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MINIREVIEWS

Role of Porins in Outer Membrane Permeability

R. E. W. HANCOCK

Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

INTRODUCTION

It is now well recognized that the outer membranes of gram-negative bacteria serve as molecular sieves which permit the passage of small hydrophilic molecules of sizes below a given cutoff, i.e., the exclusion limit (21, 27). This sieving property, which permits uptake of small substrate molecules but excludes potentially harmful enzymes and other large hydrophilic molecules, is due to a class of proteins called porins (21). Porins form trans-outer-membrane, water-filled channels, the dimensions of which determine the exclusion limit. An indication of their importance in bacteria is the observaton that *Spirochaeta aurantia*, an organism which apparently lacks lipopolysaccharide in its outer membrane (also called its outer sheath) (A. Kropinski and E. P. Greenberg, personal communication), nevertheless contains a major porin protein (19) whose properties are closely related to those of, e.g., *Escherichia coli* (7). Porins have now been identified and characterized for 32 species of bacteria (R. E. W. Hancock, *in* M. Inouye, ed., *Bacterial Outer Membranes as Model Systems*, in press; Hancock, unpublished data), representing 10 separate families of gram-negative bacteria, as well as in the outer membranes of mitochondria and chloroplasts (4) of eucaryotic cells. Given the enormous wealth of knowledge on porins, I will make no attempt in this review to describe them comprehensively, and the reader is referred to a number of recent reviews (4, 14, 15, 22, 26, 31). Rather, I attempt here to provide an overview of our understanding of the functions of porins, with emphasis on recent findings.

PHYSICAL PROPERTIES OF PORINS

In general, the physical properties of the 44 porins examined to date are quite similar. Porins have monomer molecular weights in the range of 28,000 to 48,000, are present in the membrane as oligomers (usually trimers [23]) that tend to be resistant to sodium dodecyl sulfate denaturation (23, 37), are often strongly but noncovalently associated with the underlying peptidoglycan (37) and with lipopolysaccharide, and, in the cases studied, have acidic pIs and a high content of β-sheet structure (reviewed in references 4 and 22). However, exceptions to many of these properties have been described (4, 22).

Of the four well-studied porins in *E. coli*, OmpF, OmpC, and PhoE show striking similarities in nucleotide and amino acid sequences (20), suggesting a common evolutionary origin, while LamB is less similar but contains several regions of local homology (32). Interestingly, LamB has physical characteristics almost identical to those of the other three porins (31), although it is functionally distinct (see below).

Porins usually form trimeric aggregates in their native states (23). Electron microscopy and image reconstruction studies of the *E. coli* OmpF porin have shown that the trimer apparently contains three separate openings at the surface, which coalesce into a single channel near the center of the membrane (12) (Fig. 1). In agreement with this structure, to date there has been no demonstration of a functional monomer unit of the OmpF porin. In contrast, *Pseudomonas aeruginosa* porin F, which is present in the outer membrane as an oligomer (probably a trimer [1]), is capable of functioning in model membranes after sodium dodecyl sulfate dissociation to a monomer (46, 48). Interestingly, recent evidence suggests that the *E. coli* PhoE porin may also contain three pores per trimer (10). The structures of these porins and of the phosphate-selective porin protein P are presented in schematic form in Fig. 1.

IN VIVO EVIDENCE FOR A ROLE FOR PORINS IN OUTER MEMBRANE PERMEATION

The strongest data supporting an in vivo role for porins in the permeation of substrates across the outer membranes of gram-negative bacteria have been obtained by comparing porin-deficient mutants with their isogenic wild-type strains. A method for measuring outer membrane permeability of β-lactam antibiotics, developed separately by Zimmerman and Rosselet (49) and Sawai et al. (38), was used to demonstrate that porin-deficient mutants have 10- to 100-fold-lower rates of β-lactam permeation than their porin-sufficient parental strains (Table 1). The effect of these lower rates of antibiotic permeation often is to make the porin-deficient cells more resistant to hydrophilic antibiotics (18). However, it is important to note that lower outer membrane permeability is not by itself sufficient to result in increased resistance to antibiotics. For example, a β-lactam (e.g., nitrocefin) present at 50 μg/ml in the medium can equilibrate across the outer membranes of wild-type *E. coli* and an *E. coli* porin-deficient mutant in approximately 2 and 80 s, respectively, times that are far less than the generation times of these strains. Therefore, for slow uptake to result in resistance, a secondary defense mechanism must work in synergy with slow uptake. As demonstrated by Vu and Nikaido (45) for *Enterobacter cloacae* strains, this secondary defense mechanism for β-lactam antibiotics is probably β-lactamase, which can hydrolyze β-lactams in the periplasm, thus preventing their buildup to concentrations that can result in inhibition of their cellular targets. Such considerations are responsible for the puzzling observations that porin-deficient mutants are considerably more resistant than porin-sufficient strains to some antibiotics, but only slightly, if at all, more resistant to other antibiotics (18). Thus, one must consider not only the relative rates of permeation of these antibiotics

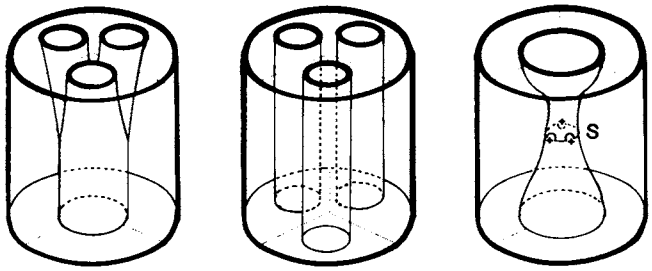

FIG. 1. Schematic three-dimensional representations of the structures of three different porin proteins. The porins are oriented such that the top of the figure is the portion exposed in the external environment, whereas the bottom is the portion extending into the periplasmic space. The plane of the outer membrane would run horizontal to these channels. These general structures are inferred from the following data: OmpF porin of *E. coli*, Fourier transform electron diffraction studies of oriented lipid bilayers stacked with OmpF porin molecules (12); PhoE porin of *E. coli*, high-voltage-induced single-channel closure experiments as well as cited but as yet unpublished low-resolution structural studies (10); protein F of *P. aeruginosa*, chemical cross-linking studies inferring a native trimer structure (1), together with the demonstration that purified monomers form functional channels with exclusion limits similar to those of outer membrane fragments (46, 48) (evidence has been presented that protein F forms two distinct populations of channels; see text); protein P of *P. aeruginosa*, black lipid bilayer measurements, chemical modification studies (15), chemical cross-linking (1), and symmetry arguments (15) suggesting a selectivity filter (S) consisting of three charged lysine molecules (+), each of which is contributed by a separate monomer subunit of the protein P trimer and each of which is proposed (15) to coordinate one of the symmetrical negative or partial negative changes on phosphate ions.

in porin-sufficient and porin-deficient strains, but also the efficiency (i.e., the K_m and V_{max}) of the secondary defense mechanism at the steady-state periplasmic concentration of antibiotic achieved through alteration of this rate in the porin-deficient mutants.

It is perhaps worth mentioning that the term porin-deficient mutant is probably a misnomer, since such mutants almost certainly contain low levels of alternative porins which are responsible for the residual permeability of the mutant outer membrane (5). As a practical corollory to the above-described research with mutants, it could be shown that *P. aeruginosa*, which is known to demonstrate substantial resistance to nearly all commonly used antibiotics, has much lower permeability than *E. coli* (24, 47) and simplistically can be considered analogous to an *E. coli* porin-deficient mutant (Table 1, footnote *a*).

By far the bulk of data obtained for porins comes from model membrane studies. It is thus important to note that the ability of a number of porin proteins to form water-filled channels has been confirmed in vivo (3, 15). In addition, a variety of properties which have been studied for model membrane systems, including channel size (Table 1), ion selectivity (for the *E. coli* OmpF, OmpC, and PhoE porins [30]), maltodextrin and phosphate specificity (for the *E. coli* LamB [13] and *P. aeruginosa* protein P [35] porins), and poor function (for *P. aeruginosa* porin protein F [24, 48]), have been confirmed in experiments with intact cells. These data strongly support the validity of model system studies in investigating the properties of porins. It should be stressed, however, that model system studies provide an oversimplified view of how porins behave in the bacterial cell. For example, while bacterial porins in model systems behave as if they are completely symmetrical (cf. mitochondrial porins [4]), it is clear that the environments of their channel openings at the surface of the cell and in the periplasmic space are completely different. The existence of these asymmetric environments (see reference 31 for a more complete discussion of periplasmic sinks) and, in at least one case, of a periplasmic substrate-binding protein which associates the LamB porin (13) creates a considerably more complex situation than that portrayed by model systems.

MODEL MEMBRANE STUDIES OF PORINS

A variety of model systems have been used to investigate the functions of porins in vitro. These systems have been described in some detail previously (4; Hancock, in press). The two most-utilized systems, liposome swelling and black lipid bilayer studies, probe the function of the porin in allowing passage of medium-sized sugars, β-lactams, and amino acids, and small- to medium-sized ions, respectively. While these different types of substrates are clearly relevant to porin function in the intact cell, it is of interest to consider whether the two model systems provide similar basic information. Perhaps the most important requirement of a good model system is that it should have accurate predictive abilities. In Table 1, the diameters of four porin channels, predicted by three different model systems, are compared. It is comforting that despite the different natures of the three model systems and the completely different equations and assumptions utilized to calculate these channel diameters (4, 28; Hancock, in press), the estimates are usually quite similar for a given porin. It should be noted that all of these calculations adopt one common and obviously incorrect assumption: that the channels are perfect cylinders. Therefore, one should consider channel size estimates to be somewhat descriptive.

Indeed, recent studies have placed doubts on the use of conductance measurements for estimating channel diameters with the formula $\bar{\Lambda}/\sigma = \pi r^2/l$ (where $\bar{\Lambda}$ is the average single-channel conductance, σ is the bulk conductance of the given salt solution, r is the channel radius, and l is the channel length [assumed to be 6 nm] [12]). Dargent et al. (10), using sustained high voltage (>124 mV) as a tool to bring about channel closure, provided strong data that the PhoE trimer consists of three separate channels (Fig. 1) with

TABLE 1. In vivo and in vitro properties of porins

Porin	Relative β-lactam permeation rate of porin-deficient mutant[a]	Estimated pore diameter (nm)[b]			
		In vivo	Liposome dilution studies	Liposome swelling studies	Black lipid bilayer studies
OmpF	≤0.002	1.16 (28)	1.13[c]	1.16 (29)	1.15 (7)
OmpC	0.05		1.13[c]	1.08 (29)	1.02 (7)
PhoE	0.09		1.13[c]	1.06 (29)	1.11 (7)
Protein F	0.16 (0.8)	>1.63[d]	2.0–2.9[d]	2.0 (48)	2.0 (7)

[a] Rate relative to a parent containing the indicated porin as the only major porin. Rates for *E. coli* porins OmpF (3, 33), OmpC (extrapolated number [29]), and PhoE (33) are for cephaloridine diffusion across the outer membrane of intact cells; the *P. aeruginosa* protein F (24) rate is for nitrocefin diffusion. The number in parentheses is the rate of nitrocefin permeation for wild-type *P. aeruginosa* relative to that of wild-type *E. coli*.

[b] Numbers in parentheses are reference numbers.

[c] Based on estimates that raffinose (diameter, 1.128 nm [36]) is the largest molecule capable of penetrating the *E. coli* outer membrane (21).

[d] Based on data measuring the exclusion limits of plasmolyzed cells (11) and protein F-containing liposomes (16), respectively (i.e., >2,000 and 3,000 to 9,000, respectively) and on the assumption that the spherical diameter of saccharides is related to the cube of their molecular weights (48).

single-channel conductances in 1 M KCl of 0.6 nS (1S = 1 Ω^{-1}). Benz et al. (7) used the conductance of the PhoE trimer (1.8 nS) to estimate the channel diameter given in Table 1. If the correct single-channel conductance were used instead, then with the formula and assumptions described above, a channel diameter estimate of 0.64 nm would result for PhoE. Earlier voltage-induced channel breakdown studies of the OmpF porin channel (39) suggested that this channel also behaves as if it contains three channels per trimer (presumably the three openings on the outer surface of the OmpF channel [Fig. 1]), giving rise to similar problems with the PhoE channel diameter estimates. In addition, discrepancies between oligosaccharide exclusion (44) and single-channel conductance (43) estimates of the *Haemophilus influenzae* porin channel diameter were observed. There are a number of possible explanations for these discrepancies. One possibility is that the channel length has been underestimated in the equation above. Second, use of this equation rests on the assumption that the ions are interacting with water molecules only and not with the walls of the channel, thus raising the possibility that the diameter estimated from this equation measures the extent only of free water in the channel. Third, Vachon et al. (43) have pointed out how such discrepancies can be explained, in part, by consideration of channel shape. In summary, the data above suggest that black lipid bilayer studies are not as good for predicting the exclusion limits of porin channels as previously assumed. However, this methodology is still a powerful tool for studying the physical and functional characteristics of individual porin proteins (8, 10, 15).

FUNCTIONAL PROPERTIES OF PORIN PROTEINS

The functions of porin channels have been the topic of a number of recent reviews (4, 22, 26, 31; Hancock, in press) are merely outlined below. Porins form large (0.6- to 2.3-nm-diameter), transmembrane, water-filled channels. The size of the channel largely determines the exclusion limit of the outer membrane for hydrophilic compounds (Table 1), and most porins demonstrate little chemical (as opposed to ion or charge) selectivity for different substrates (6, 28, 30, 31). The interior of a porin channel contains charged amino acids lining part of the channel (17, 22, 40), and it is the number and position of these charges relative to the most constricted portion of the channel which appear to be the strongest determinants of the ion selectivity of the channel (17). Porin channels can be either cation or anion selective, but generally this selectivity is weak for small ions like K^+ and Cl^- (ranging from threefold anion selective to 40-fold cation selective [7]). These ion selectivities are reflected in the sieving properties of the porins for antibiotics of different charges. For example the cation-selective OmpF channel favors the passage of zwitterionic over anionic β-lactams, whereas for the anion-selective PhoE channel this situation is reversed (30).

Porin channels seem to be neither voltage gated nor, in most cases, voltage regulated (4). However, as noted above, sustained application of high voltages can cause channel breakdown (10, 39). These voltages (>120 mV) apparently exceed the measured Donnan potential (50 to 80 mV) across the outer membrane (41) and must be considered nonphysiological. It is the tertiary and quaternary structures of the porin which apparently determine its function, and, in the cases studied, neither lipids (Hancock, in press) nor lipopolysaccharides (34) influence the function of porins.

PORINS WITH UNUSUAL PROPERTIES

Most porin proteins form simple aqueous channels which confer a molecular sieving function on the outer membrane. However, a few porin proteins demonstrate substrate specificity. The well-characterized *P. aeruginosa* P and *E. coli* LamB proteins have been shown to function in vivo in phosphate (35) and maltose-maltodextrin (13) transport, respectively, across the outer membrane. Both channels demonstrate binding sites (Fig. 1) with affinities of around 0.2 to 0.3 mM for their respective substrates (8, 15), and it has been suggested that they have channel structures that are quite different from those of general diffusion porins like OmpF and OmpC (8, 15). The PhoE porin, while having many similarities to the general diffusion porins (7), has been shown to function in polyphosphate uptake (10, 33); the *Bordetella pertussis* 40-kilodalton porin is, like protein P, anion specific (2), although its specific anionic substrate, if any, remains to be determined.

Recent data, utilizing *P. aeruginosa* protein F purified both from an *E. coli* isolate containing the cloned protein F gene and from *P. aeruginosa*, have suggested that this protein forms two types of channels (46), a predominant small channel (single-channel conductance, 0.36 nS) and a rarer large channel (single-channel conductance, 5.0 nS) that had been previously described (6, 48). The predominant small channel is probably too small to allow passage of antibiotics, including β-lactams, and the low proportion (6, 24, 47) of large channels has been proposed to be responsible for the low diffusion rate and consequent high intrinsic resistance of *P. aeruginosa* to antibiotics (26). This proposed functional heterogeneity is interesting, and my co-workers and I have recently described a model involving two alternative arrangements of the four protein F cysteine molecules in cystine disulfide bonds (R. A. Moore, W. A. Woodruff, and R. E. W. Hancock, Antibiot. Chemother., in press) to account for these observations.

NONPORIN PATHWAYS OF UPTAKE ACROSS THE OUTER MEMBRANE

Not all molecules cross outer membranes via porin proteins. Excreted proteins, transforming DNA, and bacteriocins are all too large to pass through the channels of porins, and the mechanisms of passage of these macromolecules are inadequately understood (but see reference 14). In addition, two alternative (nonporin) uptake pathways have been proposed, the self-promoted pathway for polycation (e.g., polymyxin and aminoglycoside antibiotics) uptake (14) and the hydrophobic pathway for uptake of hydrophobic substances (25–27) (e.g., in *Neisseria* and *Haemophilus* spp.). These pathways have been discussed in detail previously (14, 31). In considering outer membrane permeation, it is important not to underestimate the importance of these nonporin uptake pathways.

OUTSTANDING QUESTIONS

In the 10 years since their existence became known, there has been an enormous explosion of knowledge about porins. This has been fuelled by the relative ease with which these proteins can be purified, their stability to detergent and chemical treatments, and the development of excellent model membrane systems for their study. However there is a clear need to expand our knowledge regarding the in vivo interactions between porin proteins and other cellular com-

ponents. In addition, a number of outstanding questions remain. In particular, it is necessary to obtain three-dimensional structures for one or more porin proteins to allow structure-function relationships to be determined at the molecular level. Such studies are well under way for the OmpF porin (12). It will be of great interest to determine exactly how the substrate-specific porins, protein P and LamB, differ structurally from general diffusion porins like OmpF. These studies will be assisted greatly by models built as a result of a variety of molecular biological approaches (9, 43).

Another important problem relates to the relative importance of porin and nonporin uptake pathways in diverse organisms. While more than 40 porins from 32 different gram-negative bacterial species have been studied, these represent a small percentage of the known species and only 25% of the families of gram-negative bacteria. Thus, our current understanding of the fundamental aspects of porin function is based on a rather limited consensus (heavily biased towards medically important pathogens). It is clear, however, that future studies will have the advantage of being able to build on a rather sophisticated framework of information about porin function.

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