

## NOTES

# The Amino Terminus of *Pseudomonas aeruginosa* Outer Membrane Protein OprF Forms Channels in Lipid Bilayer Membranes: Correlation with a Three-Dimensional Model

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*Pseudomonas aeruginosa* OprF forms 0.36-nS channels and, rarely, 2- to 5-nS channels in lipid bilayer membranes. We show that a protein comprising only the N-terminal 162-amino-acid domain of OprF formed the smaller, but not the larger, channels in lipid bilayers. Circular dichroism spectroscopy indicated that this protein folds into a  $\beta$ -sheet-rich structure, and three-dimensional comparative modeling revealed that it shares significant structural similarity with the amino terminus of the orthologous protein *Escherichia coli* OmpA, which has been shown to form a  $\beta$ -barrel. OprF and OmpA share only 15% identity in this domain, yet these results support the utility of modeling such widely divergent  $\beta$ -barrel domains in three dimensions in order to reveal similarities not readily apparent through primary sequence comparisons. The model is used to further hypothesize why porin activity differs for the N-terminal domains of OprF and OmpA.

OprF is a major outer membrane protein in *Pseudomonas aeruginosa* that has been studied extensively due to its proposed utility as a vaccine component, its role in antimicrobial drug resistance, and its porin function (3, 6, 7, 13, 20). It has been shown to be required for cell growth in low-osmolarity medium and for the maintenance of cell shape (21). Through epitope-mapping experiments and linker insertion mutagenesis, we originally proposed a 16- $\beta$ -stranded membrane topology model for OprF (19). However, on the basis of deletion studies and secondary structure predictions, we recently proposed a revised model with the N-terminal half of the protein forming an eight-stranded  $\beta$ -barrel domain that is inserted into the outer membrane. The C-terminal half was proposed to form a domain that is exposed and available to monoclonal antibody binding on the cell surface (9) and binds peptidoglycan in the periplasm (15). These two domains are linked by a proline-rich hinge-and-loop region that contains two disulfide bonds.

A somewhat analogous structure has been proposed for the *Escherichia coli* outer membrane protein OmpA (5, 12, 18), and these proteins, which also share some functional similarities, are considered orthologs. Consistent with this concept, significant amino acid sequence similarity has been detected between OprF and OmpA, but only in their C-terminal domains (39% identity, 56% similarity). However, secondary structure predictions indicate that the N-terminal domains may also be similar, despite their lack of substantial sequence identity (15% identity, with no regions of similarity identified using BLAST2 with an “expect value” cutoff of 1,000). Recently, Pautsch and Schulz (12) solved a crystal structure for the N-terminal half of OmpA that was mutated at residues 23, 34, and 107 (in order to obtain crystals) and had been reconsti-

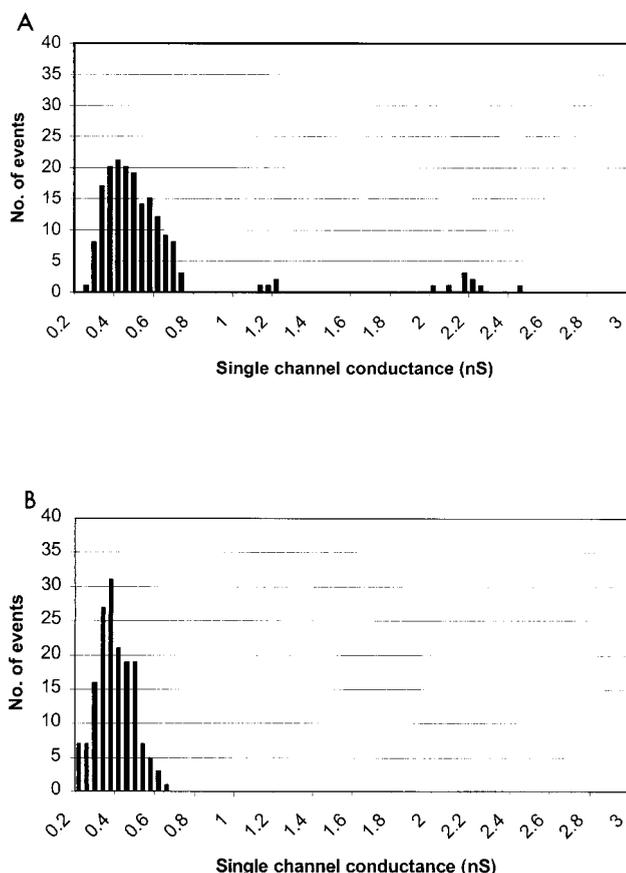


FIG. 1. Histograms of single-channel conductance measurements showing channel size distributions for OprF (A) and OprF<sub>1-162</sub> (B).

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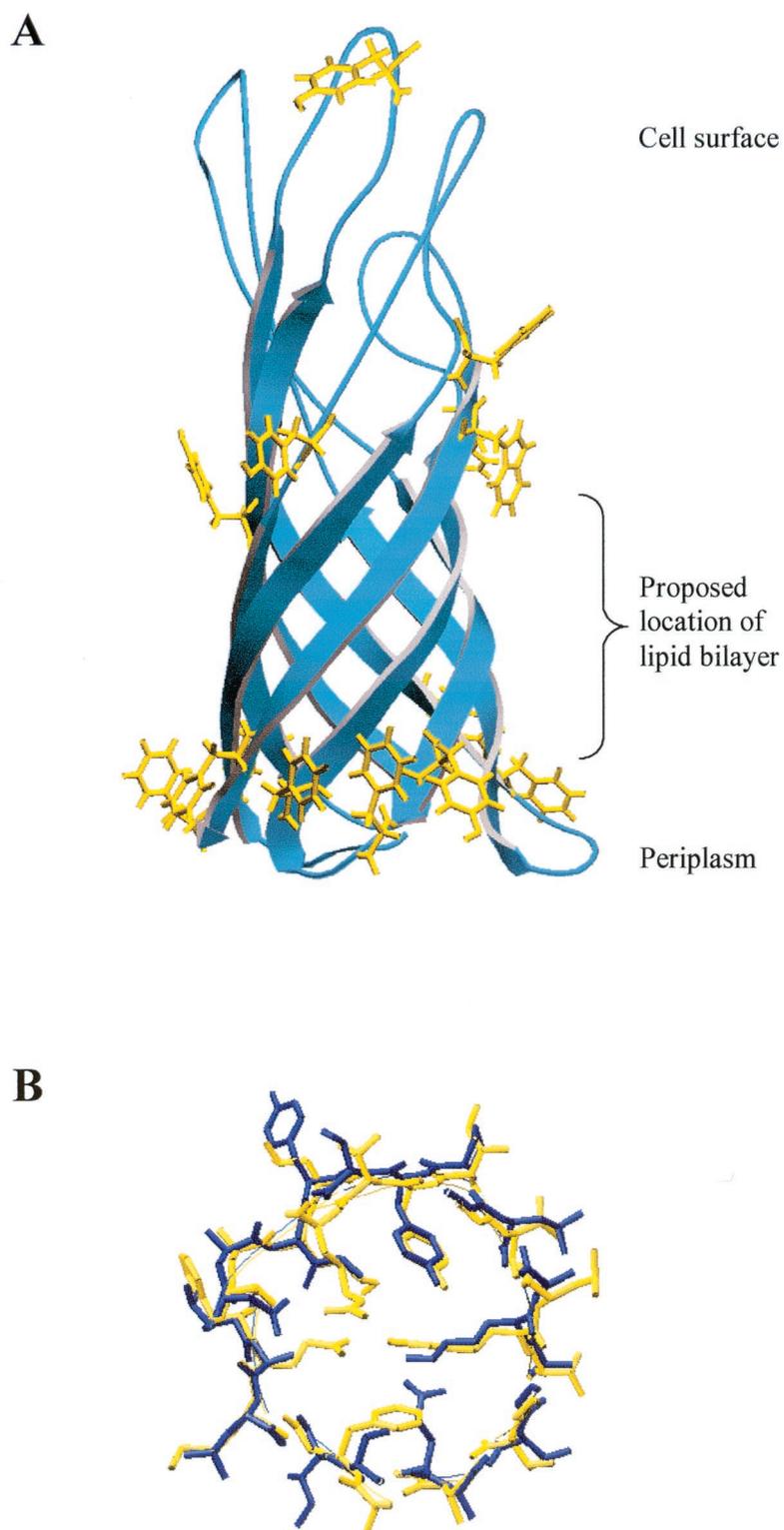


FIG. 4. Three-dimensional model of OprF<sub>1-162</sub>, constructed by threading the sequence of OprF<sub>1-162</sub> on a crystal structure of OmpA<sub>1-171</sub>. (A) Overview of the molecule, highlighting all aromatic residues on the outside of the protein (yellow). Note the striking rings of aromatic residues at the proposed water-lipid interfaces. (B) A slice horizontally through the barrel of the proposed model of OprF (blue), overlaid on the structure of OmpA (yellow), illustrating how residues previously proposed to form a barrier to pore formation in OmpA (12) are not conserved in OprF and allow for a larger channel with no salt bridge in that region.

after mock purification in the same manner as the OprF and OprF<sub>1-162</sub> proteins.

**Structural analysis by indirect immunofluorescence of surface epitopes, and CD spectroscopy.** To confirm that the OprF<sub>1-162</sub> protein was folding in a conformation similar to that of the equivalent domain in wild-type OprF in our studies and was correctly localized to the cell surface, indirect intact *E. coli*/pER163 cells expressing OprF<sub>1-162</sub> were examined by indirect immunofluorescence labeling using the monoclonal antibody MA7-1, which binds to a surface-exposed epitope, amino acids 55 to 62 in the OprF N terminus (14), as previously described (9). Cells expressing OprF<sub>1-162</sub> were highly fluorescent, consistent with surface exposure of this epitope, while cells not expressing any OprF protein sequences showed no fluorescence (data not shown).

To evaluate the secondary structure of this OprF N-terminal domain, CD spectroscopy was performed on purified OprF<sub>1-162</sub> by using a model J-70 spectropolarimeter (Jasco, Tokyo, Japan) connected to a Jasco data processor, using a quartz cell with a 1-mm path length. CD spectra were measured at 25°C, between 190 and 250 nm at a scanning speed of 10 m/min in 10 mM sodium phosphate buffer (pH 7.0) with 0.1% sodium dodecyl sulfate. The resulting spectrum (Fig. 2) was highly similar to that observed for antiparallel  $\beta$ -sheet-rich proteins (4), with a characteristic minimum at 217 nm. This is consistent with the proposal that this domain forms a  $\beta$ -barrel.

**Three-dimensional modeling.** The N-terminal half of OprF, OprF<sub>1-162</sub>, shares only 15% identity with the corresponding region of OmpA, OmpA<sub>1-171</sub>, yet secondary structure prediction algorithms, CD spectroscopy results, and other data (15) are consistent with these proteins sharing similar  $\beta$ -sheet secondary structures and thus indicate that OprF<sub>1-162</sub> may form a  $\beta$ -barrel. Similarity of OprF and OmpA in the C terminus further supports an orthologous relationship between these proteins. We therefore attempted to model the OprF N terminus using the published OmpA N-terminus crystal structure (12). We visually aligned the N-terminal 160 amino acids of OprF with the corresponding N-terminal 171 amino acids of OmpA used for crystallization (Protein Data Bank Identification no. 1BXW) (12). We used amino acid hydrophobicity, rather than identity, as a guide for constructing the alignment. The alignment was further modified after a first round of modeling revealed that one putative transmembrane  $\beta$ -sheet strand was misaligned because a charged residue was pointing out of the central barrel region into a region of the lipid bilayer (final alignment shown in Fig. 3). Previous studies of OmpA (8) and crystal structures of other outer membrane proteins strongly indicate that charged residues are not permissive in such a location in a  $\beta$ -barrel protein. Using the Insight II (version 97.2) molecular modeling program "Homology" (Molecular Simulations Inc., San Diego, Calif.), the OprF<sub>1-162</sub> sequence was threaded to the OmpA<sub>1-171</sub> structure, constraining regions that aligned with the  $\beta$ -sheet regions of OmpA and allowing more freedom in the formation of loop regions (which were not precisely positioned in the OmpA model). The entire structure was then energy minimized using the "Discover" program of Insight II (Fig. 4). The model is available from the authors as a Protein Data Bank file, and animations and other images of the model may be viewed as supplementary data at <http://www.cmdr.ubc.ca/bobh/oprfmodel.html> to aid visualization of its three-dimensional structure.

It was apparent from this model that OprF and OmpA share significant structural similarity, particularly in terms of the conservation of the hydrophobicity of residues pointing toward the outside of the barrel, and also rings of aromatic residues at the proposed lipid-water interface of the molecule (Fig. 4). In

fact, a number of residues were found to be conserved between the structures in three-dimensional space, though these residues were not in the same location in the primary sequence (for example, the underlined residues in Fig. 3). The degree of structural similarity was striking, considering the marked lack of identity between these two proteins in this domain. This disparity between structural sequence similarity in this region supports the belief that orthologous  $\beta$ -barrel structures diverge quickly in primary sequence from each other over time (relative to other common protein folds) due to a lack of primary sequence constraints while they remain structurally very similar.

There was one notable difference between the structure of OmpA<sub>1-171</sub> and the three-dimensional model of OprF<sub>1-162</sub> which we hypothesize could explain the fact that no channels, or only very small channels (0.05 to 0.08 nS), have been observed for the OmpA N-terminal domain, whereas we observed channels of predominantly 0.36 nS with OprF<sub>1-162</sub>. Residues previously implicated in blocking channel formation in the OmpA N-terminal domain (12), or at minimum providing a constriction in the pore, were noticeably not conserved in OprF, and more significantly, the residues that replaced them in OprF permitted the formation of a possible channel (Fig. 4B; see also supplementary data). The previous study reporting this barrier in the pore (12) also presented an alignment of OmpA orthologs, suggesting that this barrier was conserved and that the OmpA  $\beta$ -barrel domain was more conserved than is noted for most porins. However, their analysis was based on phylogenetically very similar organisms. Our analysis of OprF suggests that this proposed constriction is not as conserved as previously thought and that this  $\beta$ -barrel domain is not more conserved in primary sequence than has been observed for other porins.

The evidence presented here and in previous studies (15) strongly suggests that the N-terminal half of OprF can form a  $\beta$ -barrel. A three-dimensional model for the N terminus of OprF is proposed, and we support the benefit and utility of modeling proposed orthologous outer membrane proteins in three-dimensional space, even if they share little sequence identity. There is currently a need for better transmembrane  $\beta$ -strand prediction algorithms for outer membrane proteins. Based on our experience studying outer membrane proteins and on the studies of others, we propose that an amphipathicity plot that pays particular attention to the location of hydrophobic residues and to preferential placement of aromatic residues at the membrane-solvent interface, as well as some of the specific residue constraints reported by Koebnik (8), may be the most effective way to identify transmembrane  $\beta$ -strands. This is particularly important given the significant lack of sequence identity constraints required by a  $\beta$ -barrel structure.

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## AUTHOR'S CORRECTION

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Volume 182, no. 18, p. 5251–5255, 2000. It should have been noted that Sugawara et al. (E. Sugawara, M. Steiert, S. Rouhani, and H. Nikaido, *J. Bacteriol.* **178**:6067–6069, 1996) were the first to propose an OmpA-like, two-domain model for OprF.