS-PAGE.