

Pseudomonas aeruginosa Outer Membrane Protein F: Structural Role and Relationship to the *Escherichia coli* OmpA Protein

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A *Pseudomonas aeruginosa* outer membrane protein F-deficient Ω -insertion mutant strain H636, in contrast to its protein F-sufficient parent strain H103, was unable to grow on unsupplemented Proteose Peptone no. 2 broth (Difco Laboratories, Detroit, Mich.). Addition of high concentrations of NaCl, KCl, glucose, sucrose, or potassium succinate permitted growth of strain H636 at rates approaching those of the parent strain H103. Strain H636 cells were 33% shorter and had a 46% smaller cross-sectional area than did the parent strain growing at similar rates on the same medium. These properties of the *oprF*: Ω mutant were analogous to those previously observed for *Escherichia coli* *ompA* mutants in an *lpp* (Braun lipoprotein-deficient) mutant background. Therefore, we compared *P. aeruginosa* protein F and the *E. coli* OmpA protein. In addition to many similarities previously described, sequence alignment demonstrated substantial amino acid sequence homology throughout the carboxy-terminal 168 to 180 amino acids of the two proteins. Consistent with this observation, polyclonal antiserum specific for OmpA reacted on Western blots (immunoblots) with protein F. Expression of protein F from the cloned *oprF* gene in an *E. coli* *ompA lpp* double mutant resulted in a 1.7-fold increase in cell length and a 2.1-fold increase in cross-sectional area compared with values for the same mutant containing only the plasmid vector onto which the *oprF* gene had been cloned. These results favor a structural role for *P. aeruginosa* protein F and suggest that it is strongly related to the *E. coli* OmpA protein.

Outer membranes of gram-negative bacteria contain three classes of components: proteins, lipopolysaccharide, and lipids. Some of the proteins are present in very high copy number (5×10^4 to 2×10^5 copies per cell) and are therefore called major proteins (11). To date, only a limited variety of functions have been ascribed to the constitutive major outer membrane proteins of gram-negative bacteria. In *Escherichia coli* K-12, for example, there are four major outer membrane proteins found under many growth conditions. Proteins OmpF and OmpC function as porins in that they contain water-filled channels which pass through the outer membrane and constitute the major channels for hydrophilic substances smaller than the size exclusion limits of the channels. The OmpA protein has also been proposed to have a porin function (12), although this is somewhat disputed (15). It is associated with the underlying peptidoglycan (11). OmpA protein, together with the Braun lipoprotein, has been demonstrated to have a structural role in the stability and shape maintenance of *E. coli*, since *E. coli* *ompA lpp* double mutants do not grow well in medium unsupplemented by excess cations and adopt a rounded shape (19). In addition, OmpA functions in stabilizing mating aggregates with F⁺ donor cells (1, 18). The fourth major outer membrane protein, the Braun lipoprotein, is present in two forms, free or covalently bound to the underlying peptidoglycan (11). As mentioned above, it, together with the OmpA protein, apparently has a cell structural role. There is some evidence from *Salmonella* *lkyD* mutants (6) that it is the bound form of lipoprotein which has the most significant role in outer membrane stability.

Pseudomonas aeruginosa has only three constitutive proteins, F, H2, and I, present in very high copy numbers (although five other proteins are also prominent in the *P. aeruginosa* outer membrane [10]). Protein H2, the peptidoglycan-associated lipoprotein, and protein I, the Braun

lipoprotein equivalent, have as yet no ascribed functions, since no mutants lacking these proteins have been isolated to date. Interestingly, however, protein I does not have a peptidoglycan-bound form (10). Protein F has been shown to function as a porin (14, 16, 22), and there is some evidence that it constitutes the major uptake route for hydrophilic substances across the outer membrane (14, 16, 21). Each of these three outer membrane proteins is peptidoglycan associated (10), although generally speaking less strongly associated than the *E. coli* OmpA protein (in that lower temperatures of heating in sodium dodecyl sulfate [SDS] suffice to displace proteins F, H2, and I from the peptidoglycan).

A chemically mutagenized *oprF* mutant lacking protein F was previously shown to require salt supplementation to proteose peptone no. 2 (PP2) broth (Difco Laboratories, Detroit, Mich.) for growth (14). This implied that protein F might also have a structural role in *P. aeruginosa*. Unfortunately, this mutant tended to revert readily. However, by cloning the *oprF* gene, we were able to mutagenize this gene in vitro with the antibiotic resistance cartridge Ω and in vivo with transposon Tn1 and, by recombination into *P. aeruginosa*, isolate stable insertion mutants (21). As mentioned previously (21), these mutants also required salt supplementation in PP2 broth for growth. Furthermore, the recently available sequence of the *oprF* gene (5) demonstrated a region of limited homology between protein F and the OmpA protein (19 direct matches in a stretch of 30 amino acids from each of the two genes). In this study, we investigated the potential structural role of protein F in *P. aeruginosa* cells and the relationship of this protein to the *E. coli* OmpA protein.

MATERIALS AND METHODS

Bacterial strains and growth media. *P. aeruginosa* prototrophic strain H103 and its protein F-deficient derivative H636 *oprF*: Ω were described previously (21). Derivatives H103(R68.45) and H636(R68.45) were constructed by conju-

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gation of plasmid R68.45 from an auxotrophic *P. aeruginosa* donor, PAO25 *argF leu-10*(R68.45), into strains H103 and H636, respectively, and selecting for a plasmid-derived marker, encoding resistance to 200 µg of tetracycline per ml, and growth on unsupplemented BM2 succinate (14) plates to counterselect against the donor. *E. coli* K-12 strain C386 *lpp ompA tsx* (19) was obtained from U. Henning, University of Tubingen, Tubingen, Federal Republic of Germany. Plasmid pRK404 and its derivative pWW2200, containing a 2.4-kilobase-pair *PstI* insert encoding the *oprF* gene, were introduced into strain C386 by transformation as described previously (21).

Strains H103 and H636 were grown with shaking at 37°C in PP2 medium with the described supplements. *E. coli* C386(pRK404) and C386(pWW2200) were grown overnight with shaking at 37°C in LB broth (21) containing 15 mM MgSO₄ and 25 µg of tetracycline per ml (to ensure plasmid maintenance), diluted 100-fold into fresh supplemented LB broth, and grown with shaking at 37°C.

Sequence comparison. The published OmpA (2) and protein F (5) sequences were compared by using the program SEQNCE version PC3 (Delaney Software Ltd., Vancouver, British Columbia, Canada) and a FAST-P algorithm. Conservative substitutions were assessed by the minimum-mutation matrix scoring system of Dayhoff (3, 4), with a cutoff score of 0.9. By comparison, finding a conserved cysteine yields a score of 2.0, whereas conserved serines, alanines, and asparagines give a score of 1.0.

Growth and β-lactamase experiments. *P. aeruginosa* strains were grown overnight in PP2 broth containing 200 mM NaCl and then diluted 40-fold into sidearm flasks containing fresh PP2 broth with the desired concentration of NaCl, KCl, glucose, sucrose, or potassium succinate, pH 7.0. Cultures were incubated in a water bath with vigorous shaking, and cell density was monitored at hourly intervals over an 8- to 12-h period, using a Klett-Summerson photometer with a green filter.

For the shift experiments, strains H103(R68.45) and H636(R68.45) were grown with vigorous shaking at 37°C in PP2 broth supplemented with 50 mM NaCl to a density of 3×10^8 to 4×10^8 cells per ml. They were then diluted 15-fold into 30 ml of prewarmed unsupplemented PP2 broth in a sidearm flask and shaken vigorously in a water bath at 37°C. Thus, the final postshift concentration of NaCl in the PP2 broth was 3.3 mM NaCl. At the given intervals, cell density was assessed as described above, and 1-ml samples were taken for assessment of supernatant β-lactamase resulting from leakage of plasmid R68.45-encoded β-lactamase from its usual periplasmic location. Samples were centrifuged for 1 min at 9,000 × g in an Eppendorf centrifuge, and β-lactamase activity was assessed by using the chromogenic β-lactam nitrocefin as previously described (14).

Cell length and area measurements. Mid-logarithmic-phase cells (optical density at 600 nm of around 0.5 to 0.6) growing on LB broth containing 15 mM MgSO₄ (*E. coli* strains) or Muller-Hinton broth (*P. aeruginosa* strains) were heat fixed onto microscope slides and Gram stained. An important feature of these media was that they allowed nearly the same rates of growth regardless of the OmpA or protein F phenotype of the cells (2, 21). A cover slip was placed over the stained cells, and the cells were viewed under oil in a Zeiss Universal microscope, using ×100 magnification. Images obtained were digitized and analyzed with a SEM-IPS image analysis system (Kontron, Munich, Federal Republic of Germany). Briefly, gray images on a white background were digitized into 256 levels of gray and then discriminated

on the basis of their gray values into binary images, which were directly measured by computer to determine maximum diameter (length), minimum diameter (width), and total area of the image of the stained cell (delineated by the gray-to-white boundary).

Western blotting (immunoblotting). Western blotting was performed by the method of Mutharia and Hancock (13). OmpA-specific polyclonal rabbit antiserum was a kind gift of Milan Blake (Rockefeller University, New York, N.Y.). *E. coli* outer membranes were obtained from strain LE392 (*hsdR514 supE44 supF58 lacY1 GalK2 galT22 metB1 trpR55*).

RESULTS

Osmotic stabilization requirement for growth of the *P. aeruginosa oprF::Ω* mutant. We observed previously that both chemically (14) and insertion (21)-mutagenized protein F-deficient *P. aeruginosa* mutants required addition of salt to PP2 broth to permit growth. In this study, we attempted to characterize this phenomenon in more detail by comparing the growth properties of the protein F-deficient Ω-insertion mutant H636 *oprF::Ω* with those of the parent strain H103 *oprF⁺*. It should be noted that since the *oprF* gene apparently constitutes a single gene transcriptional unit (5), the effects observed in this study were related to the presence or absence of protein F rather than to polar effects on the production of other gene products. In addition, separate controls demonstrated that the effects observed were not due to the 2-kilobase-pair Ω sequences inserted into the *oprF* gene.

Addition of 50 mM NaCl to PP2 broth had little effect on growth of the wild-type strain H103 (Fig. 1A). In contrast, the *oprF::Ω* mutant H636 did not grow in PP2 broth in the absence of added salt (Fig. 1B) even after overnight incubation. However, progressively increasing amounts of NaCl up to 50 mM NaCl resulted in a progressive enhancement of growth. In PP2 broth supplemented with 200 mM NaCl, the rate of growth of strain H636 was only 20% slower than the rate of growth of strain H103 on unsupplemented PP2 broth (Table 1). A variety of other compounds were tested to see whether the reconstitution of growth of strain H636 *oprF::Ω* by NaCl addition was chemically specific. It was found that addition of KCl, glucose, sucrose, or succinate in high quantities resulted in partial or nearly complete reconstitution of the growth rate of strain H636 to a level approaching that of the wild type. This finding suggested that the requirement for NaCl for growth of strain H636 in PP2 broth was actually a requirement for an osmostabilizing substance. This result was consistent with our previous observation (21) that a rich broth with higher osmolarity, Muller-Hinton broth, supported very similar rates of growth for both strain H103 (51-min doubling time) and H636 (57-min doubling time).

In an attempt to investigate the basis for lack of growth in PP2 broth in the absence of an osmostabilizer, strains H103 and H636 *oprF::Ω* growing in the mid-logarithmic phase of growth were diluted 15-fold from PP2 broth containing 50 mM salt into PP2 broth with no added salt, to yield a final salt concentration of 3.3 mM. No substantial decrease in optical density (Table 2) or viable cells (data not shown) was observed immediately after the shiftdown. In addition, no immediate substantial increase in the level of periplasmic β-lactamase found in the supernatant was observed immediately after the shiftdown (Table 2). At longer times, there was a sixfold increase in the level of β-lactamase found in the

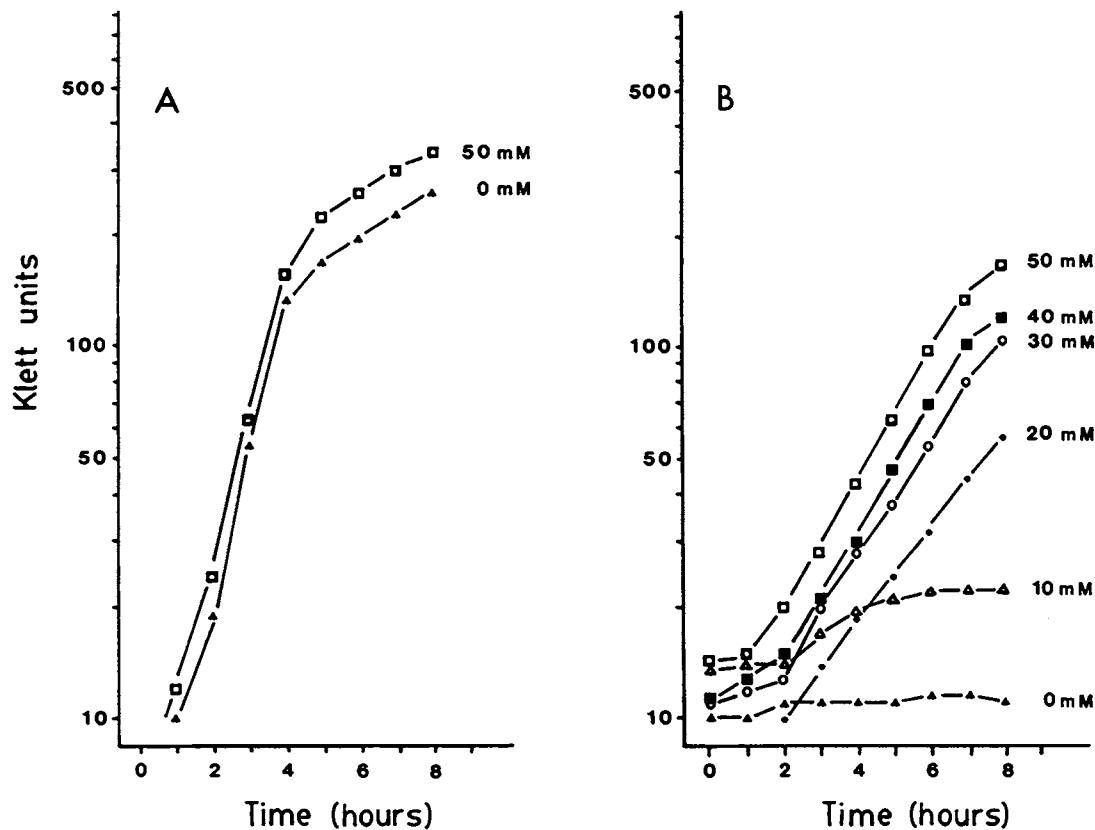


FIG. 1. Growth curves of strain H103 (A) and its derivative H636 *oprF*::Ω (B) on PP2 broth supplemented with the indicated concentrations of NaCl. For clarity, growth curves for strain H103 on PP2 broth supplemented with 10, 20, 30, and 40 mM NaCl have been omitted since they were superimposable on the growth curves shown.

supernatant of strain H636, which suggested progressive destabilization of the outer membrane of this strain. Similarly, there was a two- to three-fold increase in the level of β -lactamase in the supernatant of strain H103. This change in β -lactamase levels was not accompanied by cell lysis, as evidenced by microscopic observation and by the lack of change in the Klett reading. The most pronounced phenomenon observed in these studies was the high intrinsic leakage of β -lactamase from strain H636, i.e., 3.5-fold greater than the intrinsic leakiness of strain H103 in PP2 broth supplemented with 50 mM NaCl. The strain H103 culture resumed logarithmic growth after approximately 40 to 60 min. This

long lag period was probably due to oxygen shock experienced during the shift, since *P. aeruginosa* is an obligate aerobe. However, at 180 min strain H636 had still not started to grow, in agreement with the data in Fig. 1B.

Influence of protein F on cell morphology. Cells of the protein F-deficient mutant strain H636 appeared more rounded than those of the parent strain H103 when growing on the equivalent medium. To study this phenomenon in more detail, cells growing in the mid-logarithmic phase of growth in Muller-Hinton medium were examined microscopically and assessed by an image analyzer. This procedure

TABLE 1. Doubling times during the logarithmic phase of growth at 37°C for *P. aeruginosa* H103 and its derivative H636 *oprF*::Ω in PP2 broth culture with various supplements

Supplement	Concn (mM)	Doubling time (min)	
		H103	H636 <i>oprF</i> ::Ω
None		55	No growth
NaCl	50	47	142
	200	47	66
KCl	50	62	94
	200	55	67
Glucose	100	61	267
	200	70	107
Sucrose	100	66	103
	200	72	73
Potassium succinate	200	58	56

TABLE 2. Influence of shift from high (50 mM) to low (3.3 mM) NaCl-containing PP2 medium on the turbidity and leakage of β -lactamase from strain H103(R68.45) and its protein F-deficient derivative H636(R68.45)

Time of measurement	Normalized Klett reading ^a		Supernatant β -lactamase activity ^b	
	H103	H636	H103	H636
Preshift	27	20	6.9	25.9
Postshift				
1 min	25	21	8.6	41.2
10 min	23	21	10.7	74.6
20 min	23	21	8.6	122.6
30 min	28	23	13.7	132.4

^a Normalized by dividing the preshift Klett reading by 15 to take into account the dilution upon shifting.

^b Expressed as nanomoles of nitrocefin hydrolyzed per minute per milliliter of supernatant, normalized to a Klett reading of 100.

TABLE 3. Influence of a functional *oprF* gene on cell length and area in *P. aeruginosa* and in *E. coli* C386 *lpp* *ompA*^a

Strain	Relevant genotype	No. of cells assessed	Mean cell length (μm) \pm SD	Mean cell cross-sectional area (μm^2) \pm SD
<i>P. aeruginosa</i>				
H103	<i>oprF</i> ⁺	473	1.40 \pm 0.33	0.70 \pm 0.25
H636	<i>oprF</i> ::Ω	480	0.94 \pm 0.21 ^b	0.38 \pm 0.11 ^b
<i>E. coli</i>				
C386(pWW2200)	<i>lpp</i> <i>ompA</i> (<i>oprF</i> ⁺)	426	1.85 \pm 0.54	1.36 \pm 0.51
C386(pRK404)	<i>lpp</i> <i>ompA</i>	445	1.07 \pm 0.30 ^b	0.62 \pm 0.22 ^b

^a Cell widths were not significantly different, being an average of 0.57 μm for the *P. aeruginosa* strains and 0.72 μm for the *E. coli* strains.

^b Significantly different from the isogenic *oprF*⁺ control value; $P < 0.001$ by Student's *t* test.

demonstrated that strain H636 cells were on average 33% shorter and had a 46% smaller area than did the parent strain H103 (Table 3). There was no significant difference in the average widths of strains H103 and H636 (approximately 0.57 μm). Thus, although strain H636 was not spherical, it was certainly more rounded than strain H103. It should be noted that this morphological difference was not per se related to the osmotic effects observed above, since strains H103 and H636 grew at similar rates on Muller-Hinton broth (21).

Relationship of protein F to the *E. coli* OmpA protein. Duchene et al. (5) demonstrated that there was a stretch of 30 amino acids from the *P. aeruginosa* protein F sequence that showed strongly homology to a 30-amino-acid stretch of the *E. coli* OmpA protein in that 19 of 30 amino acids were identically placed. We searched for further homology by using a FAST-P algorithm, introducing suitable gaps in the sequences. By introduction of just five gaps, it was found that the homology between proteins F and OmpA extended over the entire carboxy-terminal halves of these proteins. Indeed, we observed 56 direct matches and 36 conservative substitutions between the carboxy-terminal 180 and 168 amino acids of proteins F and OmpA, respectively (Fig. 2). This generated a normalized alignment score of 258 (4), which was almost identical to that obtained by Doolittle in comparing similar-sized stretches of human myoglobin and human hemoglobin (4). Therefore, we considered these regions of protein F and the OmpA protein highly homolo-

gous. Interestingly, the two largest gaps introduced were in the region encompassing the four cysteines of protein F (which form disulfide bonds [22]) and in the region near the two cysteines of OmpA (which do not form a disulfide bond). No similar extensive homologs were observed at the amino termini of the two proteins, and many gaps had to be introduced to permit partial alignment of the sequences.

To confirm this apparent sequence homology, Western blots were performed with polyclonal antisera raised against the OmpA protein. Immunological cross-reactivity was observed in nine independent experiments using either whole outer membranes (Fig. 3) or purified proteins F and OmpA (data not shown).

E. coli *ompA* *lpp* double mutants were previously demonstrated to have almost spherical shapes (19). In contrast, *lpp* mutants that were *ompA*⁺ had normal rod-shaped morphology. Therefore, we decided to determine whether protein F could substitute for the OmpA protein in restoring normal morphology. Derivatives of *E. coli* K-12 C386 *ompA* *lpp* harboring plasmid pRK404 or pWW2200 were created. These plasmids differed by the presence of a 2.4-kilobase-pair *PstI* insert encoding the gene for protein F. In control experiments, we demonstrated that C386(pWW2200) produced protein F in amounts equivalent to those demonstrated previously in other genetic backgrounds (22; i.e., protein F was a major outer membrane protein).

E. coli C386(pRK404) was 43% shorter and had a 54% smaller area than did strain C386(pWW2200), which expressed protein F (Table 3). These differences were highly statistically significant and analogous to the differences observed in protein F-sufficient and -deficient *P. aeruginosa* strains (Table 3).

DISCUSSION

The data presented in this paper strongly suggest that outer membrane protein F has a structural role in *P. aeruginosa* cells and that it has substantial homology to another structural outer membrane protein, the *E. coli* OmpA protein. Thus, comparison of a defined *oprF*::Ω insertion mutant with its protein F-sufficient parent suggested a role for protein F in growth in low-osmolarity media and in maintenance during growth of elongated rods. The reason why the *oprF*::Ω mutant failed to grow in unsupplemented PP2 broth remains somewhat obscure. Clearly, growth could be promoted by substantial concentrations of both charged and uncharged compounds, which suggested that these sub-

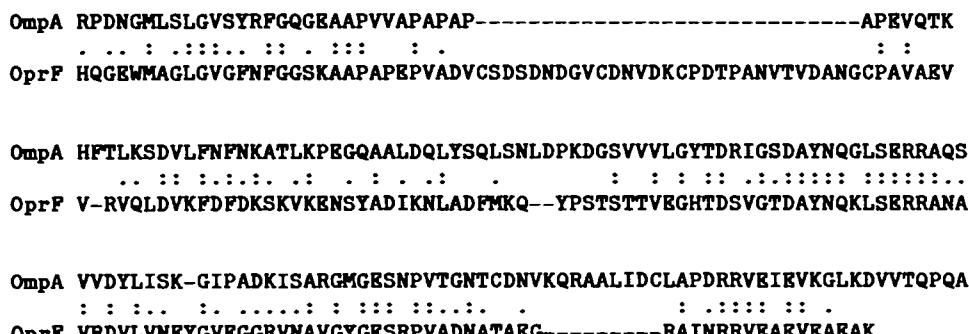


FIG. 2. Comparison of the carboxy-terminal 168 amino acids of the *E. coli* OmpA protein (residues 177 to 335) with the 180 carboxy-terminal amino acids of *P. aeruginosa* protein F (residues 146 to 326). Alignment of sequences was optimized by introduction of two large gaps and three one- to two-amino-acid gaps with the assistance of the FAST-P algorithm as described in Materials and Methods. : Direct matches. After alignment of sequences, conservative substitutions (.) between sequences were assessed by the Dayhoff minimum-mutation matrix (3, 4), using a matching score of 0.9 as a cutoff.

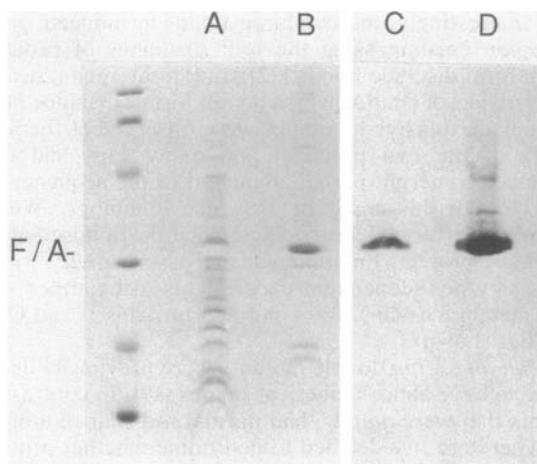


FIG. 3. SDS-polyacrylamide gel (lanes A and B) and corresponding Western blot probed with OmpA-specific antiserum (lanes C and D) of the *P. aeruginosa* H103 (lanes A and C) and *E. coli* LE392 (lanes B and D) outer membrane proteins. Samples (10 µg of protein) were solubilized at room temperature in solubilization-reduction mix without 2-mercaptoethanol before SDS-polyacrylamide gel electrophoresis and Western transfer to nitrocellulose. Under these conditions, the two proteins had similar electrophoretic mobilities (F/A). The additional minor bands and smearing in lane D are apparently artifacts resulting from lack of heating during solubilization, since they were not observed when samples were heated to 100°C for 10 min before electrophoresis. The left-most lane consists of molecular weight standards phosphorylase B (97,400), bovine serum albumin (66,200), ovalbumin (42,700), carbonic anhydrase (31,000), trypsin inhibitor (21,500), and lysozyme (14,400). Analogous data were obtained for preparations solubilized at 100°C for 5 min in solubilization-reduction mix containing 2-mercaptoethanol.

stances behaved osmotically rather than through electrostatic or specific chemical interactions with cells. However, upon shiftdown from moderate- to low-salt-containing medium, we observed no lysis, no marked decrease in viability, and no immediate increase in leakage of periplasmic β -lactamase. This finding suggested that osmoprotection was not required per se to osmotically stabilize cells. Indeed, the fourfold-higher leakage of β -lactamase from the protein F-deficient mutant H636 cells growing logarithmically on PP2 broth containing 50 mM NaCl may be related to the general property of altered outer membrane permeability in this strain. In keeping with this proposal, strain H636 was previously shown to have a substantial increase in outer membrane permeability to hydrophobic substances in comparison with the protein F-sufficient parent strain H103 (21). These two alterations in outer membrane permeability may have a common basis, since polymyxin B treatment of wild-type cells causes increased uptake of hydrophobic probes and increased leakage of periplasmic β -lactamase (16).

Previous data have suggested that the *E. coli* OmpA protein has a structural role in a *lpp* (Braun lipoprotein-deficient) mutant background (19). The structurally most important form of Braun lipoprotein appears to be the 33% of lipoprotein molecules that are covalently bound to peptidoglycan (6). However, the equivalent *P. aeruginosa* lipoprotein I is apparently not present in a covalently bound form (10). Therefore, the situation in *E. coli* *lpp* mutants may be considered somewhat analogous to that in *P. aeruginosa* wild-type cells in that deletion of OmpA on the one hand and

protein F on the other leads to similar structural perturbations. It should be pointed out, however, that although the general observation of rounding of cells was quite similar in mutants lacking either of these proteins, other differences were observed. For example, whereas *E. coli* *ompA lpp* double mutants grow poorly in dilute nutrient broth and growth can be partly restored by addition of unspecified concentrations of NaCl, divalent cation chlorides at 15 to 30 mM are the preferred salts for reconstituting normal growth rates (9). In contrast, supplementation of strain H636 with 30 mM MgCl₂ did not substantially promote growth of strain H636 (data not shown).

Nevertheless, many favorable comparisons can be made between protein F and the OmpA protein. As shown here, they have strong amino acid sequence similarities in their carboxy-terminal halves, they cross-react immunologically, and protein F can apparently partly reconstitute elongated morphology in an *ompA lpp* double mutant. In addition, it has previously been shown that both proteins are heat modifiable on SDS-polyacrylamide gels in analogous fashions, remain bound to the peptidoglycan when outer membranes are treated with SDS in the presence of high salt or EDTA (although the peptidoglycan association of protein F is more easily disrupted by elevated temperatures [10, 11]), contain substantial β -sheet structure, can be cross-linked to higher oligomers with chemical cross-linking agents, are present in high copy number, and upon treatment with proteases yield a single proteolytic cleavage product that is approximately 6,000 to 8,000 daltons smaller (10, 11, 16, 17). In addition, *E. coli* *ompA* mutants are deficient as recipients in conjugation with F⁺ or Hfr donors (18), a phenomenon that demonstrates a role for *ompA* in stabilizing mating aggregates (1). Similarly, *oprF* mutants are deficient as recipients in conjugation with donors of the P1 incompatibility group plasmids (T. Nicas, Ph.D. thesis, University of British Columbia, Vancouver, British Columbia, Canada, 1984). Protein F has been shown to be a porin (9, 16), and its porin activity is expressed by protein F purified from *E. coli* strains containing the cloned *oprF* gene (22). Similarly, some studies have suggested that the OmpA protein may function as a porin for amino acids (12), although these claims have been disputed (15) and no definitive model membrane studies on the purified OmpA protein have appeared. Nevertheless, because of the many similarities between protein F and the OmpA protein, we are now embarking on such a study. It should be mentioned that there may be a family of OmpA-like proteins in gram-negative bacteria, since the pIII protein of *Neisseria gonorrhoeae* has significant sequence homology and cross-reacts immunologically with both OmpA (8) and protein F (W. A. Woodruff, unpublished results). In addition, *Haemophilus influenzae* has an outer membrane protein that cross-reacts immunologically with the OmpA protein (20).

Three months after this manuscript was submitted, a paper was published by Gotoh et al. (7) in which the authors confirmed (14, 21; Fig. 1) the influence of salt on growth of protein F-deficient mutants and presented data on enhanced β -lactamase excretion and rounded shape of protein F-deficient mutants. However, these authors suggested that the exclusion limit of *P. aeruginosa* was a monosaccharide, in contrast to conclusions derived from similar liposome-swelling experiments (reviewed in reference 16) which suggested the exclusion limit to be much larger. They further suggested that the *P. aeruginosa* outer membrane is not permeable to a variety of β -lactams to which it is markedly

susceptible, and we find ourselves unable to agree with these conclusions.

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