Chapter 31

Function and Structure of the Porin Proteins OprF and OprP of *Pseudomonas aeruginosa*

R. J. Siehnel, N. L. Martin, and R. E. W. Hancock

In the outer membranes of gram-negative bacteria like *Pseudomonas aeruginosa*, the channels of porin proteins provide a molecular sieving function for the passage of hydrophilic solutes into the periplasm. Solutes larger than the exclusion limit of the porin channels are unable to cross the outer membrane barrier. There are several excellent reviews of broad scope concerning porins (see Benz [1988] and reviews cited therein). *P. aeruginosa* has four major and two minor outer membrane proteins that have been demonstrated to act as porins (Table 1). These proteins have general properties similar to those of porins from other gram-negative bacteria. The ultimate goal toward understanding the molecular mechanisms involved in the role of porins is a detailed knowledge of the molecular architecture of the porin proteins. In this chapter, we describe our current level of understanding of the functional and structural details of the best-studied porins of *P. aeruginosa*, protein F (OprF) and protein P (OprP) (Table 1).

FUNCTIONAL ASPECTS OF *P. AERUGINOSA* PORIN PROTEINS

Porins of gram-negative bacteria fall into two functional classes. General diffusion porins are chemically nonspecific, although they may be weakly selective, as is the case for the major porins of *Escherichia coli*. Specific porins differ in that they contain saturable binding sites for specific classes of solutes, such as LamB and Tsx of *E. coli* and OprP of *P. aeruginosa* (Benz, 1988). There are also outer membrane proteins, such as OmpA and Braun lipoprotein of *E. coli*, that have been ascribed only structural roles. We review here the current data on the role of OprF of *P. aeruginosa* as both a general diffusion porin and an integral structural protein and the role of OprP as an example of a specific porin in *P. aeruginosa*.

R. J. Siehnel, N. L. Martin, and R. E. W. Hancock • University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5.

TABLE 1
Physical properties of *P. aeruginosa* porin proteins[a]

Porin	Mol wt	Native oligomeric structure	SDS-stable oligomers	Heat-modifiable monomers	Secondary-structure prediction (%)[b]				Conditions favoring production
					H	S	T	C	
OprF	35,250	Trimer	Part	Yes	9	62	20	10	Constitutive
OprP	45,300	Trimer	Yes	No	3	65	26	6	Low phosphate
D2	45,500	—[c]	No	Yes	—	—	—	—	—
D1	46,000	Trimer	No	Yes	—	—	—	—	Glucose as carbon source

[a] Proteins C and E from *P. aeruginosa* have not been described, as we are unsure of their identity. We have not located a major protein banding in the area of protein C on SDS-gels, and two-dimensional gels have shown multiple bands located in the area of protein E (B. L. Angus and R. E. W. Hancock, unpublished results).
[b] H, α helix; S, β sheet; T, β turn; C, random coil. Values were obtained by CD at room temperature in 10 mM Tris–0.1% SDS.
[c] —, Not done.

OprF

As mentioned above, the major outer membrane protein OprF of *P. aeruginosa* is a bifunctional protein, serving both as a porin (Hancock, 1986; Nikaido and Hancock, 1986) and as a protein required for maintaining the structural integrity of *P. aeruginosa* (Gotoh et al., 1989; Woodruff and Hancock, 1988, 1989; see below). OprF purified from *P. aeruginosa* (Benz and Hancock, 1981) or from a clone in *E. coli* (Woodruff et al., 1986) has been shown to form large pores (conductance greater than 4 nS) in addition to smaller pores (0.36 nS) in artificial bilayer experiments. Quantitation of the two sizes of channels revealed the larger pores to be far less prevalent (<1%) than the smaller pores (Woodruff et al., 1986). Recently, there has been some reevaluation of the exclusion limits of *P. aeruginosa* that were previously defined by growth on pentamethionine (Miller and Becker, 1978), susceptibility to large antibiotics (Table 2), liposome swelling assays (Yoshimura et al., 1983), radioactive efflux experiments (Hancock et al., 1979), and the above-mentioned lipid bilayer conductance experiments. These

TABLE 2
Susceptibility of *E. coli* and *P. aeruginosa* to selected antibiotics

Antibiotic	Mol wt	Charge(s)		MIC (μg/ml)		Reference
		+	−	*E. coli*	*P. aeruginosa*	
Piperacillin	516	0	1	2	2	Rolinson, 1986
Ceftazidime	545	1	2	0.12	2	Neu et al., 1988
Cefpiramide	610	0	1	1.1	2.9	Rolinson, 1986
E-1040	636	1	1	0.12	0.25	Neu et al., 1988
Cefsulodin	531	1	2	64	2	Fukasawa et al., 1983
Cefoperazone	644	0	1	0.9	11	Fukasawa et al., 1983
Apalcillin	508	0	1	4	2	Rolinson, 1986
Mureidomycin C	897	1	3	>200	3	Isono et al., 1989

studies are based on plasmolysis experiments (Caulcott et al., 1984; Yoneyama et al., 1986) and liposome swelling assays (Yoshihara et al., 1988; Gotoh et al., 1989) and on the high resistance of *P. aeruginosa* to certain antibiotics (Gotoh et al., 1989). The applicability of these results to the in vivo situation is somewhat controversial, since they predict an exclusion limit of *P. aeruginosa* outer membranes of a molecular weight of approximately 180 (Yoneyama et al., 1986) to 220 (Yoshihara et al., 1988), whereas *P. aeruginosa* is clearly susceptible to antibiotics of much larger molecular weights (Table 2). Conversely, the high resistance of *P. aeruginosa* to certain antibiotics could be accounted for by the small number of large pores (and consequent small area for diffusion of antibiotics) in synergy with a secondary resistance determinant such as periplasmic β-lactamases (see Hancock and Woodruff [1988] for a discussion). It must also be noted that the results of liposome swelling assays of Yoshihara, Nakae, and collaborators are at odds with those previous liposome swelling assays which predict, in agreement with our own data, a larger exclusion limit (Fig. 1). Although we still favor the conclusion that *P. aeruginosa* has a large exclusion limit, there is some doubt as to whether OprF constitutes the large pore (Woodruff and Hancock, 1989). The exact nature of the dual pore size observed with OprF is currently being addressed by genetic manipulations of the four cysteines in OprF that have been hypothesized to be relevant to this phenomenon (Moore et al., 1987) and by investigations of antibiotic-resistant OprF-deficient (Piddock et al., 1987) or OprF-altered (Godfrey and Bryan, 1987) mutants.

OprF has been shown to have homology with OmpA of *E. coli* (see below), and the two proteins show many similarities (Table 3). *P. aeruginosa oprF* mutant strains show many defects similar to those of *E. coli ompA lpp* double mutants, including leakage of periplasmic contents, a rounded morphology, and a need for osmotic stabilization (Woodruff and Hancock, 1989; Gotoh et al., 1989; Sonntag et al., 1978). Furthermore, OprF expressed from the cloned gene could restore elongated morphology to an *E. coli ompA lpp* double mutant (Woodruff and Hancock, 1989), suggesting that the two proteins have interchangeable roles in cell shape determination. Since mutations preventing expression from the *oprF* gene of *P. aeruginosa* are pleiotropic, it is difficult to evaluate the effect of the loss of the porin function even though such mutants arise from a single gene mutation. In the case of constructed insertion mutations in the *oprF* gene, the most overwhelming effects observed were on structural properties and on nonspecific permeability, which could conceivably mask the effect of porin loss (Woodruff and Hancock, 1988, 1989). Nevertheless, such mutants showed a 1.3- to 3.4-fold increase in resistance to a variety of β-lactam antibiotics (Woodruff and Hancock, 1988), thus favoring a role for OprF as porin (although these susceptibility changes are marginal). In contrast, two other groups have published data on OprF-deficient (Piddock et al., 1987) and OprF-altered (Godfrey and Bryan, 1987) mutants with large increases in antibiotic resistance compared with the wild type. However, these mutants require further genetic characterization.

We feel that future studies should rely on point mutations or small insertions or deletions that might disrupt the pore-forming domain without severely affecting the structural domain of OprF. These mutations and their resultant phenotypes

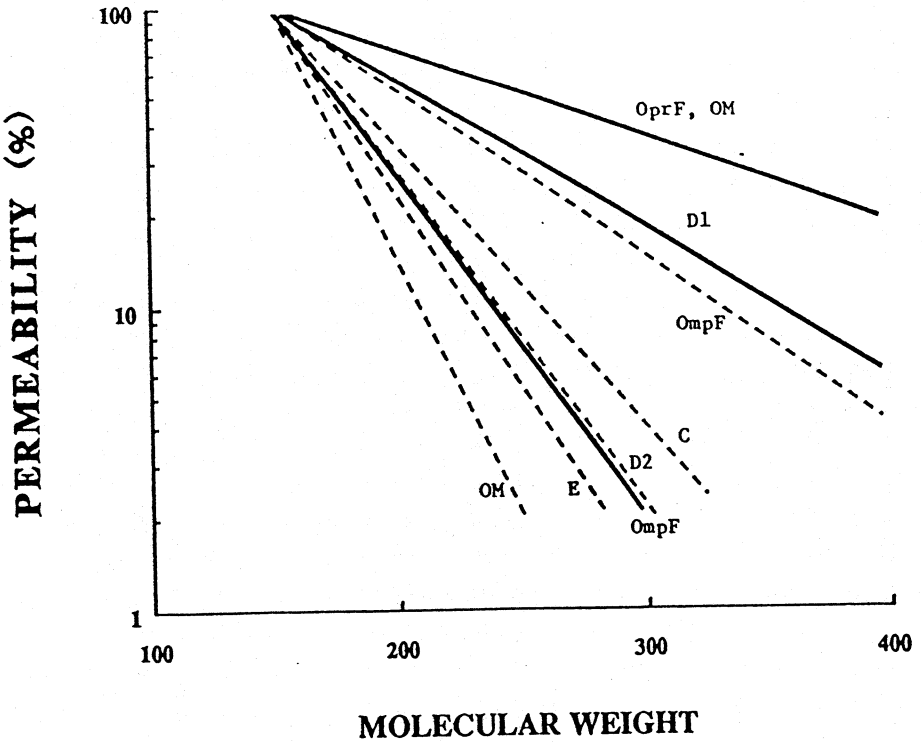

FIGURE 1. Influence of substrate molecular weight on the swelling of liposomes reconstituted with various *P. aeruginosa* porins or outer membrane (OM) preparations or with *E. coli* OmpF porin. For clarity, individual datum points have been omitted and the lines have been adjusted to give 100% swelling with arabinose (molecular weight, 150). The solid lines are data from Nikaido and collaborators as follows: OprF (=F), outer membrane, and D1 (Yoshimura et al., 1983; Trias et al., 1988); and OmpF from *E. coli* (Nikaido and Rosenberg, 1983; Yoshimura et al., 1983). The dashed lines are data from Nakae and collaborators as follows: outer membrane (Yoshihara et al., 1988); C, D2 (=D), and E (Yoshihara and Nakae, 1989); and OmpF from *E. coli* (Yoshihara and Nakae, 1989; a steeper slope was observed by Yoshihara et al. [1988]). Yoshihara and Nakae (1989) observed only weak porin activity for the OprF porin in this assay. Note that major discrepancies were observed in the slopes of the lines observed for both *P. aeruginosa* outer membrane and *E. coli* OmpF. The data for OmpF (solid line), when fit to the Renkin equation, predicted a pore diameter of 1.16 nm, and the data for OprF predicted a 2-nm pore, consistent with other data (Hancock, 1986).

should then be genetically transferred to another strain and their association with antibiotic resistance confirmed. Alternatively, if OmpA from *E. coli* could complement structural defects in *P. aeruginosa oprF* mutants, the effects on antibiotic resistance of the loss of the OprF porin might be more accurately assayed. We consider such manipulations to be absolutely necessary to unequivocally resolve the role of OprF as the major *P. aeruginosa* porin.

OprP

OprP is the porin component of a high-affinity phosphate uptake system that is induced when *P. aeruginosa* is grown in an environment deficient in phosphate

TABLE 3
Comparison of the *E. coli* OmpA protein with the *P. aeruginosa* OprF protein

Protein	Property													
	Required for growth in low-osmolarity medium	Required for rod-shaped morphology	Immunological cross-reactivity	Sequence relatedness (C terminus)	Mol wt	β structure (β sheet + β turn) (%)	Change in apparent mol wt		Peptidoglycan association	Lipopolysaccharide association	Products of chemical cross-linking	Role in conjugation	Porin function	No. of cysteines
							Upon heating	Upon trypsin treatment						
OmpA	+[a]	+[a]	+	+	35,159	77	6,000	8,000	+	+	Dimers, trimers	F plasmids	?	2
OprF	+	+	+	+	35,250	82	6,000	8,000	+	+	Dimers, trimers	P1 plasmids	+	4

[a] *lpp* mutant background.

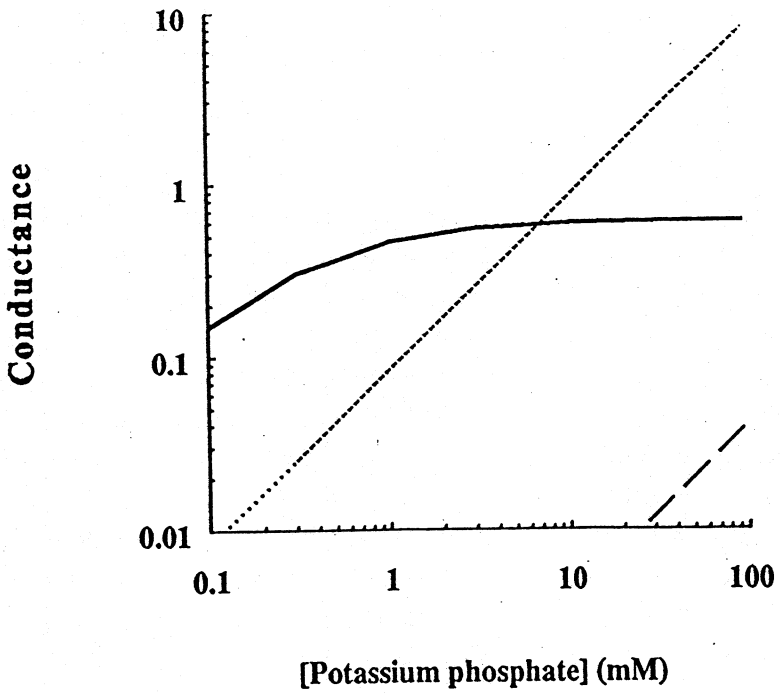

FIGURE 2. Benefit of a specific phosphate channel in the *P. aeruginosa* outer membrane. The total conductances (i.e., transmembrane flux in microsiemens) at various concentrations of potassium phosphate for 10^5 OprP channels (——), 2×10^5 small OprF channels (. . .), and 200 large OprF channels (- - -) were estimated on the basis of published single-channel conductance experiments (Benz and Hancock, 1981; Woodruff et al., 1986; Hancock and Benz, 1986). These numbers of channels represent crude estimates of the numbers of each porin species in a given cell 1 h after induction of OprP by shifting *P. aeruginosa* to phosphate-deficient medium. Note that because OprP contains a phosphate-binding site (K_d of 0.3 mM), it exhibits saturable flux, since association-dissociation at the binding site is rate limiting. At a physiologically relevant phosphate concentration (0.15 mM), at least 20 times more phosphate will pass through OprP than through both OprF channels.

(Hancock et al., 1982). This inducible system also involves a periplasmic phosphate-binding protein (Poole and Hancock, 1983, 1984) and is apparently similar to the high-affinity Pst system induced under similar conditions in *E. coli* (Willsky and Malamy, 1980). The analogous porin in *E. coli* is the PhoE protein. The regulatory mechanism that controls the expression of these regulons appears to have been well conserved. A well-characterized *cis*-acting regulatory sequence of the *pho* regulon (the *pho* box) has been identified preceding the *oprP* gene from *P. aeruginosa* and shown to function in *E. coli* (Siehnel et al., 1988b). In addition, a *phoB*-like gene and a *phoR*-like gene isolated from *P. aeruginosa* have been shown to complement, respectively, the *phoB* and *phoR* regulatory genes of *E. coli* (Filloux et al., 1988). Although this system appears to be conserved, the porins induced show marked differences. PhoE is a general diffusion porin that forms large (1.1 nm), weakly anion selective channels (Benz et al., 1984). In contrast, OprP forms constricted (0.6 nm), anion-specific channels with a saturable phosphate-binding site (Hancock and Benz, 1986; Fig. 2). OprP has been shown to have a K_d of 0.3 mM for phosphate binding at pH 7 (compared with a K_d

of 40 mM for chloride binding [Hancock and Benz, 1986]). It appears to work in conjunction with a periplasmic phosphate-binding protein (K_d of 3.4×10^{-7} M for phosphate) in vivo (Poole and Hancock, 1984; Hancock et al., 1987). Although OprP binds a variety of anions, its affinity for phosphate is at least 60 to 100 times greater than for other anions (Hancock and Benz, 1986), demonstrating its substrate-specific characteristic. Thus, although OprP has a smaller channel than PhoE and the larger OprF channel, it is a much more efficient channel for the transport of phosphate than these general porins at low, physiologically relevant, external phosphate concentrations (e.g., 0.15 mM phosphate; Fig. 2). The low numbers of the large OprF pores per cell and the small channel size of the other OprF pores preclude OprF as an important uptake system for sequestering phosphate under these conditions (Fig. 2). However, OprP is induced at higher phosphate levels in strains lacking OprF (W. A. Woodruff and R. E. W. Hancock, Ph.D. thesis, University of British Columbia, Vancouver, British Columbia, Canada, 1988), indicating that OprF influences the amount of phosphate available to interact with the primary signal controlling the *pho* regulon within the cell and may constitute a secondary phosphate uptake system across the outer membrane.

As suggested above, the saturable nature of OprP makes it amenable to kinetic studies. Since OprP can be expressed in a functional conformation in *E. coli* (Siehnel et al., 1988a), we are now using site-directed mutagenesis to investigate regulatory, functional, and structural aspects of this specific porin (i.e., the identification of the amino acids involved in phosphate binding).

STRUCTURAL ASPECTS OF *P. AERUGINOSA* PORIN PROTEINS

Porin proteins were first isolated from *E. coli*, and the OmpF (matrix protein) was the first porin from which crystals that diffracted to high resolution were grown (Garavito and Rosenbusch, 1980; Garavito et al., 1983). Although many attempts have since been made to elucidate the three-dimensional structure of OmpF and several other bacterial porins by X-ray diffraction and by electron and optical diffraction of specimens prepared for electron microscopy, the actual molecular structure of a porin is yet to be determined. Given that a definitive model of porin structure is not possible until the crystallographic data have been resolved, this section summarizes the structural data available on porins F and P from *P. aeruginosa*, compares these data with information available on other bacterial porins, and presents structural models for these proteins.

OprF

The most prominent constitutively expressed protein in the *P. aeruginosa* outer membrane is OprF. This protein is heat and 2-mercaptoethanol modifiable. Unheated OprF bands at an apparent molecular weight of 36,000, and exhaustively heated (100°C for 60 min) OprF bands at 41,000 in the presence of 2-mercaptoethanol. Without 2-mercaptoethanol, unheated OprF bands at 33,000 and heated OprF bands at 39,000 (Hancock and Carey, 1979). The stability of the

protein to boiling in sodium dodecyl sulfate (SDS) might be attributed to the strong association of β strands, since the protein has a high β content, as assessed by circular dichroism (Table 1). The ability of 2-mercaptoethanol to modify the gel mobility of OprF has been ascribed to the possession of two intrachain disulfide bonds (Hancock and Carey, 1979; see below). Small amounts of oligomeric forms of OprF have been observed on Western blots (immunoblots) reacted with monoclonal antibodies specific for OprF (Mutharia and Hancock, 1985). Chemical cross-linking has confirmed that native OprF forms an oligomeric (possibly a trimeric) structure (Angus and Hancock, 1983). Although lipopolysaccharide is normally associated with OprF and can be chemically cross-linked to OprF (Angus and Hancock, 1983), the presence or absence of lipopolysaccharide in protein samples run on SDS-gels does not seem to affect the electrophoretic mobility of OprF (Hancock and Carey, 1979).

Beher et al. (1980) previously described heat-modifiable outer membrane proteins in a large number of enteric and nonenteric bacteria, including *P. aeruginosa* OprF, which were similar to OmpA. The nucleic acid sequence of the *oprF* gene was determined by Duchêne et al. (1988), and they found a short stretch of the deduced amino acid sequence of OprF to be homologous with a 30-amino-acid stretch of OmpA from *E. coli* and from *Enterobacter aerogenes*. Further analysis has shown that the carboxy-terminal half of OprF (from residues 146 to 326) exhibits distinct similarity to the entire carboxy-terminal half (residues 177 to 335) of OmpA from *E. coli* and also to the majority of the amino acid sequence of protein III from *Neisseria gonorrhoeae* (Gotschlich et al., 1987a; Gotschlich et al., 1987b; Woodruff and Hancock, 1989). A number of the OmpA-like proteins have been sequenced, and a comparison of their amino acid sequences shows that many of these proteins are much more alike within the carboxy-terminal region than in the amino-terminal region (Klugman et al., 1989; Gotschlich et al., 1987a; Gotschlich et al., 1987b). This relatively constant maintenance of amino acid composition suggests that the carboxy-terminal portion provides an essential functional region of OmpA.

Although there is very little direct amino acid homology in the amino-terminal halves of OmpA and OprF, a comparison of their antigenic indices, which are compiled by summing several weighted measures of secondary structure (hydrophobicity, surface probability, flexibility, and the Chou-Fasman and Garnier-Osguthorpe-Robson predictive methods [Garnier et al., 1978]), shows that the tertiary structures of these proteins are probably quite similar in this region (N. L. Martin and R. E. W. Hancock, in press). Figure 3 consequently predicts OprF, by analogy to OmpA (Klose et al., 1988), to have eight membrane-spanning sequences from residues 1 to 145.

The current predictive model of OmpA from *E. coli* places amino acids 177 to 335 in the periplasm of the cell (Vogel and Jähnig, 1986; Klose et al., 1988), based primarily on the accessibility of a protease cleavage site that releases this carboxy-terminal portion of the protein and on the complete lack of cell surface-exposed areas found in the carboxy-terminal half during extensive mapping studies with spontaneous and genetically engineered mutants resistant to OmpA-specific bacteriophages (Manoil and Rosenbusch, 1982; Morona et al., 1984;

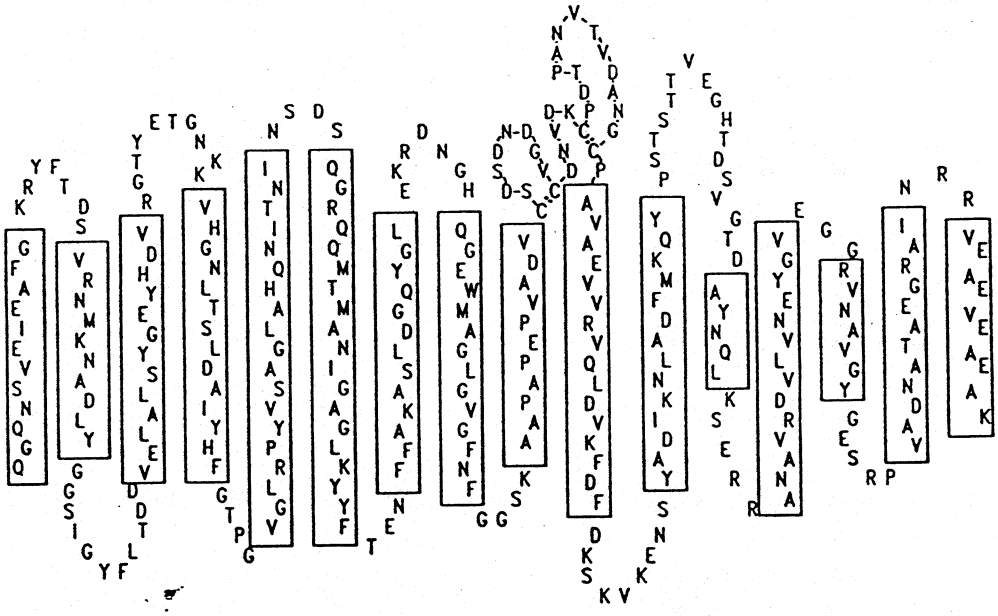

FIGURE 3. Conceptual model of the structure of OprF. Regions predicted (by the method of Paul and Rosenbusch [1985]) to have β structure are boxed and are assumed to sit within the membrane. Positions of the β strands are drawn on the basis of the model of OmpA except that the carboxy-terminal region of OprF is placed in the membrane for reasons discussed in the text, whereas the homologous (Woodruff and Hancock, 1989) region of OmpA has been previously assigned to the periplasm (Morona et al., 1984; Klose et al., 1988).

Klose et al., 1988). In contrast, the carboxy-terminal portion of OprF appears to be surface exposed. Molecular genetic manipulation of the gene for OprF has permitted localization of the surface-exposed epitope of monoclonal antibody MA5-8 to the carboxy-terminal half of the molecule (W. A. Woodruff and R. E. W. Hancock, unpublished data).

Interestingly, in the amino acid comparisons of the second halves of OmpA, protein III, and OprF, the regions that do not match at all are limited to the areas containing cysteine residues in each of these proteins (Woodruff and Hancock, 1989). The four cysteines in OprF are thought to form two disulfide bonds (Hancock and Carey, 1979). In our working model, these disulfides have been placed on the outside surface of the cell, since they may be involved in maintaining the surface-exposed, conformational epitope recognized by monoclonal antibody MA4-4, which is recognized by the antibody only in the unreduced form of OprF (Mutharia and Hancock, 1985).

On a more general level, the large amount of β-sheet structure in outer membrane proteins sets them apart from most other proteins that have been studied in detail. As a result of this predominance of β-sheet content, most existing predictive methods of protein secondary structure greatly underestimate the actual β-sheet content, since these methods were developed for soluble proteins (Garnier et al., 1978) or for membrane proteins with membrane-spanning α helices (Kyte and Doolittle, 1982). Also, these predictive methods do not take

TABLE 4
Comparison of the predicted secondary structure of OprF, using CD and the prediction method of Garnier et al. (1978)

Structure	Value (% of total)				
	CD, 1–326[a]		Prediction method		
	RT[b]	100 C[c]	1–326	1–161	161–326
α Helix	9	22	30	24	36
β Sheet	62	39	25	25	24
β Turn	20	15	22	23	21
Random coil	10	22	24	29	19

[a] Amino acid residue number.
[b] Room temperature in 10 mM Tris–0.1% SDS.
[c] 100°C for 15 min in 10 mM Tris–0.1% SDS.

into account tertiary and quaternary interactions within the native protein and between protein oligomers. Table 4 outlines secondary-structure predictions for OprF, obtained by using circular dichroism (CD) (C. M. Kay, W. D. McCubbin, E. A. Worobec, N. L. Martin, and R. E. W. Hancock, unpublished data) and the predictive method of Garnier et al. (1978). The CD values obtained after boiling the protein in 0.1% SDS most closely resemble the structure predictions from the amino acid sequence, whereas the CD values for native protein indicate a much higher β-sheet content and very little α helix. However, it should be noted that the mild boiling in SDS performed in the CD experiments shown in Table 4 was not sufficient to cause OprF to alter its migration on SDS-polyacrylamide gels (to its heat-modified position). Thus, it must retain considerable secondary structure, as also indicated by CD. We assume that boiling in 0.1% SDS probably destroys tertiary associations within the protein, rendering it most like a soluble protein and therefore corresponding more closely to predictions based on the amino acid sequence alone. The high content of β-sheet structure in native OprF is compatible with much of the protein being inserted in the membrane (Fig. 3).

OprP

The trimeric nature of a functional OprP porin implies that specific molecular interactions within the monomer and between the monomeric units form the phosphate-selective pores. To this end, OprP is a very stable protein. Although trimers are readily dissociated into monomers at 88°C in 2% SDS, dissociations within the monomer are difficult to induce. CD spectra have indicated a β-sheet content of 65%, which only drops to 55% after boiling in 0.1% SDS, along with changes from 3 to 8% for α helix, 26 to 25% for β turn, and 6 to 12% for random coil (Worobec et al., 1988). OprP trimers, even when they are lipopolysaccharide free, are also resistant to digestion with several proteases and maintain their normal SDS-polyacrylamide gel mobility and porin function when attacked by other proteases. In the latter case, one can observe alterations in gel mobility only when trimers are dissociated into monomers before electrophoresis (Worobec et al., 1988).

TABLE 5
Degree of alignment of paired amino acid sequences of bacterial porins

Porins compared	Matches	Conservative substitutions[a]	Gaps introduced
PhoE[b] versus OmpF[b]	230	49	9
PhoE versus OprP[c]	67	59	12
OprP versus P2[d]	52	63	8
OprP versus LamB[b]	76	77	18
P2 versus OmpF	95	94	35

[a] Alignment of sequences was optimized by using the FAST-P algorithm, and conservative substitutions between sequences were assessed by the Dayhoff minimum-mutation matrix (Doolittle, 1986), using a matching score of 0.9 as a cutoff.
[b] From *E. coli*.
[c] From *P. aeruginosa*.
[d] From *H. influenzae*.

Although OprP shows little amino acid sequence homology with OprF (R. J. Siehnel, N. L. Martin, and R. E. W. Hancock, manuscript in preparation), it does exhibit some similarity with porin protein P2 from *Haemophilus influenzae* type B (Vachon et al., 1987) and several regions of homology with LamB and PhoE from *E. coli* (Table 5) which often correspond to cell surface-exposed areas mapped by phage binding and monoclonal antibodies in LamB (Saurin et al., 1989). These regions of homology suggest that these proteins may have evolved from a common ancestral porin protein.

Since OprP contains a phosphate-binding site and is directly involved in the uptake of phosphate, the three-dimensional structure of this protein is expected to be somewhat different from those of the general diffusion porins such as OprF and OmpF. Chemical modification studies aimed at examining the role in ion conductance of charged ε-amino groups of lysine residues are in agreement with this proposal (Hancock and Benz, 1986; Hancock et al., 1986).

Attempts thus far to further define the three-dimensional structure by crystallography have produced crystals too small to allow X-ray analysis (Worobec et al., 1988). It is hoped that combining ongoing studies involving analyses by electron microscopy and optical diffraction with continued efforts at crystallography and analysis by genetic manipulation will provide more information on the molecular structure of OprP in the near future.

SUMMARY

The porin proteins embedded in the outer membrane of *P. aeruginosa* are involved in both nonspecific (e.g., OprF) and specific (e.g., OprP) uptake of molecules across the outer membrane. In artificial membrane systems, OprF preparations demonstrate two distinct pore sizes. Several lines of evidence suggest that OprF is the major porin of *P. aeruginosa*, although this is currently disputed in the literature. In addition to having a permeability function, OprF plays a structural role in maintaining cell shape and in permitting growth in

low-osmolarity medium. Indeed, it is both structurally and functionally analogous to the OmpA protein from *E. coli*. OprP, a phosphate-selective porin, is produced only when external phosphate concentrations necessitate a more efficient uptake system for phosphate (in which OprP plays a role). Thus, OprP is part of an adaptation by the cell to allow it to survive under new environmental conditions. Consequently, it can be considered broadly analogous to the *E. coli* LamB maltoporin, with which it demonstrates some sequence relatedness (Table 5). Although the function of porin proteins has been studied for a number of years, it is only recently that the details of the physical structure of these proteins are beginning to be understood. Various techniques have been used quite ingeniously to extract small bits of data, which have then been assembled into models for further, more rigorous testing. By adapting models for the *E. coli* OmpA and LamB proteins, we have now created secondary-structure models of OprF (Fig. 3) and OprP (Siehnel et al., in preparation), respectively. Ultimately, however, crystallographic analysis is the only technique that will provide details on the structure of porins sufficient for a complete understanding of their function. Until the puzzle of porin structure is solved at a molecular resolution, porins will remain only functionally defined as proteins containing water-filled channels that allow compounds into and out of the bacterial cell.

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