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Two-Dimensional Analysis of Proteins Specific to the Bacterial Magnetic Particle Membrane from *Magnetospirillum* sp. AMB-1

Yoshiko Okamura, Haruko Takeyama, and Tadashi Matsunaga*

Department of Biotechnology,
Tokyo University of Agriculture and Technology,
2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan,
E-mail: tmatsuna@cc.tuat.ac.jp

Abstract

We report the identification of five proteins expressed specifically on the bacterial magnetic particle (BMP) membrane of *Magnetospirillum* sp. AMB-1. These proteins are major components of the BMP membrane. The molecular weights were determined to be 12.0, 16.0, 24.8, 35.6, and 66.2 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Of these five, the 16.0-kDa protein was the most abundant in the BMP membrane. Furthermore, the 16.0-kDa protein consisted of two components each of differing pI. The 35.6-kDa protein was the second most abundant protein of the five detected.

Index Entries: Magnetic bacteria; bacterial magnetic particle; bacterial magnetic particle–specific protein.

Introduction

Magnetic bacteria synthesize intracellular magnetite, which is encapsulated by a lipid bilayer membrane. These structures are collectively termed bacterial magnetic particles (BMPs). Their size varies from 50 to 100 nm in length, and number over 10 per cell. Various proteins and regulatory factors are involved in their synthesis.

We hypothesized that proteins expressed on the BMP membrane play a direct role in the regulation of magnetite crystallization. In developing this hypothesis, we recently isolated *mag*A from *Magnetospirillum* sp. AMB-1 by transposon mutagenesis *(1)*. MagA is an iron transporter, and is the first gene involved in BMP formation to be isolated. Analysis using a *mag*A-*luc*

*Author to whom all correspondence and reprint requests should be addressed.

fusion protein localizes MagA to both the BMP and cytoplasmic membranes *(1,2)*. Various proteins associated with the BMP membrane may promote hypothetical reactions involved in magnetite generation inside BMP envelopes. These include accumulation of supersaturating quantities of iron via a reductive step from outside the envelope and oxidation of iron, or partial reduction and dehydration of ferrihydrite to magnetite *(3)*.

Interestingly, the morphology of BMPs is species dependent. It is therefore reasonable to hypothesize that species-specific biological factors located on the BMP membrane direct magnetite crystallization *(4)*. Moreover, transmission electron microscopy of *M. magnetotacticum* MS-1 reveals that BMP envelopes appear prior to crystallization of magnetite *(5)*. The mechanism of envelope formation, however, still remains unclear.

We have previously reported a technique for preparation of recombinant BMPs on which proteins were displayed by gene fusion using *mag*A as a BMP-located anchor *(2)*. We have furthermore applied such recombinant BMPs to biotechnologically important problems, including novel bioassay platforms for the medical profession *(6)* and the environmental management sector. We furthermore envisage production of fine chemicals through expression on BMPs by gene fusion followed by simple purification using magnets. For efficient display of useful proteins on BMPs, the isolation of proteins specific and abundantly located on the BMP membrane has been required. We correspondingly report the detection and partial amino acid sequence of five new proteins that are specific to the BMP membrane.

Materials and Methods

Organisms and Growth Conditions

Magnetospirillum sp. AMB-1 (ATCC 700264) was grown anaerobically at 26°C in modified magnetic spirillum growth medium at pH 6.75 *(7)*. Batch culture was carried out in a 50-mL (or 5-L) flask containing 40 mL (or 4 L) of medium.

Subcellular Fractionation

AMB-1 cells were harvested, resuspended in 20 mL of HEPES buffer (10 m*M*, pH 7.0), and then disrupted using a French Press (three cycles at 1300 kgf/cm^2). After removal of cell debris from the extract, BMPs were separated using a samarium-cobalt (Sm-Co) magnet (18 × 11 × 14 mm). BMPs collected using a magnet were washed with phosphate-buffered saline by sonication. The BMP-free extract was ultracentrifuged (100,000*g* for 1 h) to separate cellular membranes and cytoplasm. The cellular membrane fraction was washed twice with HEPES. Cellular and BMP membrane fractions were each dissolved in 8 *M* buffered urea (containing 50 m*M* Tris, 10 m*M* dithiothreitol, pH 8.5).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Sample was incubated with an equal volume of 2X sample buffer (0.125 *M* Tris-HCl, pH 6.8; 10% 2-mercaptoethanol; 4% sodium dodecyl sulfate [SDS]; 10% sucrose; 0.004% bromophenol blue) at 100°C for 5 min. SDS-polyacrylamide gel electrophoresis (PAGE) was performed on 12.5% (w/v) acrylamide gels *(8)*. Proteins were stained with Coomassie brilliant blue R-250. The molecular weight was calculated from a standard linear regression curve using low molecular weight calibration markers (Amersham Pharmacia Biotech, Uppsala, Sweden).

Two-Dimensional PAGE

BMP membrane proteins were separated in a pH gradient (3.5–10.0) using tube gels. A constant voltage (800 V) was applied for 10 h, then increased to 1000 V and held for an additional 1 h. The tube was automatically subjected to two-dimensional (2D) electrophoresis on a homogeneous SDS gel (12%) under constant voltage (300 V) for 2.5 h. The 2D gel was stained with Coomassie brilliant blue R-250. Carbamylyte™ calibration markers (Amersham Pharmacia Biotech, Uppsala, Sweden) were used to estimate the pI of the proteins.

Results and Discussion

Magnetic bacteria are unique in that they exhibit intracellular compartmentalization like eukaryotes *(5)*. Various proteins expressed on the BMP membrane are hypothetically involved in the synthesis of BMPs.

Figure 1 shows protein profiles of subcellular fractions as determined by SDS-PAGE. SDS-PAGE revealed the existence of characteristic proteins in each subcellular fraction. More than 10 cytoplasm-specific bands were observed. Many proteins were observed in the cellular membrane fraction. Almost all of these were also found in the BMP membrane fraction. However, five proteins were specific to the BMP membrane fraction (Fig. 1, arrowhead). The molecular weights of these five BMP-specific proteins were estimated to be 12.0, 16.0, 24.8, 35.6, and 66.2 kDa (a–e in Fig. 1, respectively) by SDS-PAGE. Figure 2 shows protein profiles by 2D-PAGE. 2D-PAGE revealed pIs of major components in the BMP membrane fraction. pIs of each protein were 3.6, 3.7, 6.17, 6.46, and 7.55 (b1, b2, c, and d in Fig. 2, respectively).

Of these five BMP-specific proteins, the 16.0-kDa protein was the most abundant. 2D-PAGE reveals that it consists of two components of differing pI. The 35.6-kDa protein was the second most abundant of the five BMP-specific proteins. The gene for this protein, termed *mps*A, has been cloned. *mps*A exhibits homology with the α-subunits of acetyl-CoA carboxylase of *Escherichia coli (9)* to 51.4%, and the results from the hydropathy plot suggested that MpsA is a hydrophilic protein. Therefore, MpsA is considered to be fixed by a specific-binding protein associated with the BMP

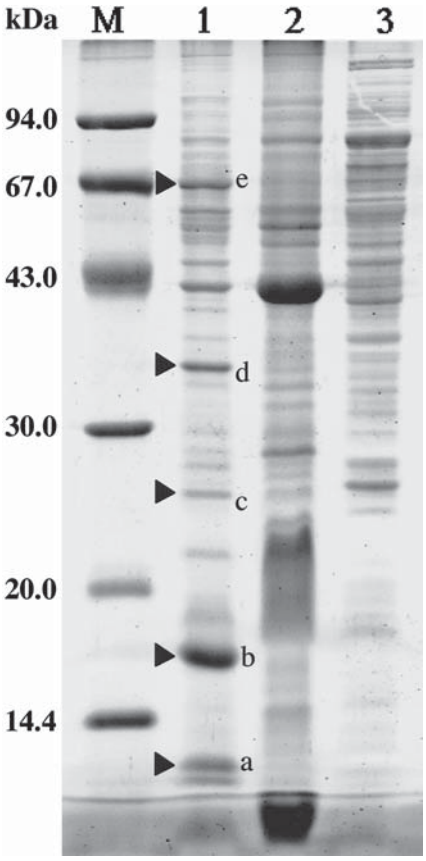

Fig. 1. Protein profiles by SDS-PAGE. Forty micrograms of protein from each fraction was applied. Lane 1, BMP membrane solubilysate; lane 2, membrane solubilysate; lane 3, cytoplasmic fraction; M, standard markers. Arrowheads indicate BMP-specific proteins. a–e correspond to blots in Fig. 2.

membrane. MagA, which is estimated to be 46.8 kDa from the deduced amino acid sequence, was not found to be a BMP-specific protein. This finding was supported by results from *mag*A-*luc* localization studies. However, the MagA band is not a major component that can be detected by 2D-PAGE, and it is probably sufficient to accumulate of iron. The 16-kDa protein exhibited a high relative abundancy in the BMP membrane as compared with other BMP-specific proteins. It is therefore a promising candidate for use as an anchor in the construction of recombinant BMPs by gene fusion.

In *M. magnetotacticum* MS-1, two BMP-specific proteins of mol wt 33 and 15 kDa *(5)*, and three of mol wt 28, 22, and 12 kDa *(10)* have already been observed. The 12-, 16-, 24.8-, and 35.6-kDa (MpsA) proteins of AMB-1 may correspond to the 12-, 15-, 22-, and 33-kDa proteins of MS-1. These four proteins may be required for BMP formation because they are not species dependent. The gene encoding the 22-kDa protein of MS-1 has been iso-

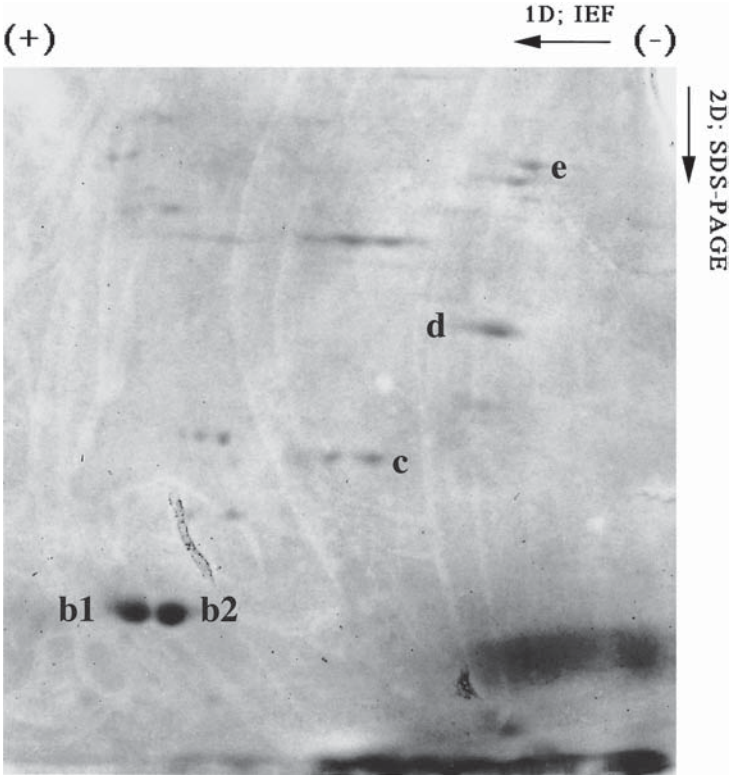

Fig. 2. 2D-PAGE analysis of BMP membrane proteins. b1, b2, and c–e correspond to bands in Fig. 1. IEF, isoelectric focusing.

lated and is termed *mam22 (10)*. It exhibits homology with the tetratricopeptide repeat (TPR) protein family. However, the function of MAM22 is still unclear. The TPR motif has been identified in several cell division cycle gene products and in proteins involved in the regulation of RNA synthesis *(11,12)*. Since AMB-1 may have this protein, RNA synthesis involved in BMP formation may be regulated by the TPR. Furthermore, BMP formation is possibly related to cell cycles.

It is necessary to analyze these proteins further not only for elucidation of the mechanism of BMP biosynthesis but also for the development of an efficient display system of useful proteins on BMPs.

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