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Outer Membrane Proteins

5

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1. INTRODUCTION

Pseudomonas aeruginosa is a gram-negative bacterium and as such has an outer membrane. The general functions of the outer membrane include size-dependent exclusion of larger molecules, permitting selective permeability of smaller molecules, uptake of large and small polycations, specific facilitated uptake of certain substrates, export of excreted molecules including proteins, secondary metabolites and siderophores, maintenance of cell shape and growth in low osmolarity medium, binding of phages, bacteriocins, and pili during conjugation, serum resistance, and surface binding of antibodies and complement. Most of these functions have been described for *Pseudomonas aeruginosa* and result from the properties of outer membrane functions. It has been 5 years since the last review of the outer membrane proteins of *Pseudomonas aeruginosa* (*Hancock et al.*, 1990). Because of the vast amount of data available on this topic, we have concentrated on the information published since the previous review, but prior to the release of the *Pseudomonas aeruginosa* genomic sequence (http://www.pseudomonas.com).

2. ROLE IN ANTIBIOTIC SUSCEPTIBILITY

It is now well established that *Pseudomonas aeruginosa* is intrinsically antibiotic-resistant and that the low outer membrane permeability of this organism contributes to this high intrinsic (background) level of resis-

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Table I. (*Continued*)

Protein name	Other names	Apparent molecular weight[a]	Sequence accession number	Map position	Production conditions	Function	Identified in follow fluorescent pseudomonads[d]
OprO		48,000	M86648	SpeI-C DpnI-N	Inducible by low phosphate, stationary phase	Pyrophosphate uptake	
OprB	D1	46,000	X77131	–	Inducible by glucose	Glucose/sugar uptake	PP, PF
OprD	OprD2, D2, D	45,500	Z14065 X63152	SpeI-F DpnI-A	Low level constitutive	Basic amino and imipenem uptake	
OprE	E1	43,500	D12711	–	Anaerobic induction	Unknown specific substrate	
E	E2	44,000	–	–	Constitutive	Structural?	
OprF	F	38,000	M94078 M18795	SpeI-L DpnI-C	Constitutive	Major nonspecific porin/structural	PP, PS, PF, PC, PST, AV, PT
OprG	G	25,000	–	–	Low level constitutive. Induced in high iron, high Mg^{2+}	Unknown	
OprH	H1	21,000	M26954	SpeI-M DpnI-A	Low Mg^{2+}, Sr^{2+}, Mn^{2+}, and Ca^{2+}	Gated porin?; polycation/EDTA resistance	PP, PS, PF, PC, PST
OprL	H2	20,500	Z50191	–	Constitutive	Structural	All
OprI	I	8,000	M25761	SpeI-V DpnI-D	Constitutive	Structural	All

[a]Apparent molecular weight on SDS-PAGE. Actual molecular weights can be obtained from the sequences. Apparent molecular weights vary according to the SDS-PAGE system used.
[b]Physical map position not determined.
[c]Gene cloned but accession number not available at press time.
[d]Abbreviations: PP - *Pseudomonas putida*; PF - *P. fluorescens*; PS - *P. syringae*; PC - *P. chloraphis*; PT - *P. tolasii*; PST - *P. stutzeri*; AV - *Azobacter vinelandii*; All - all of the above Pseudomonads.

aerobactin (Liu and Shokrani, 1978) produced by enteric bacteria, ferrioxamine B produced by *Streptomyces spp.* (Cornelis *et al.*, 1987), and pyoverdines produced by other fluorescent pseudomonads (Hohnadel and Meyer, 1988).

The induction of high molecular weight, iron-regulated, outer membrane proteins by *P. aeruginosa*, in response to the presence of non-pseudomonad siderophores, occurs with desferral, a derivative of ferrioxamine B, and ferri-enterobactin. The latter enterobactin-mediated protein has been characterized. Poole *et al.* (1990), upon studying enterobactin-mediated iron uptake by a pyoverdine-deficient strain of *P. aeruginosa*, identified a novel 80,000-Da outer membrane protein. Mutant strains incapable of enterobactin-dependent iron uptake were also lacking this protein, suggesting that this protein an enterobactin receptor. The structural gene for this putative enterobactin receptor was later cloned and characterized (Dean and Poole, 1993). The gene product was termed PfeA for *Pseudomonas* ferric enterobactin receptor. The structural gene for this putative enterobactin uptake was expressed in E. coli and was capable of complementing a mutation in the *E. coli fepA* gene which encodes the *E. coli* FepA, enterobactin receptor protein (an 81,000-Da outer membrane protein). The *pfeA* gene codes for an 81,000-Da protein which has more than 60% homology to its *E. coli* counterpart. The most significant homology is in regions involved in ligand binding. In addition, and not surprisingly, the two proteins cross-react. *P. aeruginosa* PfeA protein can replace FepA in *E. coli*, hence reinforcing the similarity between enterobactin-dependent uptake between the two organisms. This suggests the existence of analogous components in *P. aeruginosa*, such as periplasmic and cytoplasmic membrane proteins. The identification of a TonB box at the N-terminus of PfeA and Fur binding sites upstream of the *pfeA* gene also indicates the similarities between the two systems because FepA function depends on interaction with the TonB protein (Lundrigan and Kadner, 1986) and the expression of FepA is under control of the *fur* gene (Earhart, 1987). The extent of the analogy between the two systems is currently under investigation.

The concomitant loss of pyoverdine production and decreased production of a 90,000-Da IROMP in *P. aeruginosa*, led Poole *et al.* (1991) to believe that this protein is a ferri-pyoverdine receptor. In addition, a mutant strain deficient in the production of this protein shows a substantial decrease in pyoverdine-mediated iron transport. Previous to this Cornelis *et al.* (1987) assigned this function to an 80-KDa IROMP. An 85-KDa protein has also been identified as a pyocin S-receptor in a clinicalisolate of *P. aeruginosa* (Gensberg *et al.*, 1992). It is likely that these three proteins are the same. Using the above mutant strains, more re-

tance(iewed in Hancock *et al.*, 1990). It is clear that reduced outer membrane permeability, by itself, is not sufficient to explain resistance, because antibiotics equilibrate across even the weakly permeable outer membrane of *Pseudomonas aeruginosa* in a few seconds. Thus a secondary mechanism must exist that takes advantage of defective permeability. For β-lactams, inducible chromosomal β-lactamase has been proposed as such a mechanism (Bayer *et al.*, 1987), whereas for other antibiotics, efflux is involved (Li *et al.*, 1994a,b; Poole *et al.*, 1993). There is also evidence that efflux may be involved in intrinsic (basal) resistance of *Pseudomonas aeruginosa* to β-lactams, in that *mexA*, *mexB or oprM* interposon mutants are supersusceptible to many β-lactams (Li *et al.*, 1995). However, although efflux pumps have quite loose specificity, they favor hydrophobic (or amphipathic), weakly positively charged compounds, whereas β-lactams are generally anionic or zwitterionic and reasonably hydrophilic. Furthermore, β-lactams act in the periplasm and thus face only one uptake/efflux barrier whereas the usual substrates for efflux pumps must cross two membranous barriers. In addition, there are considerable disparities in the extent to which susceptibility of some β-lactams is affected by *mexA* or *oprM* deletion. Therefore in this case, it might be necessary to exclude other possible secondary effects, e.g., on β-lactamase levels or inducibility, penicillin binding protein levels, or peptidoglycan biosynthesis, before one can definitively conclude that β-lactams are subject to efflux. Nevertheless, it is clear that low outer membrane permeability is an absolute determinant in the intrinsic antibiotic resistance of *P. aeruginosa* because specifically increasing outer membrane permeability by cloning in a large channel porin (Loop 5 deletion mutant of OprD) increases antibiotic susceptibility to many antibiotics by eightfold or more (Huang and Hancock, 1996).

3. IROMPs: FpvA, FptA, PfeA

Pseudomonas aeruginosa, like most bacteria, requires iron for growth. To sequester iron from its environment, this organism produces two iron chelators or siderophores, pyochelin and pyoveridine (Cox and Adams, 1985), which are produced under conditions of iron limitation. Up to seven iron-regulated outer membrane proteins have also been identified (Cornelis *et al.*, 1987), some of which have been classified as receptors for the iron-bound siderophores and hence are involved in the first step in the entry of iron into the cell. In addition to the two aforementioned siderophores, *P. aeruginosa* can utilize siderophores synthesized by other organisms, such as enterobactin (Polle *et al.*, 1990) and

Table I. Outer Membrane Proteins of *Pseudomonas aeruginosa*

Protein name	Other names	Apparent molecular weight[a]	Sequence accession number	Map position	Production conditions	Function	Identified in following fluorescent pseudomonads[d]
FpvA	IROMP	80,000	L10210 U07379	SpeI-J DpnI-B	Iron limitation	Ferripyoverdine uptake	PP, PF, PS, PC, PT
FptA	IROMP	75,000	U03161	SpeI-A DpnI-C	Iron limitation	Ferripyochelin uptake	
PfeA	IROMP	80,000	M98033	–[b]	Iron limitation, enterochelin present	Ferrienterobactin uptake	
OprC	C	70,000	Cloned[c]	–	Anaerobic induction, copper repressible	Copper transport	PST
OprJ	OprK	54,000	Cloned	–	Derepressed by mutation	Efflux	
AlgE	alg 76	54,000	M37181	SpeI-T DpnI-H	Coexpressed with alginate exopolysaccharide	Putative export of alginate	AV, PS, PP
OprN		50,000	–[b]	–	Derepressed by mutation	Efflux	
OprM	OprK	50,000	L23839	SpeI-H DpnI-E	Constitutive low level, can be derepressed	Efflux	
OprP	P	48,000	X53313	SpeI-C DpnI-N	Inducible by low phosphate	Phosphate uptake	PP, PS, PF, PC

(continued)

cently Poole *et al.* (1993) reported cloning the gene for the putative pyoverdine receptor by complementation. The product of the *fpvA* gene is consistent with an 86-kDa mature protein. Sequence analyses revealed homology to highly conserved domains found in TonB-dependent receptors, even though nothing resembling a TonB box was found. FpvA also has regions of homology with the PupA and PupB proteins of *P. putida*. PupA and PupB are IROMPs which function as receptors for ferric pseudobactin, a siderophore which is similar to pyoverdine (Bitter *et al.*, 1991). Some homology was also found with FhuE, the *E. coli* receptor for fungal siderophores coprogen, rhodoturilic acid, and ferrioxamine A.

Two different IROMPs have been described as involved in ferripyochelin uptake by *P. aeruginosa*. Originally, a 14-KDa protein, designated the ferripyochelin-binding protein (FBP) was assigned this role (Sokol and Woods, 1985). However, Heinrichs *et al.* (1991) demonstrated that a high MW protein (75,000 Da) interacts with pyochelin and supports pyochelin-mediated iron transport at nM levels of $FeCl_3$. This group also reported that a strain deficient in this protein transports ferripyochelin at higher ferric chloride concentrations, hence suggesting a putative role of the 14,000-Da protein in a second, lower affinity transport system. They also attributed the failure of earlier studies to observe the 75-Kda protein to the induction of the protein in the late log to stationary phase. The structural gene for the high-affinity ferripyochelin receptor (*fptA*) was cloned and the expression of a 75-Kda protein (Ankenbauer, 1992) substantiated the findings of Heinrichs *et al.* (1991). The mature FptA protein has a molecular mass of 76 KDa, and sequence homology studies revealed considerable homology with FpvA of *P. aeruginosa*, PupA and PupB of *P. putida*, and FhuE of *E. coli* (ankenbauer and Quan, 1994). Like FpvA and PupB, no TonB box was identified. Pyochelin and its receptor are both regulated by the transcriptional activator PchR which in turn is iron-regulated. The *fptA* gene is preceded by a sequence matching the *E. coli* fur-binding site, indicating the importance of iron concentrations in the expression of this receptor. Heinrich and Poole (1993) described a regulator PchR for both pyochelin and FptA biosynthesis.

In their study of the uptake and function of an antipseudomonal cephalosporin antibiotic, Yamano *et al.* (1994) found that the compound was best transported via the ferric iron transport pathway. They also found that the compound was best transported via the ferric iron transport pathway. They also found that mutant strains resistant to this antibiotic lacked a 66-kDa IROMP which, as they predicted, acts as a receptor for the antibiotic. *E. coli* IROMPS Fiu and Cir have been implicated

in the uptake of similar antibiotics (Curtis *et al.*, 1988; Nikaido and Rosenberg, 1990), hence suggesting a similarity between the uptake systems and the proteins in question. No further reports have emerged regarding the role of this 66-KDa IROMP.

4. OprC

OprC is a low copy number outer membrane protein which has been shown by Yoshihara and Nakai (1989) to form slightly anion-selective, small diffusion pores. The purified protein runs on SDS-PAGE with an apparent M_r of 70,000. The results, however, from this study are not without controversy because the investigators demonstrated in the same study that the major nonselective porin OprF, of *P. aeruginosa* did not exhibit porin function. In a later study, Nikaido *et al.* (1990) repeated the work of Yoshihara and Nakai and found that in their hands the levels of OprC were so low that they were not able to assess its function accurately. OprC has also been linked to the uptake of anionic antipseudomonal β-lactam antibiotics, such as cefsulodin, piperacillin, and azetreonam (Satake *et al.*, 1990). However, the role of OprC in antibiotic susceptibility was recently reexamined by Yoneyama *et al.* (1995).The *oprC*, *oprD*, and *oprE* genes were disrupted by gene replacement, and the resulting mutant strains were examined for susceptibility to a variety of antiotics. The results demonstrated that OprC is not involved in imipenem permeability nor the permeability of any of a number of antipseudomonal antibiotics, including quinilones, cephalosporins, chloramphenicol, and various penicillin derivatives.

Yamano *et al.* (1993) demonstrated that OprC levels increase fourfold during anaerobic growth and this increase is not caused by a concomitant decrease in iron concentration in the growth medium, nor reduced growth rate in a minimal medium. It was proposed that the increased production of this protein along with OprE aids in the growth of *P. aeruginosa* by enhancing the rate of entry of essential nutrients into the cell during oxygen stress. More recently, Nakae and collaborators sequenced OprC and showed that it is 65% homologous with NosA of *P. stutzeri* (Lee *et al.*, 1989), an outer membrane porin required for producing copper-containing nitrite reductase. Like NosA, it was shown that OprC is made only anaerobically and is repressed by high Cu^{2+} concentrations in the medium. Nitrite reductase is a key element in the anaerobic respiratory process termed denitrification. The predominant single-channel conductance increments of the OprC and NosA porins are virtually identical.