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Chapter Four

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Function and Structure of the Major Components of the Outer Membrane of Gram-Negative Bacteria

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In this review we have briefly outlined the major functional and structural aspects of the components of the outer membrane of Gram-negative bacteria. The functions of the major classes of proteins in the outer membrane are discussed, as are more general features such as antibiotic permeation pathways, receptors, protein excretion, and cell surface interactions. Structural information of a basic nature has been presented on the major proteins and other constituents of the cell wall. Included wherever possible are comparisons of the information available on *Brucella* with that of other Gram-negative species. We have chosen to present functional aspects of the Gram-negative cell wall first, leaving the more recent information concerning the structure of various outer membrane components to the second half of this review.

The Typical Gram-Negative Outer Membrane

The Gram-negative outer membrane profile (Figure 4-1), derived largely from studies of *Escherichia coli* and *Salmonella typhimurium*, serves as a standard for comparison of other enteric and non-enteric Gram-negative bacteria. The apolar (hydrophobic) region of the membrane, with a thickness of 4.5 nm,[1] provides an anchor for proteins and forms a structural and functional barrier between the periplasm and the exterior of the cell. The outer membrane is supported by an underlying layer of peptidoglycan. Current research suggests the peptidoglycan is a hydrated mesh approximately three molecular layers thick.[2] Approximately one-third of the Braun's outer membrane lipoprotein in *E. coli* is covalently attached to the peptidoglycan and thereby anchors and stabilizes the outer membrane.[3] However, it is not exposed to the external surface.[4] The remaining two-thirds of the Braun's lipoprotein in the cell is embedded in the outer membrane, but not covalently attached to the peptidoglycan.
Other outer membrane proteins are noncovalently associated with the peptidoglycan. One of these proteins, OmpA, also plays a role in stabiliz-

ated. General porins function as channels to the interior of the cell for the diffusion of compounds below a limiting molecular weight (the exclusion limit) and thus determine the molecular seiving function of the outer membrane. Other porins demonstrate selectivity for specific solutes. Other proteinaceous components of the outer membrane include proteases,[6] phospholipase A,[7] pili,[8] flagella, and proteins induced under specific conditions such as the divalent cation-regulated protein, H1, in *Pseudomonas aeruginosa*,[9] and the iron-regulated receptors for iron-siderophore complexes.[10]

Two major classes of molecules present in the outer membrane, lipopolysaccharide (LPS) and phospholipids, are asymmetrically arranged. The LPS is present only in the external monolayer of the outer membrane and the majority of lipids are located on the periplasmic side of the outer membrane bilayer. For enteric bacteria, this arrangement of lipids seems wise as the bacteria exist in an environment full of bile salts and lipases in the intestine.[5] The phospholipids have only two fatty acid chains connected to polar head groups while LPS has six or seven fatty acid chains linked to a diglucosamine phosphate backbone.[11] Also, LPS molecules have many negatively charged groups in the rough core oligosaccharide and on membrane-proximal sugars such as 3-deoxy-D-manno-octulosonic acid (KDO) as well as chains of repeating sugar units extending various lengths into the environment surrounding the cell.[11]

Function of Outer Membrane Components

Structural Proteins

Two classes of outer membrane proteins have been demonstrated to be involved in both outer membrane and cell structure and growth in low-osmolarity media. These are represented in *E. coli* by Omp A and Braun's lipoprotein.[12] Loss of both of these proteins confers upon the cell a rounded shape and a growth defect in certain media (without any observable changes in penicillin-binding protein 2). Either protein will reverse both properties such that single mutants are rod-shaped and grow well in most media.[13] Interestingly, it is the covalently peptidoglycan-associated form of lipoprotein that appears to be most important for maintaining structural stability in *E. coli*. In *P. aeruginosa* PAO1, the Braun lipoprotein-equivalent is not apparently covalently peptidoglycan-associated and it seems that cell shape is maintained, at least in part, by OprF. *Pseudomonas aeruginosa* PAO1 *oprF::W* mutants demonstrate rounded morphology and a growth defect in low-osmolarity media.[14] This structural role of OprF andthe fact that OprF and OmpA have substantial homology throughout their C-terminal halves, cross-react immunologically, and have many common physical properties,[14,15] lends additional support to OprF having an important role in maintaining cell shape and stability. In addition, OprF

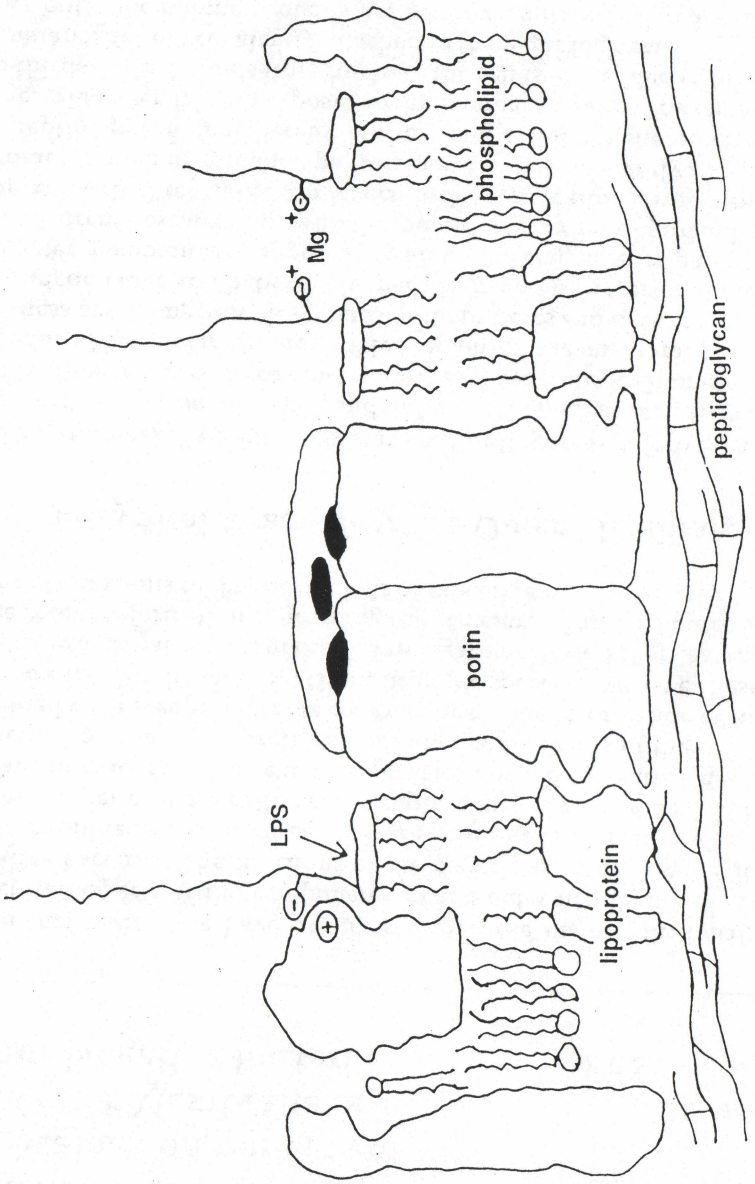

Figure 4-1. Schematic Diagram of the Gram-Negative Outer Membrane.

expressed from the cloned *oprF* gene will restore elongated morphology to an *E. coli* strain devoid of OmpA and Braun lipoprotein.[14]

In the *Brucella* spp. a peptidoglycan-associated lipoprotein that cross-reacts im-munologically with Braun lipoprotein has been demonstrated.[16] While there have been no specific studies of proteins related to OmpA in *Brucella*, it is possible that the group 3 proteins are the OmpA-equivalent in these species. In addition to *P. aeruginosa*, both *Haemophilus influenza* and *Neisseria gonorrhoeae* contain proteins that cross-react immunologically with the *E. coli* OmpA protein.[14,17]

One other outer membrane protein found in a variety of species is the peptidoglycan-associated lipoprotein,[18] equivalent to protein H2 of *P. aeruginosa*. The function of this species is unknown.

General Porins

To date, porins have been found in every Gram-negative species in which they have been sought. The porins are usually identified by their ability to reconstitute channels in lipid bilayers by one of four reconstitution methods discussed in previous reviews.[6,19] Their substantial structural stability and resistance to detergent denaturation have been extremely helpful in this regard. However, it must be stressed that these reconstitution methods are technically difficult. Based on such techniques, there is currently a dispute as to the channel size and nature of the major porin (OprF) of *P. aeruginosa*.[6,20] The problem may stem in part from the relatively low outer membrane permeability of *P. aeruginosa* compared to *E. coli*. Techniques for the examination of *E. coli* must be suitably adjusted to allow them to be applicable to *P. aeruginosa*. We feel that such disputes can only be solved by genetic experiments which confirm the nature of an outer membrane protein as a porin for antibiotics, for example. Thus, if a defined protein alteration (preferably a point mutation, or small deletion or addition to the gene) can be definitively associated with antibiotic resistance and/or a loss of *in vitro* porin activity, and these properties can be genetically cotransferred to another strain, this would represent proof of porin function. With the application of sophisticated molecular genetics to studies of porins, such mutations can be created *in vitro* and then recombined into the chromosome.[15] However, in the case of the OprF protein from *P. aeruginosa*, transposon or interposon insertion into the oprF gene, while causing modest increases in antibiotic resistance, was not entirely satisfactory due to the substantial effects of the deletion of OprF on the structure[14,20] and non-specific permeability[15] of *P. aeruginosa*.

With the above limitations, model membrane studies have allowed one to build up a very detailed picture of how general porins function.[21,22] They contain channels which are weakly selective for cations over anions, or vice versa, due to the presence of charged amino acid residues.[23] There are some suggestions that certain porins are voltage regulated,[24] but these have been disputed.[19,25] With respect to *Brucella* porins (group 2 proteins), the available evidence suggests that they fit into the Gram-negative "norm" in that they are apparently oligomeric, SDS-resistant, peptidoglycan-associated porins with pore sizes similar to *E. coli* porins.[26,27] Their strong peptidoglycan association and heterogeneous banding on SDS-PAGE represent variations on the general porin theme.

Specialized Porins

There are a limited number of known "specialized" porins. These are proteins which have channels containing specific binding sites for given molecules (Figure 4-2). The two best-studied cases are the phosphate-selective protein P (OprP) of *P. aeruginosa*[19,23,28,29] and the maltose/maltodextrin-selective LamB protein of *E. coli*.[23,30] In each case, these channels have some permeability towards other solutes, but the possession of specific binding sites allows substantially enhanced uptake at a low substrate concentration of molecules which bind, compared to molecules that do not bind (Figure 4-3). Indeed, at these low, physiologically relevant concentrations the specialized porins are orders of magnitude more effective in the uptake of their particular substrate than the general porins which possess substantially larger channels. Important features of the above two channels are that their production is regulated by their specific substrates and they are coregulated with a complex transport system.[23,31]

Other specialized porins have been less well studied. They include the glucose-selective protein D1[32] and the imipenem-selective protein D2[33] of *P. aeruginosa*, as well as the nucleoside-selective tsx protein of *E. coli*.[23,34] In addition, the iron-regulated outer membrane proteins of *E. coli* contain binding sites for specific iron-siderophore complexes, but there is no definitive data demonstrating that they are porins.[23,35] The NosA protein of *P. stutzeri* apparently contains a copper-binding site and is a porin, but the porin channel is not copper selective.[36]

Antibiotic Permeation Pathways

In most Gram-negative bacteria, porins constitute a major permeation pathway across the outer membrane for hydrophilic antibiotics. The exclusion limit and activity of the porin channels determine the efficiency of the porin pathway (also called the hydrophilic pathway). For example, it has been suggested that *P. cepacia* is antibiotic resistant due to its low outer membrane permeability, which is caused by the small size of its major porin channels.[37] Similarly, the majority of *P. aeruginosa* protein F channels are small and presumably impermeable to antibiotics, though a small percentage (<1%) have been proposed to be large and antibiotic permeable (but see above and references 6,20,22,and 38 for discussion). In *E. coli* the OmpF channel represents the major conduit for β-lactam antibiotics.[11,39] However, even in this case considerations such as the frictional

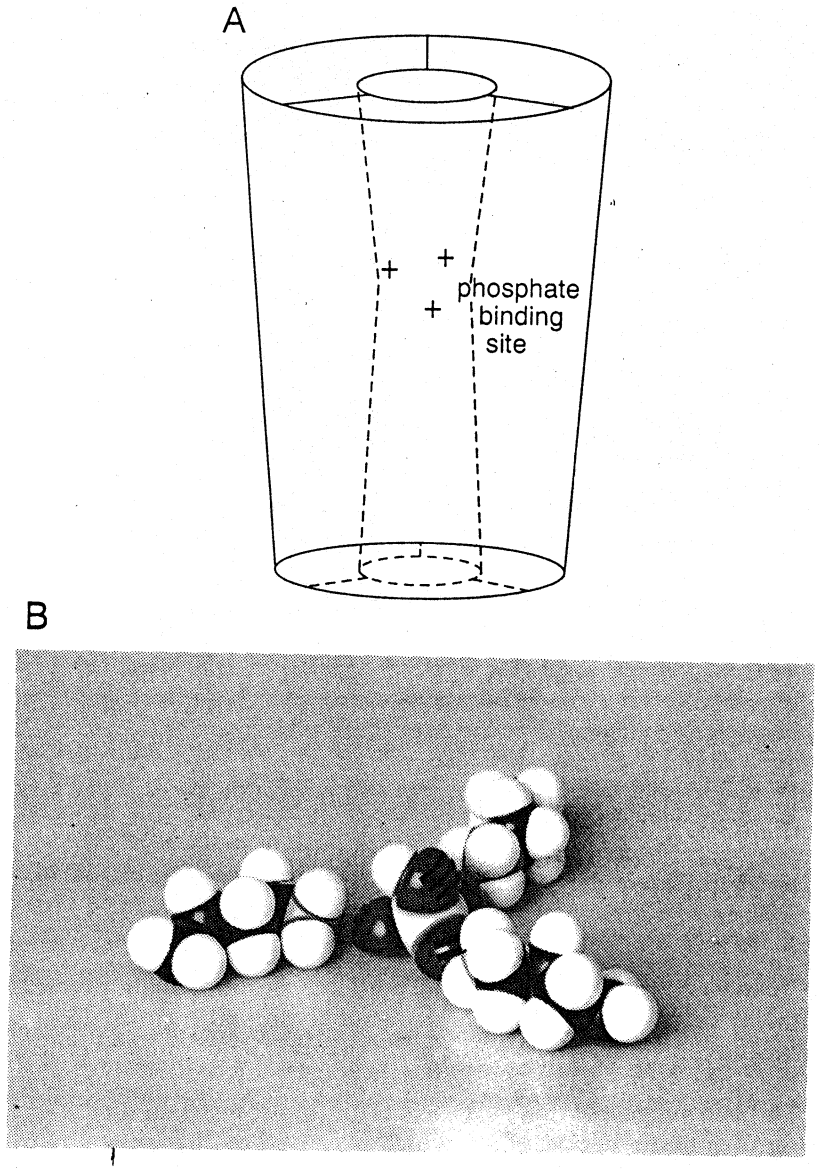

Figure 4-2a & 2b. Schematic Diagram of OprP. A. Showing the phosphate binding site. The binding site B, as diagramed here, is proposed to consist of three lysine side chains, one amino acid residue being from each monomer, which extend into the channel forming a positively charged cloud shell that would effectively bind HPO_4^{2}. There is a 3-fold symmetry of the HPO_{42}- centered around the phosphate atom.[29]

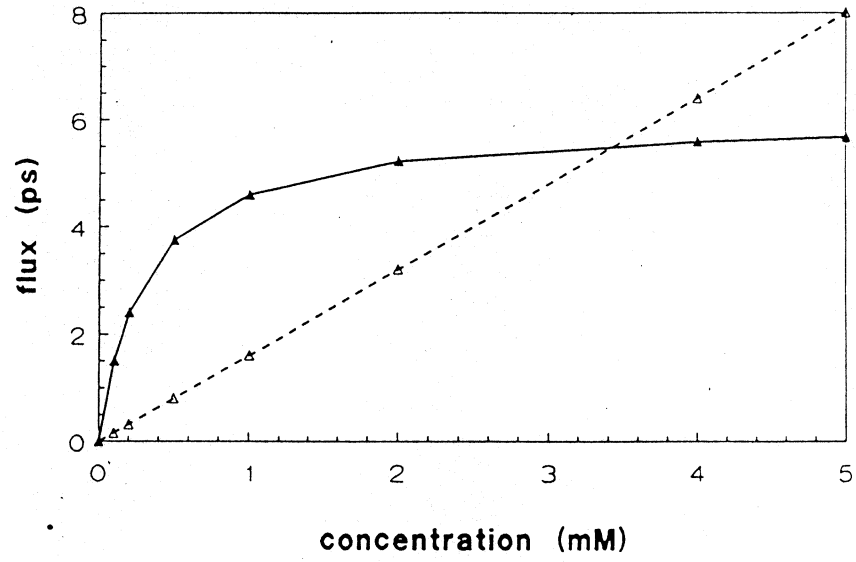

Figure 4-3. Phosphate Flux as a Function of the Concentration of Phosphate for OprP ▲ and PhoE △. The half-saturation constant Ks is 0.30 mM for OprP.

interactions between the sides of the OmpF channel and the permeating β-lactams, as well as the relatively small proportion of total outer membrane surface area that represents porin channels, means that the outer membrane reduces the rate of passage of β-lactams into the periplasm.[11,19] Thus, together with secondary defences like periplasmic β-lactamases, the molecular sieve nature of the outer membrane contributes substantially to the intrinsic resistance of Gram-negative bacteria to antibiotics.[39] The moderate susceptibility of *Brucella* spp. to hydrophilic antibiotics is consistent with model membrane studies showing similar porin sizes for *Brucella* and *E. coli*.[27]

There are two other well-defined antibiotic pathways across the outer membranes of Gram-negative bacteria, the hydrophobic pathway and the self-promoted uptake pathway.[9,11,40] The hydrophobic uptake pathway involves the uptake of hydrophobic or amphipathic molecules by direct passage through the outer membrane bilayer. Gram-negative bacteria like *S. typhimurium, E. coli,* and *P. aeruginosa* wild-type strains do not have a predominant hydrophobic permeation pathway.[6,41]

Studies with mutants of these bacteria (often LPS-altered) that are supersusceptible to hydrophobic agents, and with divalent cation chelators or polycations that increase the permeability of wild type strains to antibiotics, have indicated that the exclusion of hydrophobic antibiotics is mediated by the outer membrane in these strains.[9,41,40] It has been suggested that the outer membrane is stabilized by the strong interaction of LPS (the major, if not sole, lipidic component of the outer monolayer of the outer membrane) with itself, via divalent cation crossbridging, and with outer membrane proteins.[9,11,40] In contrast to these above bacteria, however, several pathogens, including *N. gonorrhoeae, N. meningitidis, H. influenzae,* and *B. pertussis,* have outer membranes which do take up hydrophobic compounds.[9] Symptomatic of a hydrophobic permeation pathway is high susceptibility to moderately hydrophobic agents including erythromycin and rifampicin. Based on the high susceptibility of *Brucella* spp. to these antibiotics we can assume that *Brucella* also possesses a relatively efficient hydrophobic permeation pathway.[42]

The other well-defined outer membrane permeation pathway is the self-promoted uptake pathway.[9,43] In this pathway, polycationic agents or chelators competitively displace or remove divalent cations from sites on the outer membrane where these divalent cations cross-bridge adjacent LPS molecules. The consequent destabilization of the outer membrane has been proposed to permit the enhanced uptake of the destabilizing compound, hence the name self-promoted uptake. The pathway is utilized by polycationic antibiotics such as polymyxins and, in some bacteria, amioglycosides.[9,43] Polycationic peptides called defensins (which are part of the non-oxidative killing arsenal of phagocytic cells),[44] and the fluoroquinolone antibiotic fleroxacin also use the pathway.[45] Self-promoted uptake, and consequent killing by these agents, can be inhibited by excess divalent

cations in the medium, by LPS alterations possibly in negatively charged phosphate residues, or by induction of an LPS-associated protein which has been proposed to replace divalent cations in stabilizing outer membranes.[46] Also, *P. fluorescens* cells grown under phosphate-limiting conditions are resistant to polymyxins and produce large amounts of an ornithine amine lipid in contrast to *P. fluorescens* grown in a phosphate-rich medium.[47]

Brucella outer membranes are resistant to the destabilizing effects of the divalent cation chelator EDTA[48] and *Brucella* spp. are also resistant to the polycation polymyxin B.[32] Thus, we can assume that *Brucella* does not have a self-promoted uptake system. *Brucella* spp. have been shown to contain a high content of an ornithine lipid (17 to 32% of total lipid)[49] and we propose that this molecule replaces divalent cations as the chief outer membrane stabilizing agent, thus explaining the resistance of *Brucella* to polycations and EDTA. Since *Brucella* is a facultative intracellular parasite that can survive in phagocytic cells, we assume that this property is required for *Brucella's* resistance to the polycationic peptides and proteins of neutrophil granules.[50]

In addition to the above, we have recently argued, based on data in mutants, that there are other potential non-porin pathways.[9] The above-mentioned imipenem-selective protein, D2 of *P. aeruginosa,* also creates a precedent for the existence of a selective porin for a given group of antibiotics. Another group of β-lactams contain catechol groups and are thought to be taken up and across the outer membrane by iron-siderophore uptake systems.[51]

Receptor and Enzymatic Functions

Outer membrane macromolecules, both various proteins and LPS, also serve as cell surface receptors for adsorption of phages and bacteriocins.[5] Since this results in killing of cells, we can assume these are not the normal physiological functions of these molecules. In addition, outer membrane molecules are involved in binding of conjugative pili in genetic transfer.[5] A class of high molecular weight, iron-regulated, outer membrane proteins that have been identified in most bacteria examined function as receptors for iron-siderophore complexes and in subsequent permeation of these complexes across the outer membrane.[52] Such proteins are considered important in pathogenesis since it is generally held that bacteria grow *in vivo* under iron-deprived conditions.[53] Similarly, other outer membrane proteins in *Neisseria* sp. and *H. influenzae* function in binding and subsequent removal of iron from iron-loaded transferrin or lactoferrin.[54] The btuB protein serves as a receptor for vitamin B-12 as part of the vitamin B-12 uptake pathway of *E. coli*.[5] All of these receptors have been reasonably well characterized with regards to their binding function and in many cases mutants lacking these proteins have a clearly defined loss of uptake of the substate that binds to this receptor. However, little is known about

the actual mechanism of translocation of the substrates across the outer membrane. Outer membranes have also been shown to contain proteins with a variety of enzymatic functions including phospholipase A1, esterase, and proteases.[1,5]

Role in Protein Excretion

A feature of many Gram-negative bacteria is their ability to excrete a variety of different proteins, including certain exotoxins, proteases, lipases, phospholipases, nucleases, haemolysins, etc, into the external medium. It was once assumed that such excretion might involve outer membrane breakdown and release of the enzyme from a periplasmic pool, but this is now known not to be generally true.[55,56] At least four pathways have been proposed for the mechanism of transit of excreted proteins across the outer membrane. These include secretion into the periplasm as a proprotein, followed by proteolytic removal of the "pro" sequence during passage across the outer membrane, secretion into the periplasm in a native form followed by release across the outer membrane, excretion of specific proteins associated with blebs of outer membrane material, and excretion through Bayer adhesion zones.[56]

Interaction with Environmental Surfaces

Various cell surface molecules have been described as being involved in adhesion to environmental surfaces (including adhesion to eukaryotic cells). Such adhesins include cell surface polysaccharides, fimbriae or pili, and fibrillar adhesins. However, only recently has there been good evidence to suggest a presumptive role for outer membrane proteins in adhesion. This evidence arose from genetic studies of *E. coli* P fimbriae, which like other fimbriae or pili, are anchored in the outer membrane. P fimbriae mediate binding of *E. coli* to the globoside receptor on epithelial cells.[57] It has been demonstrated that the receptor binding ligand for these fimbriae is not contained on the papA pilin protein that makes up the shaft of the P fimbriae, but rather is contained on a pair of proteins, papF and papG, which are usually located at the tip of the fimbriae. Mutants lacking the fimA protein, which presumably express the papF and papG proteins on the surface of the outer membrane, are still able to bind to globoside receptors. Although direct evidence is lacking, this creates a precedent suggesting that outer membrane proteins may be specifically involved in binding to environmental surfaces.

Structure of the Components of the Outer Membrane

Structural Proteins

It is interesting to note that predictive models of the *E. coli* OmpA protein based upon its primary structure[58] and mapping of surface exposed regions[59] suggest that only the N-terminal portion of this protein is embedded in the membrane.[60] Searches for similarities between the *P. aeruginosa* major outer membrane protein, OprF, and other proteins have found that the C-terminal half of OprF is very similar to the C-terminal half of OmpA from *E. coli* and *Enterobacter aerogenes*, and the pIII protein from *N. gonorrhoeae*.[14,61] It is this C-terminal region of OmpA which is thought not to be exposed on the surface of, or embedded in the outer membrane.[59] This is not true in the case of OprF since molecular genetic manipulation of the gene has permitted localization of the surface-exposed epitope of monoclonal antibody MA5-8 to the carboxy terminal half of the molecule (Woodruff, W.A. and R.E.W. Hancock, unpublished data). Interestingly, comparison of the antigenic index, which is calculated by summing several weighted measures of secondary structure (hydrophilicity, surface probability, flexibility, and the Chou-Fasman and Robson-Garnier predictive methods), shows a better correlation between OmpA and OprF in the N-terminal half of these proteins than in the C-terminal half which is more closely related at the primary sequence level (Figure 4-4). Group 3 proteins from *Brucella*, the proposed OmpA equivalent,[16] do not have any porin activity,[27] but no further studies on this particular group of proteins have been carried out in order to conclusively determine their structure or functional properties.

Another protein offering structural stability to the outer membrane, the Braun's lipoprotein, is not essential for growth, but mutants lacking lipoprotein produce increased amounts of outer membrane vesicles and release periplasmic enzymes.[65] A third of the lipoprotein present in the cell wall is covalently attached to the peptidoglycan through the ε-NH_2 group of the C-terminal lysine.[3] The protein portion of the molecule is mostly α-helical[4,63] and the N-terminal cysteine residue is substituted with a diglyceride on the sulfhydryl group and its α-NH_2 group is substituted with an amide linked fatty acid residue.[4] In *Brucella* the covalently peptidoglycan linked lipoprotein has an amino acid composition which is similar to that of *E. coli*[64] and seems to share antigenic epitopes with *E. coli* lipoprotein.[16]

Porins

General diffusion pores appear to be constitutively expressed, but the amount of expression seems to vary with the cell's needs. OmpF has long been regarded as the major porin of *E. coli*, however, expression of the *ompF* gene is regulated in response to the osmolarity of the medium and is predominant only under conditions of low osmolarity. At high osmolarity OmpC is predominantly expressed. Recent studies have shown that at intermediate salt concentrations there are actually OmpF/OmpC heterotrimers formed which cannot be distinguished from homotrimers by SDS-PAGE, but can be separated by anion exchange chromatography.[65] Considering the large degree of homology of the amino acids between OmpF and

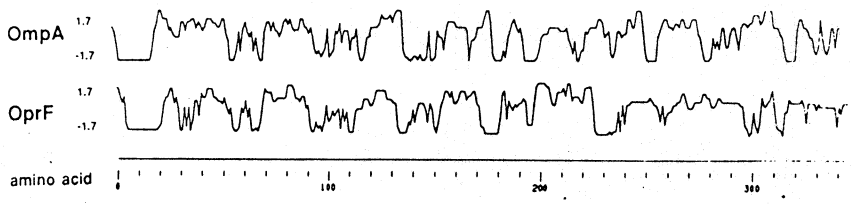

Figure 4-4. Plot of the Antigenic Index for Proteins OmpA and OprF. Note the similar peaks and valleys in the first 150 amino acids with much less similarity in the remaining C-terminal portions of the two proteins.

OmpC,[66] it seems feasible that the monomers would be interchangeable to form heterotrimers. This work, along with data which suggests that porin trimers are assembled via a dimeric intermediate,[67] suggests that bacterial cells are capable of "fine tuning" the outer membrane permeability characteristics by structurally altering porin types within the membrane.

A large amount of research has been directed towards establishing a molecular model for bacterial porins in the past few years. The crystallization of a porin and the concommitant resolution of that crystal structure will provide eagerly anticipated answers concerning the nature of the pore within these proteins, but technical problems such as protein purity (i.e. homogeneity), the need to crystallize in a detergent solution, and the difficulty of obtaining isomorphous heavy metal derivatives have proven to be significant obstacles. Nevertheless, a number of alternate techniques have been utilized to derive informative models of porin structure. Regions of cell surface exposed protein have been extensively mapped by molecular genetic studies of mutants selected using both antibody techniques and bacteriophages specific for PhoE[68,69] and LamB.[70,71,72] In addition, a series of hybrid genes generated by *in vivo* recombination between the *phoE* and *ompC* genes were characterized with respect to the binding of PhoE and OmpC specific bacteriophages and monoclonal antibodies raised against PhoE.[73,74] For PhoE, the data was consolidated and a model formulated in which eight hydrophilic regions are exposed on the external surface of the protein. Each of these regions is separated by approximately forty amino acids. These are stretches of amino acids long enough to cross the membrane twice, for a total per PhoE molecule of sixteen transmembrane segments.[68] The data for LamB suggest a folding model with eighteen membrane spanning segments.[68] These membrane spanning segments are thought to be arranged in β-pleated sheet conformation in porin proteins. There are none of the long segments of hydrophobic residues which have been shown to form membrane spanning α-helices in other membrane proteins.[75] The absence of hydrophobic segments could reflect a necessity for translocation to the outer membrane in Gram-negative bacteria. MacIntyre et al.[76] have shown that the addition of a segment of 16 to 18 hydrophobic residues inserted between amino acids 153 and 154 of OmpA blocked translocation, leaving this protein anchored in the cytoplasmic membrane.

Analyses of circular dichroism data on various bacterial porins,[77,78] infrared absorption and high angle x-ray diffraction,[79] Raman spectroscopy,[80] x-ray diffraction,[81] and Fourier transform infrared linear dichroism,[82] have all indicated a high content of β-sheet structure in OmpF, LamB, OprP, OprF,and the N-terminal 177 amino acids of OmpA. A model for the orientation of these anti-parallel β-pleated sheet structures[79] has been proposed by Nabedryk et al.[82] They suggest that the porin monomer consists of at least two β-sheet domains, both with planes perpendicular to the membrane. The strands of one sheet are lying nearly parallel to the

membrane normal and the strands of the other are inclined at a small angle away from the membrane plane.

Extensive studies using electron microscopy have also provided a great deal of structural information about porins. Dorset et al.[83] examined the structure of OmpF trimers from *E. coli* by forming two-dimensional crystals of protein packed into lipid bilayers followed by reconstruction of optical diffraction patterns. Since the resolution of these experiments was limited to 2.2 nm, the shape of the transmembrane channels could not be determined, but they found lattice constants in one crystal form to be similar to those obtained from three-dimensional crystals.[81] They also found that the amount of phospholipid associating with the protein in a small hexagonal crystal form was comparable to the amount of LPS bound to membranous sheets generated by SDS extraction of undisassociated outer membrane from *E. coli*[84,85] indicating that porin packing in two-dimensional crystals is similar to the arrangement of porin in native outer membranes. To date, electron microscopy via both optical and electron diffraction techniques has been used to generate three-dimensional images from two-dimensional specimens of OmpF,[83,86,87,88] LamB,[89] and PhoE[90] to a maximum reconstructed resolution of approximately 0.6 nm. At this resolution it has been reported that there are three channels per trimer on the external surface of the protein which merge to form one channel at the periplasmic side for proteins OmpF and LamB, or merge, but do not converge, in the case of PhoE. It now seems likely that the OmpF channel arrangement is similar to PhoE (R.M. Garavito, personnel communication) in that the channels do not actually merge, but rather narrow and bend closer together at the periplasmic side of the membrane (Figure 4-5). A similar structural arrangement among the porins of *E. coli* would be consistent with their extensive homology at the primary level.

Other methods used to determine porin structure include chemical modification of specific amino acids and analysis of the resulting changes in solute permeability through the porin. For example, it has been demonstrated that OprP from *P. aeruginosa* has a fixed, anion binding site within the channel[28,29] and that the channel has an effective sieving diameter of approximately 0.5 - 0.6 nm[29] (Figure 4-2). Various methods were used to modify charged groups within the channel and the resulting conductance was analyzed via black lipid bilayer studies. Similar studies on PhoE from *E. coli* suggested that this protein does not have a specific binding site for phosphate.[91] These results have been confirmed recently by using PhoE mutants and black lipid bilayer techniques.[92] Chemical modification has also been used to demonstrate that porin channels have constrictions[28] and that charged amino acid residues are responsible for the weak ion selectivity of general diffusion pores.[29] Modification with bulky reagents such as trinitrobenezenesulphonate does not alter the exclusion limit of the OprP channel, which suggests that the charged residues responsible for selectivity are not located in the most constricted part of the channel.[93]

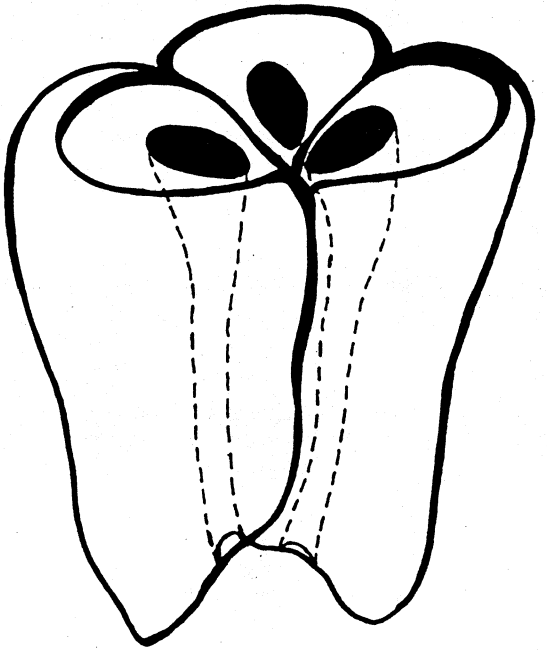

Figure 4-5. Schematic Diagram of a General Diffusion Porin with Three Separate Channels.

The properties of *Brucella* porins have been studied by Douglas et al[27] using liposome swelling assays. They found a range of pore sizes in different strains of *Brucella* with *B. canis* having the largest pores (larger than OmpF from *E. coli*), a middle range of pore sizes in several smooth and rough strains of *B. abortus*, and the smallest pore size in *B. melitensis* group 2 proteins. Except for the *B. canis* group 2 protein which had a lower mobility on SDS gels,[27] all of these proteins run as multiple bands at a molecular weight of 37,000-42,000.[27] All of these multiple bands give similar patterns in peptide mapping experiments. Explanations for the differing mobilities may be the heat modifiability of these proteins or the possibility of having different amounts of LPS remaining tightly associated with the proteins and causing them to run at slightly different positions on SDS gels.[94] The unusually strong association between protein and LPS is well documented for both smooth and rough *Brucella* strains[26,49,95,96] and seems not to be mediated by divalent cations[26] as it is in *E. coli* and other Gram-negative bacteria.[92,98,99,100]

Lipopolysaccharide

In addition to proteins, a major portion of the exterior surface area of the Gram-negative outer membrane consists of LPS. Most Gram-negative bacteria, including *Brucella*, produce both the rough and smooth types of LPS. Most enteric organisms have LPS structures consisting of similar lipid A, core oligosaccharides, and O-poly-saccharides, but *Brucella* LPS has a very different lipid A structure from that of enteric bacteria.[101] In *E. coli* all of the fatty acid chains attached directly to the disaccharide backbone of the LPS are 3-OH-tetradecanoic acids (primarily 3-hydroxymyristic acid). There are additional fatty acid residues linked to these 3-hydroxy groups forming a characteristic 3-acyloxyacyl structure.[11] *Brucella* lipid A contains amide linked, acyloxyacyl residues, 3-O(16:0)12:0, 3-O(16:0)13:0, 3-O(16:0)14:0, 3-O(18:0)14:0, 3-OH-16:0, and 3-OH-14:0, and an unusual 2,3-diamino-2,3-dideoxy-D-glucose as a backbone sugar which is similar to the lipid A of several photosynthetic bacteria.[102] There seems to be no phosphate associated with the core region although there is KDO. The lack of phosphate is interesting in light of the unusually strong association between LPS and proteins in *Brucella*. It is generally thought that LPS-protein interactions in many other bacteria may be mediated by charged residues on the LPS molecule.[11] In addition, the presumed low phosphate content of *Brucella* LPS is consistent with the lack of a self-promoted uptake pathway (see above).

In summary, the outer membrane of the Gram-negative cell wall must be traversed by every compound entering into or exiting from the bacterial cell as well as serving as a barrier against potentially harmful compounds. In this respect, the elements of the outer membrane are both functionally and structurally specialized. This review discusses the functions of the major proteins of the outer membrane in the context of permeability,

structural stability, protein excretion, and cell surface interactions. In order to understand the function of a membrane at a molecular level it is necessary to know its structure. To this end, the structure of the major proteins, lipopolysaccharide, and lipoproteins are briefly discussed. As most of the information presented here is derived from studies of *Escherichia coli* and *Pseudomonas aeruginosa*, the available research on *Brucella* spp. is included for comparison wherever possible.

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