Bacterial Porins

The outer membranes of Gram-negative bacteria are size dependent molecular sieves. Permeation of hydrophilic molecules below a certain exclusion limit occurs through the water-filled channels of a class of proteins called porins. Porins control the influx of nutrients and the efflux of metabolites and constitute a permeation barrier against certain antibiotics and other noxious chemicals. They protect pathogenic organisms from host defence factors. Porins are now well understood due to their relative ease of purification and subsequent examination in model membrane systems.

By Robert EW Hancock and Melissa H Brown

Gram-negative bacteria have adopted a two-tiered approach to the handling of chemicals in their environment. For such chemicals to enter the cytoplasm, they must first cross the outer membrane and, after passage through the periplasm, the cytoplasmic membrane. The cytoplasmic membrane is a selective barrier comprising a continuous lipid bilayer studded with proteins. Many of these proteins form parts of specific transport systems. Passage across this membrane is limited to compounds for which specific transport systems exist as well as amphiphilic or hydrophobic compounds that can pass directly across a lipid bilayer. In contrast, the outer membrane of Gram-negative bacteria constitutes a sophisticated molecular sieve. The fabric of the sieve is so constituted in many bacteria that both hydrophilic and hydrophobic compounds are excluded, whereas the holes of this sieve comprise a class of channel-forming proteins called porins. Thus, access to the Gram-negative interior is largely restricted to chemicals that are small, reasonably hydrophilic (to permit passage through outer membrane porin channels) and specific (to permit access to available cytoplasmic membrane transport systems). Intracellular pathogens are an exception to this rule. They have outer membranes that permit passage of more hydrophobic compounds. In addition, polycations (including aminoglycoside and polymyxin antibiotics) from many sources can attack sites where the outer membrane is stabilised by divalent cations, and pass across the outer membrane via the self-promoted uptake pathway. With these exceptions, passage across the outer membrane is governed by the properties of porins.

The outer membrane

The outer membranes of Gram-negative bacteria contain a few species of "major" proteins present in high copy number (10^4–10^5 or more per cell) and several minor polypeptides, embedded in an asymmetric lipid bilayer (Figure 1). The outer monolayer of the outer membrane contains lipopolysaccharide (LPS) as the predominant lipidic molecule, whereas the inner monolayer usually consists of a restricted variety of phospholipids. The outer membrane is stabilised by the strong non-covalent or, occasionally, covalent association of specific proteins with the underlying outer membrane and by the crossbridging, by divalent cations, of adjacent negatively-charged LPS molecules in the outer membrane.

The outer membrane serves a variety of functions (reviewed in [1]), including a permeability barrier which is selectively permeable to molecules in the environment of the cell. For example, the outer membranes of wild type enteric bacteria exclude hydrophobic compounds, including detergents, bile salts and degradative enzymes, as well as hydrophilic compounds above a given size range, known as the exclusion limit. In contrast, despite similar size-dependent penetration of hydrophilic molecules, several prominent pathogens, including *Haemophilus influenzae, Neisseria* sp. and *Bordatella pertussis,* have outer membranes that are quite permeable to hydrophobic compounds.

The exclusion limit is determined by the size of the porin channels in the outer membrane. For *Escherichia coli* it has been suggested that tetrasaccharides or pentapeptides are excluded whereas trisaccharides or tetrapeptides can cross the outer membrane. Coincidentally the major *E. coli* porins OmpF and OmpC show the same sieving properties when isolated and studied in model membrane systems [2]. Recently, we devised a new experimental system for estimating channel size *in vivo* by introducing a 'raffinose-using' operon on a broad host range vector into *P. aeruginosa* [3]. *Pseudomonas* is unable to grow on saccharides larger than monosaccharides. The introduction of the 'raffinose-using' operon permitted growth of *P. aeruginosa* on disaccharides and trisaccharides in an outer membrane-limited fashion (ie, sugar concentration and size-dependent and influenced by mutations in the major porin OprF). The channel size of OprF can be estimated by measuring the relative rate of permeation of di- and trisaccharides (based on the growth rate on these substrates at a single growth limiting concentration) and applying the Renkin correction (a correction based on engineering considerations regarding how a compound of given size will interact with

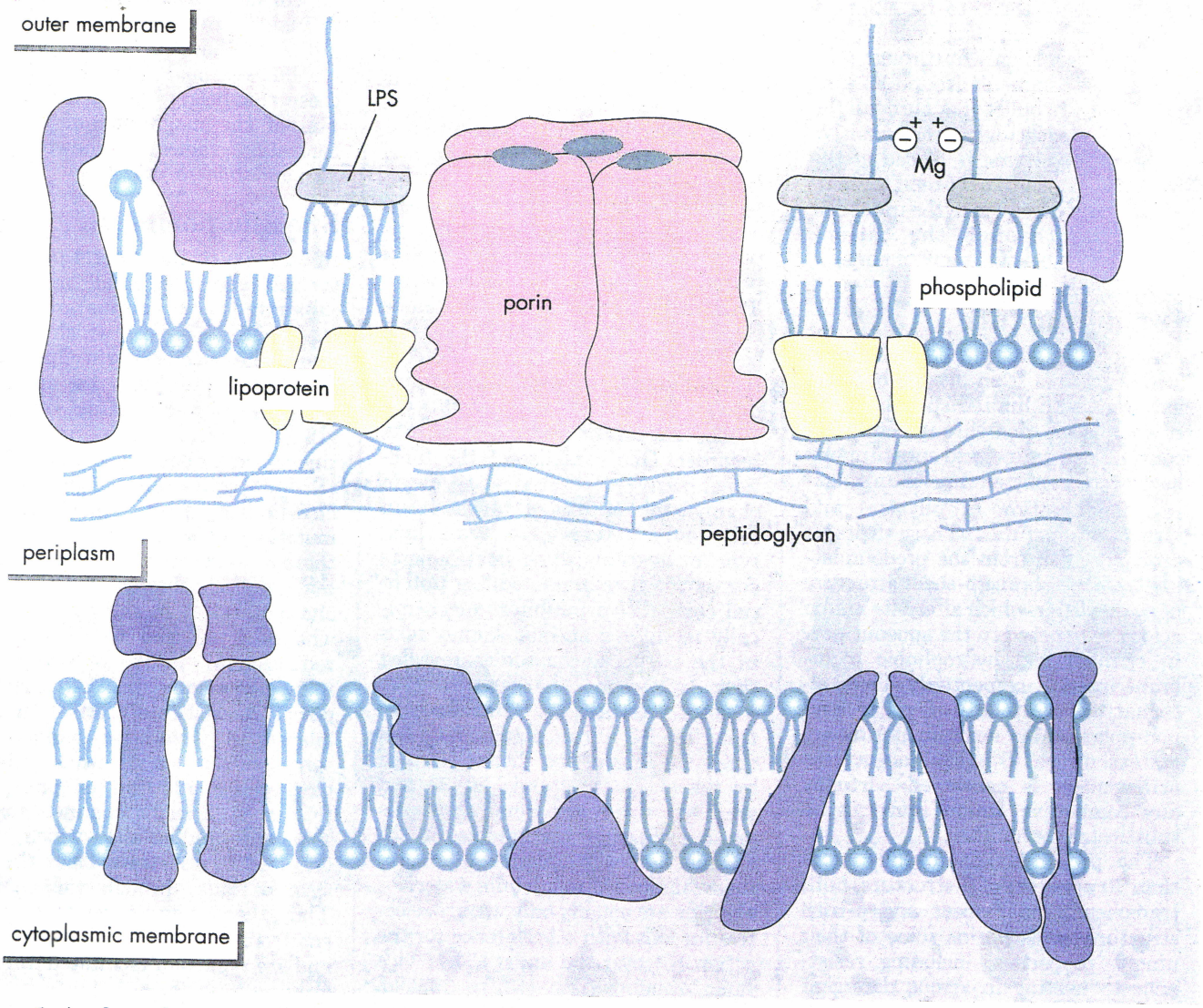

Figure 1. A schematic representation of the cell envelope of a typical Gram-negative bacterium.

a channel that is only a little larger). These data give an estimated diameter of 1.56 nm cf. an *E. coli* OmpF diameter of 1.1 nm, in general agreement with model membrane studies.

The effect of mutations defective in certain porins, on uptake of various compounds, provides the best evidence for the importance of porins in intact cells. Simple manifestations of these effects on uptake are the resistance of porin-deficient mutants to certain antibiotics. For example *E. coli* OmpF-deficient mutants are between two and 32 fold more resistant to most β-lactams which are consequently thought to be taken up via porins. However, measurement of the actual influence of porins on antibiotic uptake have been technically more difficult. The major breakthrough in this area was the recognition by Zimmermann and Rosselet [4] that upon addition of a β-lactam to cells, an equilibrium is established between the rate of diffusion of the β-lactam across the outer membrane and the hydrolysis of β-lactam in the space where cellular β-lactamase is found, the periplasm (as expressed by Michaelis-Menten kinetics). In practical terms these relationships are reduced to the simplified equation $V = P.A. (So-Si)$ where V = the rate of hydrolysis in intact cells, P = the permeability coefficient, A = the area of the outer membrane and So and Si are the external and periplasmic concentrations, respectively. To measure V, cells are loaded with periplasmic β-lactamase (by conjugating a β-lactamase encoding plasmid into the cells) and the rate of conversion of β-lactam to acidic products is measured by titration of the acidic product, or measurement of β-lactam ring breakage as a change in UV adsorbance or measurement of a reduction in β-lactam concentration in the supernatant.

Recently Bellido, Pechere and Hancock [5, 6] devised an HPLC method to measure the rate of uptake of β-lactams across the outer membrane of *Enterobacter cloacae*. The measurement showed that the rate of uptake was linear over a 100-fold concentration range for most β-lactams and was substantially reduced in a porin-deficient mutant. These observations confirmed that β-lactams pass across the outer membrane by simple diffusion through porins.

Of great interest was the *in vivo* measurement of the relative rates of permeation of the related third and fourth generation cephalosporins, cefotaxime and cefpirome, respectively. Cefpirome differs from its third generation relative, cefotaxime, by possession of a fixed positive charge in the 3-lateral chain. This positive charge leads to a six-fold higher rate of permeability across the outer membrane [6]. Examination of the three

dimensional structure of these antibiotics reveals them to be ellipsoid molecules (1.5 nm long and 0.8 nm wide) that would only fit through porin channels (diameter around 1.1 nm) if they passed 'bullet-like' through the porins. Interestingly, the positive charge in cefpirome is placed at the tip of the ellipsoid and would serve to orient and thus enhance the uptake of cefpirome through the cation-selective (see below) OmpF channel.

Porin structure

Attempts at aligning porin amino acid sequences from diverse bacteria have met with limited success [7, 8]. It would seem that through evolution considerable amino acid substitutions have been permitted despite an amazing conservation of physical and structural features. These features probably arise from the predominating transmembrane β-sheet structure of porins [7] in which alternate amino acids are exposed to the aqueous porin channel and hydrophobic membrane interior, respectively. Thus, alternating stretches of polar and non-polar amino acids in porins are not exceptional [9] and as long as this arrangement is conserved, virtually any compatible amino acid can be substituted for another.

The predominance (usually more than 70 per cent) of β-structure, both transmembrane β-sheet and β-turn structure, gives porins some of their unique properties, including resistance to heating in strong detergent solution, strong non-covalent associations with the underlying peptidoglycan and resistance to proteases. Other common properties of porins include a restricted molecular weight range (usually 28,000–48,000 kDa), an acidic pI, native trimeric structure and non-covalent association with LPS.

Recently the crystal structure of the porin from *Rhodobacter capsulatus* was solved [10] (Figure 2). Unfortunately the paucity of sequence identity with other porins has restricted the use of molecular replacement methodologies for solving other porin crystals.

Porin channels

Due to their intrinsic resistance to strong detergents and their strong association with the peptidoglycan, porins are relatively easy to purify. Functional characterisation of these proteins can be accomplished by one of three model membrane systems, liposome exclusion [12, 13], liposome swelling [13, 14] or black lipid bilayer analysis [13, 15]. Our laboratory uses the last method in which porins in detergent solution are added to the aqueous salt solution bathing a planar lipid bilayer (Figure 3). Individual porin molecules then spontaneously insert in a time-dependent fashion into the membrane, an event that can be measured by a step increase in the conductance (current divided by applied voltage) between two electrodes placed either side of the membrane. This method has the rather unique property of having single molecule sensitivity since amplifying the current through a single channel forming unit, by 10^9–10^{10} fold, results in events that can be read out on a chart recorder. One can dissect the functional parameters of a given porin channel by the use of variations in salt concentrations, salt type, lipid type, or by establishing salt gradients across the membrane, adding potential competitive inhibitors or chemically modifying specific amino acids in the porin. Such studies revealed that:

- porin channels are large, varying from 0.7–1.5 nm in diameter (ie 2-4 times the size of the channel formed by the antibiotic gramicidin A) and are filled with water. Small chemicals passing through the middle of the channel diffuse in a manner similar to their diffusion through bulk water.
- porins are usually only weakly selective for ions with a preference for the cation K^+ over the anion Cl^-, or vice versa, ranging from two to 30 fold. This selectivity is due to amino acids lining the mouth of the channel [10, 13].
- with the exceptions noted below porins are usually not chemically selective; and porins are not voltage gated or regulated.

This last point is controverial with different authors arguing that high voltages "turn-off" porin channels or result in voltage-induced breakdown depending of the authors' perspective. However *in vivo* data [16] and the finding that porins function normally in liposome exclusion experiments [12, 13] in which no transmembrane voltage is applied, seem to argue against physiologically-meaningful voltage regulation.

Despite the existence of a proteinaceous lining, uptake through porin channels fits most of the criteria for the process of simple diffusion. Since there are no binding sites involved in passage through the channel, and therefore no abrupt changes in energy levels of the porin or compound during its passage through the channel, we consider that porins are simple diffusion, rather than facilitated diffusion, channels. Thus, the movement of the small compounds through these channels will be a direct function of the magnitude of the concentration gradient, as shown both *in vivo* and *in vitro* [13].

Specific porins

In addition to the general porins described above, a small number of substrate-specific porins are known, the best studied of which are the phosphate-specific porin OprP of *P. aeruginosa* [17] and the maltodextrin-specific porin LamB of *E. coli* [18]. In general these porins are distinguished by both their *in vivo* or *in vitro* properties. The disciminating *in vivo* property is the observation that mutations knocking out production of these proteins specifically prevent uptake of the substrate for the given channel (cf. general porins which when deleted lead to a pleiotropic reduction of uptake of several unrelated compounds). *In vitro,* specific porins reconstitute channels in lipid bilayers in a stepwise manner. The salt conductance of which can be blocked by addition of phosphate (Figure 4). Specific channels contain substrate binding sites which, when occupied by substrate, block the passage of ions through the channel. Thus, these channels are quite different from the general porin channels and are typical of facilitated diffusion channels with substrate-binding affinities in the order of those found for facilitated diffusion channels of the red blood cell membrane (ie 10^{-4} M).

Porin regulation

The regulatory mechanisms for porin synthesis are complex. *E. coli* produces a number of outer membrane proteins including the porins OmpF and OmpC, which allow passive diffusion of hydrophilic molecules, but are regulated in a reciprocal fashion by a variety of environmental conditions including osmolarity and temperature. In nutrient broth or minimal media both OmpF and OmpC are expressed. In high osmolarity media OmpC is preferentially expressed and OmpF is repressed. Altering the relative amounts of OmpC and OmpF porins in the bacterial outer membrane may result in elevated resistance to certain antibiotics, including tetracycline, chloramphenicol and β-lactams, due to the OmpF porin pos-

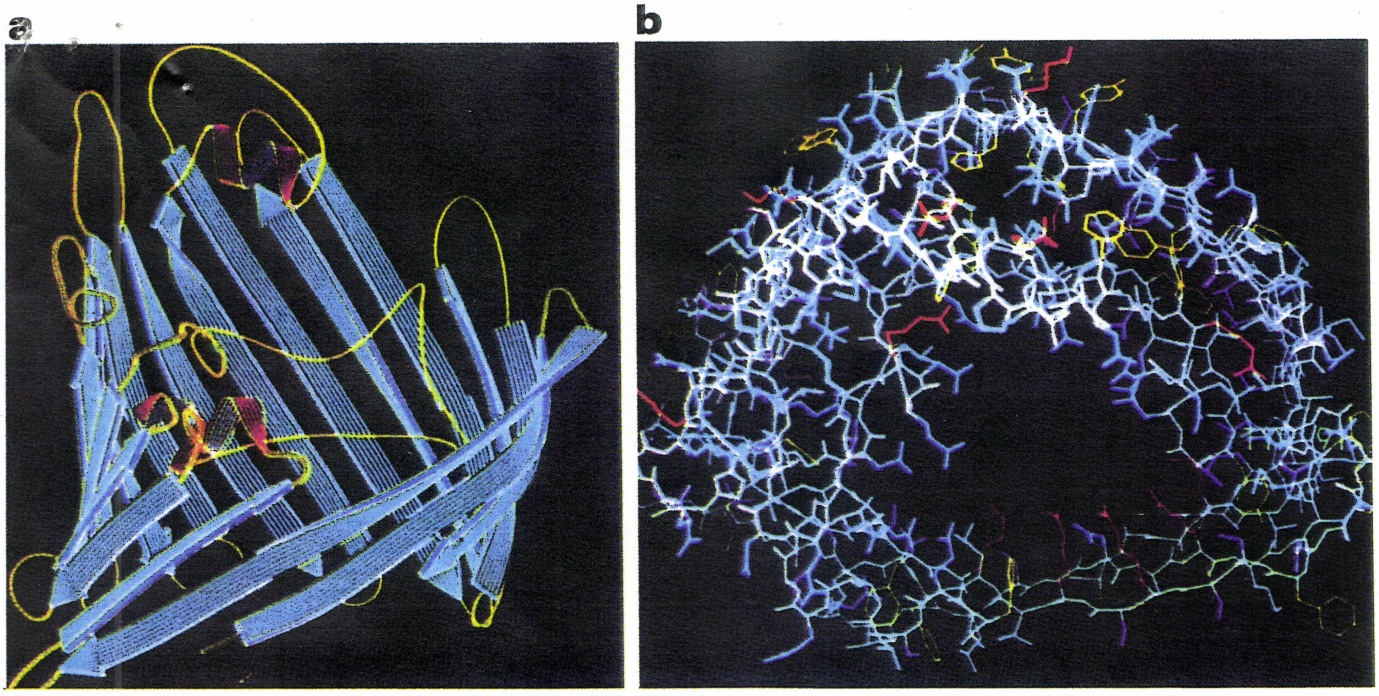

Figure 2. Two views of one subunit of the poring of *Rhodobacter capsulatus* such that (a) is a side-on view with the shorter β-sheet region (see below) internal in the trimer form, and (b) is a top view of the monomer looking straight down the centre of the pore. Three identical monomers are required to form the trimeric porin structure, which appears to be somewhat crater-like with a raised outer lip tapering to where the three channels meet. Each monomer comprises 16 β-sheet regions (as depicted in (a) by arrows) varying from 6 to 17 amino acids in length and only two short, but not membrane spanning, α-helices. The rest of the structure comprises β-turn and random coil structure. The actual channel has two surprising features. First it is not nearly as wide as might have been predicted by model membrane studies, being 0.7 nm in diameter (as opposed to predictions of 1.5 nm) [11]. Secondly, as illustrated in view (b) of the monomer, there are stretches of negatively charged amino acids lining the mouth on one side of the channel, and positively charged amino acids lining the other side. Negatively charged, positively charged, aromatic, polar and nonpolar side chains are coloured blue, red, yellow, purple, and light blue respectively.

sessing the major conduit for β -lactam antibiotics [19]. For example, growing *E.coli* in the presence of salicylate results in a decrease in the amount of OmpF in the outer membrane due to decreased *ompF* translation, via increased *micF* transcription [20]. This subsequently results in decreased sensitivity to some antibiotics and a decreased permeability of the outer membrane to cephalosporins. Thus, the host condition (ie, high osmolarity), leading to OmpF derepression, should be considered when devising antibiotic therapy.

The regulation of OmpF and OmpC expression is at both the level of transcription, involving two genes *ompR* and *envZ*, a single operon encoded by the *ompB* locus, and at the level of translation, by the participation of *micF*, an antisense RNA molecule. OmpR and EnvZ are members of a family of signal transduction proteins. OmpR is a cytoplasmic DNA binding protein, which recognises and interacts with sequences in the promoter region of both the *ompF* and *ompC* genes [21]. The EnvZ protein spans the cytoplasmic membrane and has both periplasmic and cytoplasmic domains. It possesses both kinase and phosphatase activities to phosphorylate and dephosphorylate OmpR. These two proteins have served as a prototype for so-called two-component regulatory systems, where the EnvZ protein is the sensory component and the OmpR protein is the effector component, which permits cells to respond transcriptionally to environmental conditions. The actual method of regulation performed by OmpR is still to be resolved. It has been postulated that the OmpR protein exists in three states: an inactive state: a low osmolarity state, which activates *ompF* transcription, but has no effect on *ompC* expression; and, a high osmolartiy state, which activates the *ompC* promoter and represses *ompF* transcription [22]. EnvZ may be involved in the formation of the high and low osmolarity species of OmpR, presumably by phosphorylation and dephosphorylation.

Regulation of *ompF* also involves integration host factor (IHF) [23]. This small histonelike protein appears to be involved in bending the DNA into the correct configuration for the protein interactions to occur and may help in repression of *ompF* by the high osmolarity state OmpR. The 93 nucleotide antisense RNA *micF* down-regulates OmpF production by the formation of a stable duplex with the 5′ end of *ompF* mRNA, thereby decreasing the levels of *ompF* mRNA in response to temperature increase and other stress conditions [24].

Phosphate, an essential cell nutrient, can diffuse through OmpC and OmpF porins when *E. coli* is grown in sufficient phosphate. However, another porin, PhoE, is induced in response to photphate deprivation, as is a periplasmic phosphate binding protein (PhoS), a periplasmic alkaline phosphatase (PhoA), and a cytoplasmic membrane transport system [25] termed the photphate-starvation-inducible (Pst) system. The pores of the PhoE porin are anion selective, (but interestingly not phosphate specific) in contrast to the cation-selective OmpF and OmpC pores [26].

The *phoE* gene is part of the *pho* regulon, and as such is controlled by a two component regulatory system similar to that of the *ompB* locus. It is comprised of PhoB, the positive transcriptional regulator, which in turn is regulated by PhoR, either in a positive fashion, when phosphate is limiting, or in a negative fashion when phosphate is nonlimiting. This system is more complex than the *ompB* locus as two other regulatory genes *phoM* and *phoU* are also involved, with PhoU meditating in the conver-

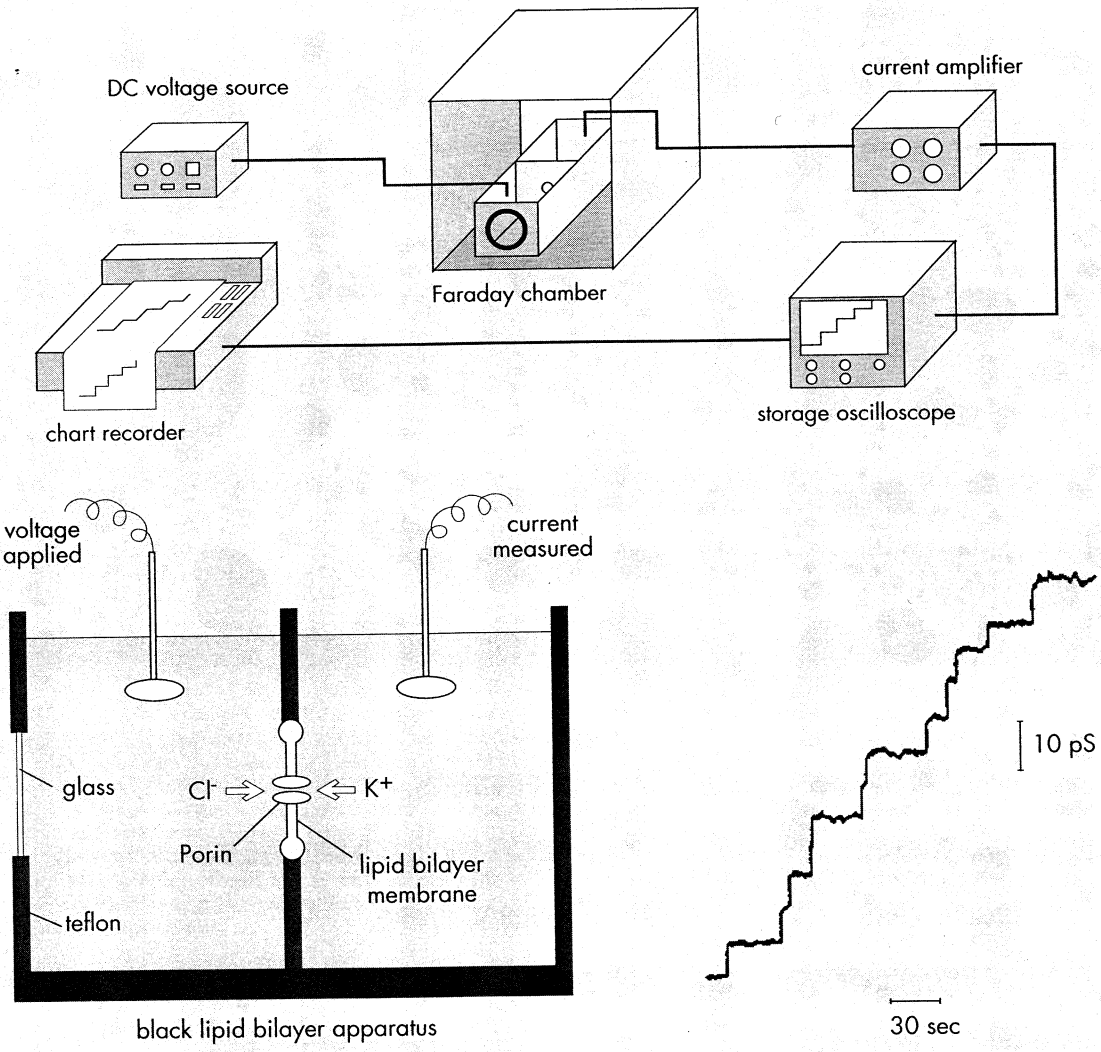

Figure 3. A schematic representation of the equipment required to measure membrane permeability using the black lipid bilayer apparatus.
A more detailed look at the actual black lipid apparatus. This chamber is divided into two components be a teflon divider containing a small hole. This chamber is filled with a solution onto which the electrodes dip. A lipid membrane forms across the hole in the divider, and reconstitution of single porin molecules into the membrane can be followed by current increases, due to movement of ions (K^+ and Cl^-) through the channels.
An example of the stepwise increases in conductance of the lipid bilayer membranes, following addition of protein P to the aqueous phase bathing the membrane.

sion of PhoR from the activator form to the repressor form, and PhoM activating PhoB, but not influenced by phosphate levels. It appears that the PhoB protein interacts with a sequence located in analogous regions upstream from the structural genes, known as the 'pho' box [25].

A protein analogous to PhoE has been found in *P. aeruginosa*, OprP [27]. There are some differences between the mechanistics of these two porins, in relation to the size of the channels, with OprP forming smaller phosphate specific channels, and also possessing a saturable phosphate binding site [17]. However, genetically these two systems have similarities in their regulation, in that expression of OprP is also regulated by a two component system analogous to and genetically interchangeable with, the *E. coli* Pst system [28].

The *E. coli lamB* gene encodes the LamB protein, a maltose inducible outer membrane protein, also known as the maltoporin or lambda receptor. The *lamB* gene is part of an intricate network involved in the transport of maltose and maltodextrins, comprised of three operons contained within two loci, *malA* and *malB* [29]. The transcription of these operons is induced by maltose and mediated by an activator encoded by the *malT* gene. In the presence of maltose the *malT* product stimulates the expression of all genes in the regulon except *malT* itself. The *malT* gene product binds to an asymmetric nucleotide sequence, and its activity requires both ATP and maltotriose as cofactors [30]. The induction of the *mal* operons is inhibited by glucose, thereby providing a further regulatory mechanism in the form of catabolite repression.

The LamB porin may interact with the periplasmic maltose binding protein (MalE), which subsequently interacts with other proteins involved in maltose transport, comprising a multicomponent binding protein dependent system. Thus, this specific transport system for maltose use is positively regulated by the channel substrate itself. A similar inducible system involved in glucose-uptake has been described in *P. aeruginosa* [31]. This system involves a protein analogous to Lamb, the *oprB* gene product, and a periplasmic glucose-binding protein similar to MalE.

Porins in bacterial pathogenesis

As predominant surface proteins in Gram-negative bacteria, it is no sur-

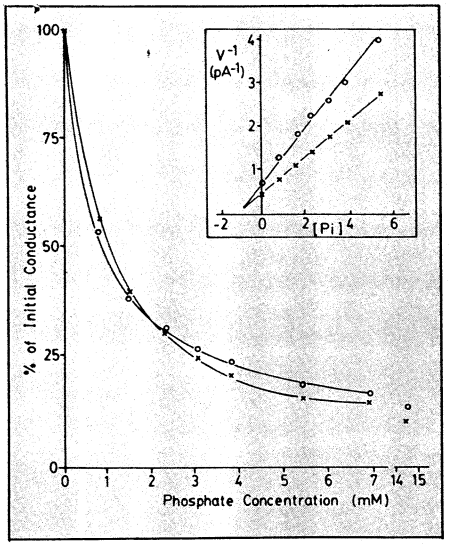

Figure 4. Kinetics of phosphate inhibition of macroscopic chloride conductance. Figure reproduced from *BBA* 860:699.

prise that porins have received substantial attention in studies of disease-causing organisms. Some of these studies relate to a role in antibiotic uptake, since few serious bacterial diseases go without antibiotic treatment. Two examples, mentioned above are the down regulation of *E. coli* OmpF proin *in vivo*, resulting in reduced antibiotic susceptibility, and the development in certain cases of clinical antibiotic resistance of porin deficient mutants. Another prominent example involves the mutation of *P. aeruginosa* to resistance to the broad spectrum β-lactam antibiotic imipenem. These mutants frequently arise during therapy. They are resistant only to imipenem and lack OprD, a specific porin with an imipenem binding site in its channel.

As antigens porins can induce vigorous immune responses during infections. However they have been used with little success in vaccine development because they tend to be antigenically heterogeneous. This is caused by amino acid sequnce variations in the surface-exposed regions of different isolates.

Where they may find use is as an adjuvant. They are easy to purify, abundant [32] and are good mitogenic stimulators of B cells. In certain applications peptide epitopes can be inserted between adjacent transmembrane β-strands to create tailored cell surface epitopes.

In addition to the roles in bacterial disease described above, the porins of *Legionella* and *Chlamydia* are thought to be involved in adherence to macrophage receptors, a major event in pathogenesis of these diseases.

Conclusions

With the availability of the first molecular structures of porins [10, 11, 34] we are now in a position to exploit a wide variety of functional information about them. Using molecular genetic techniques in combination with sophisticated functional studies and model building, it should be possible to identify those amino acids which determine pore function, and by corollary learn how to design with greater ability to penetrate these pores.

Porins are directly involved in β -lactam uptake into Gram-negative bacteria. Mutants lacking specific porins are thus resistant to certain antibiotics. Alternatively, it has been demonstrated that the ability of a β-lactam to penetrate through porins can strongly influence the effectiveness of these antibiotics [5, 6]. However, to date, resistance to bacterial β-lactamases and not optimised penetrability, have been the most important design feature for semi-synthetic β-lactams. Nevertheless, we feel that we are now approaching a situation in which chemists can rationally engineer a β-lactam for improved uptake. Since β-lactams are the single most used group of antibiotics in hospitals, with a world market in excess of 10 billion dollars, it would seem that introduction of such design features would constitute a competitive edge for companies in this marketplace.

> As antigens porins can induce vigorous immune responses during infections.

Understanding porin structure/ function relationships will have many other potential benefits. Porins have an intimate role in the lifestyle of Gram-negative bacteria. In a sense it is the molecular sieving nature of the Gram-negative bacterial outer membrane that definitively discriminates them from Gram-positive bacteria.

Thus, understanding porins and their role in Gram-negative bacteria might help us to understand why the bacterial kingdom has evolved into two groups with or without outer membranes.

Acknowledgements

The researchers' own research on outer membranes has been generously supported by grants from the Networks of Centres of Excellence to the Canadian Bacterial Diseases Network, the Canadian Cystic Fibrosis Foundation and the Medical Research Council of Canada. R Hancock wishes to acknowledge Peter Reeves who started him off on his quest for knowledge about outer membranes.

Bob Hancock and Melissa Brown did their PhDs in the Department of Microbiology and Immunology at the University of Adelaide and are currently at the Department of Microbiology, Univeristy of British Columbia, Vancouver, BC, Canada V6T 1Z3.

References

1 Hancock REW (1991) *ASM News* 57:175.
2 Nikaido H, Rosenberg E (1983) *J Bacteriol* 153:241.
3 Bellido F, Martin NL, Siehnel RJ, Hancock REW (1992) *J Bacteriol*, 174:5196.
4 Zimmermann W, Rosselet A (1977) *Antimicrob Agents Chemother* 12:368.
5 Bellido F, Pechere JC, Hancock REW (1991) *Antimicrob Agents Chemother* 35:78.
6 Bellido F, Pechere JC, Hancock REW (1991) *Antimicrob Agents Chemother* 35:73.
7 Siehnel R, Martin NL, Hancock REW (1990) *Molec Microbiol* 4:831.
8 Jeanteur D, Lakey JH, Pattus F (1991) *Molec Microbiol* 5:2153.
9 Paul C, Rosenbush J (1985) *EMBO J* 4:1593.
10 Weiss MS. Kruesch A, Schiltz E, Nestell U, Wette W, Weckesser J, Schulz GE (1991) *FEBS Lett* 280:379.
11 Woitzik D, Weckesser J, Benz R, Stevanovic S, Jung G, Rosenbush JP (1990) *Z Naturforsch* 45:576.
12 Nakae T (1976) *J Biol Chem* 251:2176.
13 Hancock REW (1986) Model membrane studies of porin function. In *Bacterial outer membrane as model systems* (Inouye, M, ed) pp 187-225. John Wiley and Sons.
14 Nikaido H, Rosenberg E (1981) *J Gen Physiol* 77:121.
15 Benz R, Woitzik D, Flamman HT, Weckesser J (1987) *Arch Microbiol* 148:226.
16 Sen K, Hellman J, Nikaido H (1988) *J Biol Chem* 263:1182.
17 Hancock REW, Benz R (1986) *Biochem Biophys Acta* 860:699.
18 Benz R, Schmidt A, Vos-Scheperkeuter GH (1987) *J Memb Biol* 100:21.
19 Nikiaido H, Vaara M (1985) *Microbiol Rev* 49:1.
20 Rosner JL, Chai T-J, Foulds J (1991) *J Bacteriol* 173:5631.
21 Mizuno T, Mizushima S (1990) *Mol Microbiol* 4:1077
22 Slauch JM, Silhavy, TJ (1989) *J Mol Biol* 210:281.
23 Tsui P, Helu V, Freundlich M (1988) *J Bacteriol* 170:4950.
24 Anderson J, Delihas N (1990) *Biochem* 29:9249.
25 Shinagawa H, Makino K, Amemura M, Nakata A (1987) Structure and function of the regulatory genes for the phosphate regulon in *Escherichia coli* . In *Phosphate Metabolism and Cellular Regulation in Microoragnisms.* Toriani-Guino a, *et al* (eds). Washington, DC ASM press, pp 20-25.
26 Benz R, Darveau, Hancock REW (1984) *Eur J Biochem* 140:319.
27 Hancock REW, Poole K, Benz R (1982) *J Bacteriol* 150:730.
28 Siehnel RJ, Worobec EA, Hancock REW (1988b) *Mol Microbiol* 2:347.
29 Davidson AL, Nikaido H (1990) *Biol Chem* 265:4254.
30 Richet E, Raibaud O (1989) *EMBO* 8:981
31 Hancock REW, Carey AM (1980) *FEMS Microbiol Lett* 8:105.
32 Bessler W, Henning U (1979) *Zblt Immunitatsforsch* 155:387.
33 Charbit A, Boulain JC, Ryter A, Hofnung M (1986) *EMBO J* 5:3029.
34 Cowan SW, Schirmer T, Rummel G, Steiert M, Ghosh R, Paupit RA, Jansonius JN, Rosenbark JP (1992) *Nature* 358:727.