Structural Study of MPN387, an Essential Protein for Gliding Motility of a Human-Pathogenic Bacterium, *Mycoplasma pneumoniae*

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ABSTRACT

Mycoplasma pneumoniae is a human pathogen that glides on host cell surfaces with repeated catch and release of sialylated oligosaccharides. At a pole, this organism forms a protrusion called the attachment organelle, which is composed of surface structures, including P1 adhesin and the internal core structure. The core structure can be divided into three parts, the terminal button, paired plates, and bowl complex, aligned in that order from the front end of the protrusion. To elucidate the gliding mechanism, we focused on MPN387, a component protein of the bowl complex which is essential for gliding but dispensable for cytodherence. The predicted amino acid sequence showed that the protein features a coiled-coil region spanning residue 72 to residue 290 of the total of 358 amino acids in the protein. Recombinant MPN387 proteins were isolated with and without an enhanced yellow fluorescent protein (EYFP) fusion tag and analyzed by gel filtration chromatography, circular dichroism spectroscopy, analytical ultracentrifugation, partial proteolysis, and rotary-shadowing electron microscopy. The results showed that MPN387 is a dumbbell-shaped homodimer that is about 42.7 nm in length and 9.1 nm in diameter and includes a 24.5-nm-long central parallel coiled-coil part. The molecular image was superimposed onto the electron micrograph based on the localizing position mapped by fluorescent protein tagging. A proposed role of this protein in the gliding mechanism is discussed.

IMPORTANCE

Human mycoplasma pneumonia is caused by a pathogenic bacterium, *Mycoplasma pneumoniae*. This tiny, 2- μ m-long bacterium is suggested to infect humans by gliding on the surface of the trachea through binding to sialylated oligosaccharides. The mechanism underlying mycoplasma “gliding motility” is not related to any other well-studied motility systems, such as bacterial flagella and eukaryotic motor proteins. Here, we isolated and analyzed the structure of a key protein which is directly involved in the gliding mechanism.

Mycoplasma members represent a group of parasitic and, occasionally, commensal bacteria that lack a peptidoglycan layer and have small genomes (1, 2). *Mycoplasma pneumoniae* is a causative pathogen of human bronchitis and walking pneumonia. Outbreaks of *M. pneumoniae* infections occur frequently in many parts of the world, and the increase in the incidence of macrolide-resistant *M. pneumoniae* infections is a growing problem (3–5). *M. pneumoniae* exhibits “gliding motility” in the direction of the leading-edge cell protrusion at a maximum speed of 1 μ m (one-half of its cell length) per second (6–9). Detailed analyses of gliding in *M. pneumoniae* have shown that the gliding cells move continuously and pivot around the protrusion (7). This motility, combined with the ability to adhere to epithelial cells, is involved in the infection process, enabling the bacteria to translocate from the tip of bronchial cilia to the host cell surface (10). Previous studies, including genome analyses, have shown that this motility is not related to other known mechanisms of bacterial movement and does not involve motor proteins known to be associated with eukaryotic cell motility (6, 11–16). The gliding machinery is located at a membrane protrusion called the attachment organelle, which is composed of nap-like surface protrusions and an internal rod-shaped core (6, 17–22). The core can be divided into three parts—the terminal button, the paired plates, and the bowl (wheel) complex—from the front to the back, respectively (17, 18, 22). Mutants lacking the core cannot form the attachment organelle; hence, the core may serve as an important support structure (23,

24). Fifteen protein components, i.e., four for the surface structure, two for the terminal button, three for the paired plates, and six for the bowl complex, have been reported. MPN387, a component of the bowl complex, has been identified as a protein that is essential for force generation but dispensable for cytodherence, based on the identification of binding-positive and gliding-negative mutants (22, 25). This suggests that MPN387 is directly involved in force generation or transmission, because most component proteins are essential for cytodherence. In the present study, we expressed and purified MPN387 as a recombinant protein and analyzed its structure by circular dichroism (CD) spectroscopy, gel filtration, analytical ultracentrifugation, electron microscopy (EM), and partial proteolysis. The results showed that MPN387 is

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a dumbbell-shaped homodimer that is about 42.7 nm in length and includes a 24.5-nm-long central parallel coiled coil.

MATERIALS AND METHODS

Sequence analyses. Coiled-coil and α -helix regions were predicted by COILS (http://www.ch.embnet.org/software/COILS_form.html) and PSIPRED (<http://bioinf.cs.ucl.ac.uk/psipred/>), respectively. Sequence similarity was searched by PSI-BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) against NCBI nonredundant protein sequences. The sequences of homologs were aligned by T-COFFEE (<http://tcoffee.vital-it.ch/apps/tcoffee/do:regular>).

Protein expression and purification. The DNA sequence of MPN387 was codon optimized to be expressed in *Escherichia coli* and synthesized (GenScript, Piscataway, NJ). The DNA coding MPN387 was inserted into pET15b (Novagen, Madison, WI) through NdeI-BamHI sites to create the pYK387 plasmid, in which MPN387 is fused by an N-terminal hexahistidine tag and a thrombin site (recombinant MPN387 [rMPN387]; MGSS-6 $\times$ His-SSGLVPRGSH-MPN387). A 5-ml overnight culture of BL21(DE3) pLysS carrying pYK387 was inoculated into 1 liter of LB medium (10 g Bacto tryptone, 5 g yeast extract, 10 g NaCl per liter) containing 50 μ g/ml ampicillin and 30 μ g/ml chloramphenicol. The cells were grown at 37°C until the culture density reached an optical density at 600 nm (OD_{600}) of 0.5. Expression of rMPN387 was induced with IPTG (isopropyl β -D-1-thiogalactopyranoside) at a final concentration of 0.1 mM, and the cultivation was continued for 3 h at 30°C. The cells were harvested by centrifugation (4,000 $\times$ g, 10 min, 4°C) and washed twice with a TN buffer consisting of 20 mM Tris-HCl (pH 8.0) and 500 mM NaCl and then stored at -80°C. The cell pellet was thawed, suspended in a binding buffer (20 mM Tris-HCl [pH 8.0], 500 mM NaCl, 50 mM imidazole-HCl [pH 8.0]) containing 1 mM phenylmethylsulfonyl fluoride (PMSF), and sonicated with a US-600 sonicator (Nihoneiki, Tokyo, Japan). The cell lysates were centrifuged (30,000 $\times$ g, 30 min, 4°C) to remove undispersed cells and inclusion bodies. The supernatants were loaded onto a HisTrap HP column (GE Healthcare, Milwaukee, WI) equilibrated with the binding buffer. The column was washed with the binding buffer, and proteins were eluted with a linear gradient of 50 to 500 mM imidazole-20 mM Tris-HCl-500 mM NaCl at pH 8.0. Fractions were monitored by SDS-PAGE with Coomassie brilliant blue (CBB) stain. Fractions containing rMPN387 were collected and dialyzed against TNED buffer (20 mM Tris-HCl [pH 8.0], 500 mM NaCl, 1 mM EDTA-NaOH, 1 mM dithiothreitol [DTT]) and loaded onto a HiLoad 16/600 Superdex 200 preparation-grade column (GE Healthcare) equilibrated with the TNED buffer at a flow rate of 1 ml/min. The purified rMPN387 was concentrated using an Amicon Ultra 10K spin filter (Millipore, Bedford, MA). A codon-optimized special enhanced yellow fluorescent protein (EYFP) gene with a dressmaker-pin-like-structure (26) was amplified by PCR and inserted into pET15b using an Infusion-EcoDry kit (Clontech, Mountain View, CA). This EYFP (marked EYFP [mEYFP]) contains an α helix at both ends, forming a coiled coil that facilitates the identification of the fused protein under EM (26). mEYFP, mEYFP-MPN387, the N-terminal fusion, and MPN387-mEYFP, the C-terminal fusion, were expressed and purified using the procedure described above.

The mEYFP-MPN387/MPN387-mEYFP hybrid dimer was prepared as follows. Approximately the same numbers of *E. coli* cells overexpressing mEYFP-MPN387 and MPN387-mEYFP were mixed together, washed twice with a binding buffer containing 1% Triton X-100, and resuspended in the binding buffer with 6 M urea to a 25-fold-higher cell density. The cells were sonicated and ultracentrifuged (145,000 $\times$ g, 30 min, 4°C). The supernatant was applied to the HisTrap HP column. Pooled fractions were dialyzed against the binding buffer and applied to another Ni affinity chromatography column.

A fragment consisting of 95 to 261 residues (named fragment v) was purified as follows. The purified rMPN387 was digested with chymotrypsin (1/100 [wt/wt] relative to rMPN387) in the TN buffer at 25°C for 60 min. The solution was applied serially to a HisTrap HP column, and size exclusion chromatography was performed with a HiLoad 16/600 Super-

dex 75 preparation-grade column (GE Healthcare) equilibrated with the TN buffer at a flow rate of 1 ml/min. Purified fragment v was concentrated using an Amicon Ultra 3K spin filter (Millipore) to a concentration suitable for CD spectroscopy.

Gel filtration chromatography and analytical ultracentrifugation. To examine the degree of self-association, analytical gel filtration chromatography was performed with a Superdex 200 Increase 10/300 GL column (GE Healthcare) equilibrated with TNED buffer at a flow rate of 0.5 ml/min at 4°C (27). All data were obtained using an Akta purifier chromatography system (GE Healthcare). Thyroglobulin (669 kDa), γ -globulin (158 kDa), ovalbumin (44 kDa), and myoglobin (17 kDa) were used as the standards for Stokes radii of 8.6, 5.1, 2.8, and 1.9 nm, respectively (28, 29). The analytical ultracentrifugation was performed using an XL-I analytical ultracentrifuge equipped with an An-60Ti rotor (Beckman-Coulter, Fullerton, CA). rMPN387 at 0.72 mg/ml was centrifuged in TNED buffer at 30,000 rpm and 4°C for 1,000 min and scanned for absorbance at 280 nm at 10-min intervals. The sediment coefficient [C(s)] distribution was analyzed using SEDFIT, version 12.52 (30), and the dimensions of the molecule were calculated using Sednterp, version 1.09.

CD spectroscopy. Far-UV CD spectra (wavelengths of 200 to 260 nm) were collected on a Jasco-720 spectropolarimeter with a Peltier-type cell holder (Jasco International Co., Tokyo, Japan), which allows temperature control. Cylindrical fused quartz cells with a 1-mm path length were filled with 0.2 mg/ml protein in the TN buffer. Spectra were obtained by averaging five successive accumulations with a wavelength step of 0.5 nm at a rate of 20 nm/min, response time of 8 s, and bandwidth of 2.0 nm. Measurements were performed three times for each protein at 20°C, and the obtained spectra were averaged. The contents of the protein secondary structure were estimated from the CD spectra by the use of K2D3 (<http://cbdm-01.zdv.uni-mainz.de/~andrade/k2d3/>).

Partial proteolysis of rMPN387 by the use of chymotrypsin and trypsin. For the domain analysis of MPN387, purified rMPN387 was digested with chymotrypsin (1/100 [wt/wt] relative to rMPN387), or trypsin (1/10 [wt/wt] relative to rMPN387) in the TNED buffer at 25°C for various times (0, 1, 5, 10, 15, 30, and 60 min). The digests were analyzed by SDS-PAGE and peptide mass fingerprinting (PMF) using an autoflex speed system (Bruker Daltonics, Billerica, MA) as reported previously (27, 31, 32). Briefly, in PMF, protein bands in SDS-PAGE were digested by the use of a residue-specific protease, i.e., trypsin. The resulting peptide mixture was extracted and subjected to mass spectrometry. The combination of mass measurements of many peptides and the genome database allowed identification of the corresponding open reading frame (ORF) for each protein band. In cases where the peptide band from SDS-PAGE was part of the protein, identification of the corresponding position in the whole sequence was possible based on the covering regions of peptides detected by the mass spectrometry and the MASCOT search engine (Matrix Science, London, United Kingdom).

Rotary-shadowing EM. Protein samples (15 μ g/ml mixed with 500 mM ammonium bicarbonate and 50% [vol/vol] glycerol) were sprayed onto a freshly cleaved mica surface as previously described (27, 33, 34). Images of the particle were picked up and averaged by EMAN, version 2.07 (<http://blake.bcm.tmc.edu/EMAN2/>) (35), and the lengths of molecular images were analyzed using ImageJ 1.45s (<http://imagej.nih.gov/ij/>).

RESULTS

Sequence analysis of MPN387. Sequence alignment of MPN387 with MG_269, an ortholog of the closely related species *Mycoplasma genitalium*, is shown in Fig. 1A. The comparison showed 175 identical and 39 similar amino acid residues in total sequences of 358 and 340 residues of MPN387 and MG_269, respectively. Searches in databases for proteins similar to MPN387 performed by PSI-BLAST using the MPN387 and MG_269 sequences resulted in identification of 33 similar proteins with E values ranging from 0.05 to 1.5, but the sequences did not show obvious similarities. The amino acid sequence of MPN387, which was analyzed by

FIG 1 Sequence analyses of MPN387. (A) Similarity between MPN387 (upper sequences) and an ortholog, *M. genitalium* MG_269 (lower sequences). Identical and similar amino acids are marked by black and gray boxes, respectively. (B) Prediction of coiled-coil region by an algorithm, COILS, for MPN387 (solid line) and MG_269 (dotted line). The y axis represents the predicted coiled-coil probability analyzed using a window of 28 amino acids. The region of amino acids 72 to 290 of MPN387 that scored over 0.5 probability is marked by black bars in panel A.

the use of COILS to predict coiled-coil regions, revealed that amino acids spanning residues 72 to 290 in MPN387 were likely to form a coiled coil with a predicted length of 31.9 nm (Fig. 1B) (36).

Isolation of recombinant MPN387. To characterize the MPN387 protein, three constructs of recombinant proteins were designed; one was tagged with a histidine hexamer at the N terminus, and the other two were fused with an mEYFP protein at the N terminus or C terminus and tagged with a histidine hexamer at the N terminus. mEYFP is a protein marked by a coiled-coil structure for detection under EM (see Fig. S1 in the supplemental material) (26). The protein lysates extracted from *E. coli* cells showed solubility of more than 70% with respect to rMPN387 by centrifugation, suggesting that the MPN387 protein itself is a soluble protein. Isolated protein fractions analyzed by SDS-PAGE were not found to contain any other major bands in the gel (Fig. 2A). Finally, 1.5 mg rMPN387, 0.2 mg mEYFP-MPN387, and 0.2 mg MPN387-mEYFP were obtained from 1 liter of *E. coli* cultures.

Analysis by hydrodynamics and CD spectroscopy. Gel filtration chromatography was applied to the purified rMPN387 protein (Fig. 2B). The elution pattern showed a single peak with an

FIG 2 Isolation and gel filtration profile of rMPN387 proteins. (A) The rMPN387 was purified through three steps as described in Materials and Methods. Lane 1, *E. coli* lysate without plasmid; lane 2, *E. coli* lysate without IPTG induction; lane 3, *E. coli* lysate induced to produce the rMPN387; lane 4, supernatant of lysate after centrifugation; lane 5, pellet of lysate after centrifugation; lane 6, peak fraction of HisTrap HP column chromatography; lane 7, peak fraction of gel filtration. Protein fractions were subjected to SDS-12.5% PAGE and stained with CBB. Molecular masses are shown on the left. mEYFP, mEYFP-MPN387, and MPN387-mEYFP were purified through the same procedure and analyzed by SDS-10.0% PAGE. Lanes 8, 9, and 10 represent peak fractions of gel filtration results for mEYFP, mEYFP-MPN387, and MPN387-mEYFP, respectively. (B) Gel filtration assay. The isolated rMPN387 was applied to gel filtration at a flow rate of 0.5 ml/min, with absorbance monitored at 280 nm. Standard proteins were analyzed in the same way, and their peak positions were plotted against their known Stokes radii (8.6, 5.1, 2.8, and 1.9 nm). The Stokes radius of rMPN387 was estimated to be 5.8 nm.

estimated Stokes radius of 5.8 nm. Analytical ultracentrifugation revealed that 62.6% of the protein sedimented with a sedimentation constant of 1.9 S_{20,w} (sedimentation coefficient standardized to 4°C in water) and with a Stokes radius of 5.8 nm (Fig. 3A and B [left]). Based on the results of analytical ultracentrifugation, the mass of rMPN387 was calculated to be 87,500 Da, which was in agreement with the 89,560.2-Da mass of the protein dimer calculated from the amino acid sequence. Therefore, it was concluded that MPN387 behaves as a homodimer in solution. The molecular dimensions were estimated as a cylinder 40.9 nm in length and 1.6 nm in diameter.

We measured the CD spectrum for the purified rMPN387 protein and observed a spectrum typical of a coiled-coil structure (37). The contents of the α helix and β strand were estimated by the K2D3 method as 66.5% and 3.1%, respectively (Fig. 3C) (38). The estimated helix content was slightly more than the coiled-coil content (61.1%, calculated as 219 residues of a total of 358 predicted by COILS) (Fig. 1B). However, the value of 66.5% agreed with the helix content predicted by PSI-PRED (67.9%), because MPN387 is predicted to contain helix in regions other than the central coiled coil.

FIG 3 Analytical ultracentrifugation. (A) Absorbance of rMPN387 scanned at 10-min intervals. Twenty representative traces with 50-min intervals are shown. The x axis shows the position from the rotational center. (B) C(s) distribution of S_{20,w} of rMPN387 (left) and mEYFP-MPN387 (right). (C) Far-UV CD spectra of isolated rMPN387 (blue) and fragment v (red) at 0.2 mg/ml in the TN buffer. Mol.CD, molar circular dichroism.

Rotary-shadowing EM. To observe the molecular images of rMPN387 by EM, purified samples were subjected to rotary-shadowing EM (Fig. 4; see also Fig. S2 in the supplemental material). Rotary shadowing is advantageous for visualization of dimeric coiled-coil regions whose diameter is on the order of 2 nm, because the diameter of thin molecules becomes greater as a consequence of metal coating in rotary shadowing (39), whereas another high-contrast method, negative staining, gives images of lesser contrast as a consequence of the penetration of the stain into the space within molecules. Many filamentous particles that were approximately 40 nm in length were observed in the microscopy field (Fig. 4A; see also Fig. S2A). The frequency of their appearance

changed with the protein concentration, indicating that these particles were the rMPN387 molecules. Most particles appeared to be dumbbell shaped, with globular parts at both ends of the central rod. The images of 136 particles were classified into five class averages (Fig. 4C). All five class-averaged images showed similar features, the clearest of which was the average of 47 particle images. The particle in the image was 42.7 nm long and was divided into three parts, consisting of globular parts that were 9.1 nm in diameter at both ends and were connected to the central rod, 24.5 nm in length (Fig. 4D). These geometries are in good agreement with the average total length of 42.8 ± 4.2 nm determined on the basis of the measurement of individual molecules ($n = 300$) (Fig. 4B). This is also consistent with the size of a cylinder that is 40.9 nm in length and 1.6 nm in diameter, as estimated by analytical ultracentrifugation (Fig. 3A and B). However, it should be noted that rotary-shadowing EM is not applicable for thickness measurements in this range because of the undefined metal thickness caused by the shadowing (27). The central rod may correspond to the coiled-coil region predicted for amino acid residues 72 to 290, which is predicted to be 31.9 nm long. The actual coiled-coil region may be shorter than predicted. According to the results of hydrodynamics analyses, the molecule is composed of two polypeptides that form a dimer of coiled coils in the central part.

To examine the polarity alignment of two peptides, we examined MPN387 fused with mEYFP at the N terminus (mEYFP-MPN387) or the C terminus (MPN387-mEYFP). The analytical ultracentrifugation of mEYFP-MPN387 showed that 95.7% of the protein molecules were monomeric (Fig. 3B, right), whereas MPN387-mEYFP molecules were aggregated under the same conditions. The rotary-shadowed image of the former showed that most molecules appeared to be labeled by a dressmaker-pin-like structure (Fig. 4G and I), similarly to the image of mEYFP (Fig. 4E and F; see also Fig. S2B in the supplemental material), which is consistent with the result of analytical ultracentrifugation. Although the α helices at the N and C termini of mEYFP were designed to form a coiled coil (Fig. 4E and F; see also Fig. S1 and S2B), mEYFP fused to MPN387 appeared to not form a coiled coil because an additional stalk was found on the mEYFP at the other side of the MPN387 in most images (Fig. 4G and H). Among 252 and 332 images of the mEYFP-MPN387 and MPN387-mEYFP particles, only 14 and 32 images were observed to have two spherical parts at the ends, respectively, suggesting that the MPN387 protein might be a parallel dimer (Fig. 4H and J; see also Fig. S2C and D). The fusion with mEYFP on the end might have affected the formation of the coiled coil through steric hindrance. To confirm this hypothesis, reconstitution of these fusion proteins was performed. The protein mixture was denatured, purified, renatured, and then observed by rotary-shadowing EM. The images of 232 molecules showed 108 rods with one globule at each end, 106 rods with a single globule, and 18 rods with two globules at one end (Fig. 4K to M; see also Fig. S2E). The fraction of the rod with one globule on each end was much higher than that of the rod with two globules on one end in mEYFP-MPN387 and MPN387-mEYFP, in agreement with the hypothesis that MPN387 forms a parallel dimer and that the dimerization might be hampered by steric effects (Fig. 4N).

Regions sensitive to chymotrypsin in the rMPN387 molecule. To characterize the domain constitution of the molecule, the purified rMPN387 molecule was partially cleaved with chymotrypsin (Fig. 5A). Four major bands appeared with an increase in

FIG 4 Rotary-shadowing EM images of MPN387 constructs. All EM images are presented at the same magnification, except for those in panels D and F, as indicated by scale bars. (A) Field image of rMPN387. (B) Length distribution. The average ($n = 300$) length of rMPN387, 42.8 ± 4.2 nm, is shown by a triangle. (C) Averaged images from 136 particles grouped into five classes. (D) Magnified image of rMPN387 averaged for 47 of 136 particle images, with the length of each part indicated. This is an enlarged version of the leftmost image in panel C. (E) A picked image of mEYFP. (F) Magnified image of mEYFP molecule composed of mEYFP at the MPN387 and at the other sides, respectively. (G) Selected mEYFP-MPN387 images, featuring an mEYFP. White and black triangles show additional stalks on mEYFP at the MPN387 and at the other sides, respectively. (H) mEYFP-MPN387 images, featuring two mEYFPs indicated by white and black triangles. (I) MPN387-mEYFP images, featuring an mEYFP. (J) MPN387-mEYFP images, featuring two mEYFPs. (K to M) Selected images of the dimer (K), monomer (L), and parallel dimer (M) molecules reconstituted from the mixture of mEYFP-MPN387 and MPN387-mEYFP. (N) Fraction of particles that are single headed (white), double headed on one end (black), and single headed on both ends (hatched) for mEYFP-MPN387 ($n = 252$), MPN387-mEYFP ($n = 332$), and a mixture of the two ($n = 232$), marked N, C, and N/C, respectively.

digestion time. The molecular masses of these bands were 42.2, 28.4, 21.1, and 17.4 kDa for the four bands from ii to v, respectively. These protein bands were subjected to PMF and were shown to cover at least the regions of amino acids 1 to 275, 1 to 256, 83 to 256, and 101 to 256, with length coverages of 60.1%, 57.0%, 36.3%, and 33.2%, respectively (Fig. 5B). Considering the residue specificity of chymotrypsin, the region most resistant to chymotrypsin was assigned to amino acids 95 to 261, which was predicted to be 25.1 nm long, a prediction that was in very good agreement with the length of central rod part observed in the rotary-shadowing EM. Partial proteolysis using trypsin showed a pattern very similar to that obtained with chymotrypsin. These results support the hypothesis that the central rod of MPN387 forms a coiled coil.

We then isolated fragment v through proteolysis and the three purification steps (Fig. 5A). The contents of the α helix and β strand were estimated as 87.4% and 0.5%, respectively, from the CD spectrum, consistent with the assumption that the region from amino acid 95 to amino acid 261 forms a coiled-coil structure (Fig. 3C) (38).

DISCUSSION

Molecular structure and positioning in the attachment organelle. Taking all the data together, a description of the whole MPN387 molecule is provided (Fig. 6A). Two globular parts of about 9.1 nm in diameter at both ends of the polypeptide are connected by a coiled-coil portion, 24.5 nm in length, that corresponds to amino acid residues 95 to 261. Two MPN387 molecules form a parallel homodimer

FIG 5 Partial proteolysis of rMPN387. (A) Protein profiles of purified rMPN387 digested by chymotrypsin for various reaction times: 0, 1, 3, 5, 10, 15, 30, and 60 min. The digested protein fractions were subjected to SDS–12.5% PAGE. The resultant peptide fragments, marked ii to v, were analyzed by PMF. i, full-length rMPN387. Isolated fragment v is shown in the rightmost lane. (B) Mapping of products on the amino acid sequence of rMPN387. A hexahistidine tag and a thrombin recognition site derived from the vector are shown by gray boxes. Protein bands i to v were assigned on the sequence based on the results of the PMF analyses. The positions of peptides detected in PMF are marked with white dots. The sequence regions of peptide bands i to v were assigned using both the evident residue numbers from PMF and the estimated band sizes. The stable region from amino acid 95 to 261 is marked by closed triangles, and the region predicted for the coiled coil from amino acid 72 to 290 is marked by a hatched box and open triangles.

through the coiled-coil interaction. The localization of 15 component proteins in the attachment organelle has been determined by fluorescence microscopy (22), and most of them are essential for cytoadherence. The N terminus of MPN387 has been mapped around the front

end of the bowl complex in the core of the attachment organelle (Fig. 6B) (22). The schematic diagram of MPN387 featuring the central coiled-coil domain as shown in Fig. 6A might be assigned onto the image of the core (Fig. 6C) (12, 22).

FIG 6 Schematic illustration of MPN387 molecule, which is composed of three domains, including a parallel coiled coil at the center (A), and possible assignment of their positions onto core images as revealed by EM (C). (B) Schematic illustration of the attachment organelle with localization of the protein. An image of the whole cell is shown at the top of the panel, and the trapezoid area is magnified in the middle of the panel, with a side view of the core. The suggested force transmission is indicated by an arrow. A schematic of an isolated core with dimensions is presented in the bottom of the panel. The mean position of the N terminus of MPN387 is marked by a triangle and a green line. (C) The molecular shape of MPN387 is superimposed onto the image of the isolated core structure. The possible protein assignments on the side and top views of core structures are shown in green in the schematics in the upper and lower parts of the panel, respectively. The N-terminal position of MPN387 along the attachment organelle determined from the fluorescence signal of EYFP is indicated by a yellow triangle. (Panels B and C were adapted from reference 22.)

Possible role in gliding mechanism. Among the 15 component proteins of the attachment organelle (22), only MPN387 and P200 (MPN567), localizing around the bowl complex region, are more related to motility than to cytodherence. Conceivably, the force for gliding may be generated in the bowl complex and transmitted to the paired plates fixed to the cell front through the terminal button. Iterative extension and retraction of the organelle would generate gliding movement. The P1 adhesin complexes, somehow linked to the internal core, repeat a catch-pull-release cycle with sialylated oligosaccharides on the host surface. The organelle would then complete a gliding cycle by pulling forward the other parts of the cell connected to the back end of the bowl complex (22, 40). The force generated in the bowl complex may be transmitted through MPN387 to the paired plates. This hypothesis is consistent with the recent observation that the lack of HMW2 decreased the gliding speed by 112-fold (41). In the absence of HMW2, the force generated around the bowl complex may still be transmitted to the P1 adhesin much less efficiently than in the presence of HMW2, possibly through a distortion of the whole machinery. This hypothesis may also be supported by a recent observation based on electron cryotomography results showing that the flexible thick plate can move along the rigid thin plate (40). However, the consequence of the lack in MPN387 may not be simple, because the amounts of several component proteins of the attachment organelle were much reduced in the MPN387 mutant, although the strain retained the cytodherence activity (25). We need more experimental data to reach a conclusion regarding the validity of the hypothesis outlined above.

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