Structure and Function of Pore-Forming β-Barrels from Bacteria

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Abstract

Crystallographic studies of the past ten years have revealed that many outer membrane proteins and bacterial toxins are constructed on the β-barrel motif. Two structural classes can be identified. The first class, represented by the porins, includes monomeric or multimeric proteins where each β-barrel is formed from a single polypeptide. The second class features proteins where the β-barrel is itself a multimeric assembly, to which each subunit contributes a few β-strands. In addition to structural investigations, much work has also been devoted to the functional aspects of these proteins, and to the relationships between structure and function. Here we present a review of the structural and the functional properties of some of the best-studied examples of these various classes of proteins, namely the general-diffusion, specific and ligand-gated porins, multidrug efflux proteins and the staphylococcal toxin α-hemolysin.

The β-barrel is a common feature for integral membrane proteins of bacterial origin. In particular, proteins involved in transport across bacterial outer membranes share this structural motif. Much attention has recently been devoted to these proteins, as atomic resolution structures have flourished in the past few years (Table). The reader is directed to recent reviews dealing with the more structural aspects of the research carried out on these proteins (Schirmer, 1998; Buchanan, 1999; Koebnik et al., 2000; Schultz, 2000). Although, the discussion of some structural features is unavoidable here, this review will be centered on the more functional or dynamic aspects of these proteins. The last couple of years have seen an explosion of articles on porins from a great variety of microorganisms. For sake of conciseness, this review is essentially focused on Escherichia coli, with mention of a handful of other organisms. This somewhat slanted view is not meant to mitigate the importance of other studies.

General Diffusion Porins

Members of the general diffusion porin family, such as the Escherichia coli OmpF, OmpC and PhoE proteins and the Klebsiella pneumoniae OmpK36 (Figure 1), are examples of the best characterized β-barrel channels at the functional level. Still, the molecular basis and physiological relevance of many of their properties, such as voltage-dependence, cooperativity and modulation, have remained elusive.

These proteins form trimers in the outer membrane of Gram-negative bacteria, where they play the major role of molecular sieves. They allow for the fast flow of hydrophilic solutes of molecular weight less than 600 through the highly hydrophobic protective barrier formed by the outer membrane and its lipopolysaccharide layer (Nikaido, 1996). A major breakthrough in porin research occurred when the crystal structures of OmpF and PhoE were solved in 1992 (Cowan et al., 1992). Various structural aspects of the OmpF porin are illustrated in Figure 2. Each monomer is a barrel of 16 β-strands connected by long extracellular loops and short periplasmic turns (Figure 2C). The stability of the trimeric assembly is provided by extensive polypeptide chain contacts between the three monomers, and by the so-called “latching” loop L2 (highlighted in darker color in Figure 2A). This loop emerges from each monomer and extends sideways, crossing over the barrel wall of the adjacent subunit and making ionic contacts with barrel residues of this adjacent subunit. The importance of this loop for the stability of the protein trimer has been demonstrated experimentally (Phale et al., 1998).

The β-barrel of each monomer forms a hollow, hydrophilic cylinder that is constricted at mid-height by the inwardly folded L3 loop. This region, the constriction zone or eyelet, is decorated with charged residues that fall into two groups: negatively charged residues on L3 (residues D113 and E117 on OmpF in Figure 2B) and a cluster of positive charges on the opposite barrel wall (residues K16, R42, R82 and R132 on OmpF in Figure 2B). This charge constellation establishes an intrinsic electrostatic potential within the pore itself, that may play an important role in driving ionic movement and in ionic selectivity, as variations in the potential profile have been found between cation- and anion-selective porins (Karshikoff et al., 1994; Zeth et al., 2000).

These porins were the first ion channel types for which a three-dimensional structure was available, and this finding paved the way for numerous investigations of structure-function relationships (Saint et al., 1996a; Eppens et al., 1997; Phale et al., 1997, 1998; Van Gelder et al., 1997; Bainbridge et al., 1998; 2002 Horizon Scientific Press

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Liu and Delcour, 1998a; Liu et al., 2000). Most of these studies combined site-directed mutagenesis with electrophysiology, either by measurements on planar lipid bilayers (also known as black lipid membranes or BLM’s) or by patch clamp (see Delcour, 1997 for a comparison of the two techniques).

The planar lipid bilayer studies established the importance of the constriction zone for permeation and ionic selectivity (Bauer et al., 1989; Lakey et al., 1991; Saint et al., 1996a). They also shed some light on the role of charged residues of the eyelet in voltage sensing. It has been long known that membrane potentials greater than a certain threshold trigger porin inactivation, leading to a substantial decrease in the current passing through a porin-containing membrane. The mutation of negatively charged residues of L3 into neutral ones was shown to render OmpF less voltage-sensitive, but PhoE more voltage-sensitive (Van Gelder et al., 1997). On the other hand, increased voltage-sensitivity was conferred to OmpF by the removal of charges from the cluster of positively charged amino acids located in the barrel wall directly opposite to L3 (Saint et al., 1996a). This intriguing observation led to the suggestion that the positive residues in the barrel are the voltage sensors in the anion-selective PhoE, while the negative charges on the L3 loop are responsible for voltage sensing in the cation-selective porins, such as OmpF and OmpC (Van Gelder et al., 1997). This difference between the two porin types was later correlated to their opposite voltage-dependence (Samartzidou and Delcour, 1998). The position of the L3 loop across the pore makes it a prime candidate for the role of a gate that might swing across the pore to bring about porin inactivation and closure. Recent experiments strongly disputed a gross movement of the L3 loop during voltage-dependent gating, because no change in voltage-sensitivity is observed when the loop is tethered to the barrel by engineered disulfide bridges (Eppens et al., 1997; Phale et al., 1997; Bainbridge et al., 1998). But there remains the possibility that more subtle or localized movements, or a breathing of the two branches of the L3 loop with respect to each other, take place during the putative conformational change underlying voltage-dependent porin inactivation.

Black lipid membranes studies on porins have tended to focus on conductance measurements or macroscopic properties of populations of channels. Because higher filter corner frequency and faster sampling are typically used in patch-clamp studies, rapid small transitions in current values, usually masked in traces obtained from BLMs, are detected with the patch clamp technique (Berrier et al., 1992, 1997; Delcour, 1997; Liu and Delcour, 1998a; Liu et al., 2000). These fast transitions between closed and open states suggest that the porins can undergo a spontaneous form of gating, that is distinct from the voltage-induced inactivation. Although one might be

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Table: Bacterial β-barrel transport proteins with solved oligomeric crystal structures at high resolution. Low-resolution X-ray structures, models, AFM and electron microscopy images are not listed.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Bacterium</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porin</td>
<td><em>Rhodobacter capsulatus</em></td>
<td>General diffusion porin</td>
<td>Weiss et al. (1991)</td>
</tr>
<tr>
<td>Porin</td>
<td><em>Rhodopseudomonas Blastica</em></td>
<td>General diffusion porin</td>
<td>Kreusch et al. (1994)</td>
</tr>
<tr>
<td>OmpF</td>
<td><em>Escherichia coli</em></td>
<td>General diffusion porin</td>
<td>Cowan et al. (1992)</td>
</tr>
<tr>
<td>PhoE</td>
<td><em>Escherichia coli</em></td>
<td>General diffusion porin</td>
<td>Dutzler et al. (1999)</td>
</tr>
<tr>
<td>OmpK36</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>General diffusion porin</td>
<td>Cowan et al. (1992)</td>
</tr>
<tr>
<td>Omp32</td>
<td><em>Comamonas acidovorans</em></td>
<td>General diffusion porin</td>
<td>Zeth et al. (2000)</td>
</tr>
<tr>
<td>LamB</td>
<td><em>Escherichia coli</em></td>
<td>Specific porin</td>
<td>Schirmer et al. (1995)</td>
</tr>
<tr>
<td>ScrY</td>
<td><em>Salmonella typhimurium</em></td>
<td>Specific porin</td>
<td>Forst et al. (1998)</td>
</tr>
<tr>
<td>FepA</td>
<td><em>Escherichia coli</em></td>
<td>Ligand-gated porin</td>
<td>Buchanan et al. (1999)</td>
</tr>
<tr>
<td>FhuA</td>
<td><em>Escherichia coli</em></td>
<td>Ligand-gated porin</td>
<td>Fergusson et al. (1998)</td>
</tr>
<tr>
<td>TolC</td>
<td><em>Escherichia coli</em></td>
<td>Multidrug efflux system</td>
<td>Locher et al. (1986)</td>
</tr>
<tr>
<td>α-Hemolysin</td>
<td><em>Staphylococcus aureus</em></td>
<td>Pore toxin</td>
<td>Song et al. (1996)</td>
</tr>
</tbody>
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Liu and Delcour, 1998a; Liu et al., 2000). Most of these studies combined site-directed mutagenesis with electrophysiology, either by measurements on planar lipid bilayers (also known as black lipid membranes or BLM’s) or by patch clamp (see Delcour, 1997 for a comparison of the two techniques).

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Figure 1. X-ray structures of three representative members of the β-barrel families described in the text. Extracellular side is at the top. The structures were drawn with Rasmol.
tempted to visualize the functional protein as the beautiful rigid open pore pictured in the X-ray structure, it would be naive to consider the protein as a perfectly motionless body once in its native environment. We have proposed that thermal fluctuations, or possibly electrostatic oscillations linked to ionic permeation, might trigger small conformational changes in the protein, that give rise to many distinct functional states. The spontaneous oscillations between these states would be manifested in the observed spontaneous gating activity. Mutations that disrupt the interactions of the L3 loop with the adjacent or the opposite barrel wall were found to lead to distinct modifications in this gating activity (Liu and Delcour, 1998a; Liu et al., 2000). The locations of the mutated residues are shown in Figure 2B. These observations suggest that H-bonding and ionic interactions between specific residues of the barrel walls and L3 play important roles in the stability of the functional states and the kinetics of conversions between these states. One of the most interesting aspects of this dynamic behavior of porins is that it allows for modulation by physico-chemical parameters that might stabilize specific conformations and thus tip the equilibrium between open and closed states to one or the other direction.

Electrophysiological experiments have provided evidence for modulation of porin function by transmembrane voltage (Schindler and Rosenbusch, 1978; Dargent et al., 1986; Samartzidou and Delcour, 1998), acidic pH (Buehler et al., 1991; Todt et al., 1992; Saint et al., 1996b; Liu and Delcour, 1998b), pressure (Le Dain et al., 1996), membrane-derived oligosaccharides (Delcour et al., 1992), and polyamines or poly-anions (delaVega and Delcour, 1995; Iyer and Delcour, 1997; Samartzidou and Delcour, 1999a). In all cases, porin inhibition (in the form of inactivation or increased tendency to close) was observed. It is unlikely that these forms of modulation are mere artifacts, as they have been observed by many different laboratories working with various techniques and preparations. In addition, measurements of antibiotic permeation in live
cells have confirmed porin inhibition by polyamines (delaVega and Delcour, 1996), pH and extracellular ionic strength (our own unpublished observations). The molecular mechanisms for these forms of modulation are not yet clearly established. With the use of atomic force microscopy, Müller and Engel (1999) reported a conformational change of the extracellular domain of OmpF in response to very acidic pH (3) or high voltages (500 mV). They proposed that the protrusions formed by the extracellular loops might rotate about a hinge at the rim of the β-barrel, thus collapsing into the vestibule and effectively closing the channel entrance (Figure 2D). The proposal that the extracellular loops may form a lid whose movement would control some forms of gating is intriguing, and certainly deserves further attention. Experimental evidence for the involvement of surface-located loops in voltage gating has been recently presented for the Hib porin of Haemophilus influenzae (Arbing et al., 2000). The authors showed that the succinylation – and thus loss of positive charges – of surface-exposed lysines of loops #1 and #4-7 lead to an increase in voltage sensitivity. An interesting implication of this work is that the voltage dependence of porin function may not necessarily require movements of charges within the transmembrane field, but possibly a sensitivity to surface potential, which must be considerable at the level of the lipopolysaccharide layer.

OmpC and OmpF are inhibited by the polyamines spermine, spermidine and cadaverine in a concentration- and voltage-dependent manner. The kinetic effects are complex and include both the suppression of channel openings and the enhancement of channel closures, as well as the promotion of blocked or inactivated states (Iyer and Delcour, 1997). The mechanisms are likely to involve multiple sites of interaction, as shown for polyamine action of eukaryotic channels, where some effects, but not all, are suppressed in some porin mutants (Liu et al., 1997; Iyer et al., 2000). Although polyamines are small enough to permeate the pore, it appears that they interact at a specific site inside the pore, that involves acidic and polar residues of the L3 loop and the barrel wall. A recent study, combining site-directed mutagenesis and the analysis of a large number of polyamine analogs, proposes a model whereby spermine would enter the pore in a head-on conformation and effectively bridge the periplasmic and extracellular branches of the L3 loop and connect them to the adjacent barrel wall (Iyer et al., 2000). This saddling over the L3 loop would bring about the allosteric modulation leading to stabilization of closed states and porin inhibition.

Although strains lacking the major general diffusion porins are viable and grow well in rich laboratory media, they tend to experience modifications in outer membrane composition, such as the appearance of previously repressed or cryptic porin-like channels (Blasband et al., 1986; Misra and Benson, 1989; Fajardo et al., 1998; Conlan et al., 2000). These observations illustrate the importance of such pathways in conferring an optimal outer membrane permeability. Porin expression studies have established that cells take great care at controlling outer membrane permeability in various conditions. Although their physiological relevance is unclear, modifications in OmpF and OmpC expression have been noted in response to osmolarity, temperature, nutrient content, pH and population density (Pratt et al., 1996; Liu and Ferenci, 1998; Liu et al., 2000; Sato et al., 2000). The shift from an OmpF-containing membrane to an OmpC-containing one can lead to a substantial decrease in overall outer membrane permeability. In some cases, it is not just the ratio of OmpF to OmpC that is changed, but rather the total amount of porin is decreased (Samartzidou and Delcour, 1999b). Thus, there are conditions where it is presumably advantageous for cells to limit their outer membrane permeability. These events take place on a minute time scale and one might envisage that faster processes, such as those modulating function rather than expression of porins, would exist to provide even more rapid emergency mechanisms in stressful situations.

One challenge facing bacteria is the need to adapt to changes in medium acidity. A well-characterized pH-dependent response is the induction of the cadBA operon at acidic pH (Olson, 1993). The operon encodes a lysine decarboxylase (cadA) and a lysine-cadaverine antiporter (cadB). Upon lowering external pH, the CadC protein of the cytoplasmic membrane binds the cadBA promoter, and activates the operon. Increased levels of CadA and CadB lead to the acid-induced synthesis of cadaverine from lysine and the subsequent excretion of the polyamine through the lysine-cadaverine antiporter. This response has been assumed to result in neutralization of the external pH, thus protecting the cell from the acidic conditions, but this is not the case (Samartzidou and Delcour, 1999b). However, the secretion of cadaverine produces a decrease in outer membrane permeability because cadaverine closes a number of porins (Samartzidou and Delcour, 1999b). This is the first case of porin modulation shown to have direct physiological consequences in vivo. Thus, cadaverine inhibition of porin may play a role in the adaptive response to acidic conditions. This hypothesis appears substantiated, as cadaverine-resistant porin mutants are more sensitive to low pH than wild-type cells (our own unpublished results). These types of studies need to be extended to a range of stimuli, as more porin mutants become available, in order to confirm the general notion that fast regulation of porin activity can provide bacterial cells with survival strategies.

The OmpA Controversy

The N-terminal membrane-spanning domain of the monomeric OmpA protein is an 8-stranded β-barrel (Figure 1) (Pautsch and Schulz, 1998). Cross sectional views reveal that the barrel is constructed as an inverted micelle, lacking a well-defined pore, although water cavities do exist. Because the structural data and some electrophysiological studies argue against
OmpA forming a pore, we have not listed this protein in the Table. It is important, however, to mention that several laboratories have found evidence that OmpA has ionophoretic properties (Saint et al., 1993; Sugawara and Nikaido, 1994; Arora et al., 2000). It is possible that the pore conformation is rare, or is not the one adopted during crystal formation, or might depend on the protein being intact versus truncated (Arora et al., 2000). Interestingly, the ability of OprF, a porin from Pseudomonas aeruginosa which is an ortholog of OmpA, to form small and large channels is also dependent on it being intact (Brinkman et al., 2000; Saint et al., 2000). In addition, a three-dimensional model of the N-terminal half of OprF shows a 8-stranded β-barrel whose pore residues have smaller side chains that the homologs in OmpA, and thus allow for a wider channel than in OmpA (Brinkman et al., 2000). An architectural similarity for the N-terminal domains of OmpA and OprF has also been proposed by others (Saint et al., 2000), although no significant conductance differences between the two proteins were detected experimentally.

Specific Porins

Members of this class of porins display selectivity for the transported solute. For example, the E. coli LamB and the Salmonella ScrY transport maltodextrins and sucrose, respectively. Each protein forms a trimer of 18-stranded β-barrels (Schirmer et al., 1995; Forst et al., 1998). As in the general diffusion porins, the L3 loop folds inward to form the constriction zone, but the channel entrance is also partially obstructed by the L1 and L6 loops. A functional consequence of this architecture is a narrower pore than OmpF, and a reduced conductance to ions (Dargent et al., 1987; Berrier et al., 2000). However, when multiple proteins are reconstituted into lipid bilayers, the ionic current passing through all these pores can be quite large. As increasing concentrations of maltodextrins are added, a progressive reduction in macroscopic current occurs and is accompanied by an increase in current noise (Andersen et al., 1995). Such a behavior indicates that a binding event takes place in the channel upon addition of sugars. The numerical values of various transport parameters (such as flux, rate constants, and Km) were obtained from noise analysis, and showed that sucrose flux through LamB was 200 times slower than maltose, despite similar binding affinities. This was due to much smaller binding and unbinding rate constants, suggesting that sucrose fits poorly inside the LamB channel. Enteric bacteria, however, can grow on sucrose as sole carbon source if they contain a plasmid expressing the sucrose regulon, including the sucrose-specific porin ScrY (Hardesty et al., 1991). As for LamB, noise analysis experiments have been performed with ScrY (Andersen et al., 1998). The kinetics parameters obtained with maltodextrins were similar for LamB and ScrY, but the binding of sucrose to ScrY was much more rapid than to LamB.

Differences in the substrate specificity of the two porins, have been attributed to the configuration of the constriction zone. Both porins make use of a “greasy slide”, a hydrophobic path of five (ScrY) or six (LamB) aligned aromatic residues, for threading sugar molecules across the channel (Dutzler et al., 1996, Forst et al., 1998). But the side chains of some L3 loop residues that make up the constriction zone are longer in LamB than ScrY, resulting in a narrower pore, unable to accommodate a sucrose molecule (Figure 3). By substituting one of the residues (D201) in ScrY by the homologous tyrosine residue of LamB (Y118), Ulmke and colleagues (1999) were able to convert ScrY into a more Lamb-like pore, with a Km for sucrose much closer to that of LamB than of wild-type ScrY. Conversely, the fusion of the N-terminal periplasmic domain of ScrY confers to LamB the ability to permeate longer maltooligosaccharides (Dumas et al., 2000). This three-stranded coiled-coil domain offers binding affinity to sucrose, and has been proposed to act as an “extended sugar slide” (Dumas et al., 2000).

Figure 3. Comparison of the constriction zone of LamB and ScrY. The cross-sections are viewed from the periplasmic side. In order to get a clearer picture of the pore region, some of the extracellular loops have been clipped from the plane of view. The β-carbon backbone is shown as a ribbon, with residues depicted as sticks. Three residues that play an important role in substrate discrimination are labeled by the respective amino acid one-letter codes (from left to right): D121, R109 and Y118 for LamB, and N192, F204, D201 for ScrY. It is clear that these residues project further into the LamB pore than into the ScrY pore, thus restricting sucrose entry in maltoporin. The crystal structures are rendered with the Insight II program.
for phage DNA injection into the cell, but the mechanism of this transport is still unclear. It has been shown recently that the incorporation of Shigella sonnei LamB porin that had been incubated with λ phages results in the appearance of two types channels: typical LamB channels, and a novel channel type that is much larger than LamB channel and displays a characteristic voltage-dependent form of gating (Berrier et al., 2000). Interestingly, both channel types are resistant to block by maltodextrins added to the same side as the phages, a result that had also been documented for E. coli LamB (Van Gelder et al., 2000). It is not yet clear whether the novel channel is a modified LamB protein, or is formed by insertion of phage proteins. It is possible that the interaction of the λ phage with LamB has triggered a conformational change that leads to the displacement of residues that limit channel entrance, as if binding of the phage led to opening of the pore. This type of phage-dependent activity has also been suggested for the interaction of phage T5 with the FhuA porin, as described below.

**Ligand-Gated Porins**

The passage of iron-complexed siderophores across the outer membrane requires specialized porins, the TonB-ExbB-ExbD complex and an energized inner membrane (Moeck and Coulton, 1998). These proteins form a complex machinery that links the outer and the inner membrane, at least in a functional way, and draws on the electrochemical potential of the cytoplasmic membrane for the uptake of iron. The molecular details of the functioning of this fascinating device are still poorly understood. The recently solved crystal structures of the E. coli ferric enterobactin receptor FepA (Buchanan et al., 1999) and ferrichrome receptor FhuA (Fergusson et al., 1998; Locher et al., 1998) show that these 22-stranded β-barrels are completely occluded by a central plug formed by the N-terminal globular domain. These amazing structures add their own bit of mystery to the molecular puzzle of iron uptake.

As expected, a plugless mutant forms large pores, allowing passage of ferrichrome, but also several antibiotics (Braun et al., 1999). Surprisingly, an equally large channel can also be obtained by removing a much smaller, 34-residue long fragment (Δ322-355), which we now know is not part of the central plug, but constitutes one of the external loops (L4) (Killmann et al., 1993). It is not known whether the L4 deletion has created a misassembled protein with a displaced plug, or another conformation that mimics the active open state. However, the likelihood for the latter scenario is strengthened by the following observation. FhuA is a receptor for phage T5, which binds precisely on the L4 loop. Bonhivers and colleagues (1996) made the interesting observation that channel opening occurs when reconstituted purified FhuA is exposed to phage T5. The authors proposed that the binding of T5 to the L4 loop has triggered a conformational change leading to pore opening, which is mimicked in the ΔL4 deletion mutant. Thus, the L4 loop may act as a gating loop, at least in the case of T5-dependent channel opening. This role of L4 is difficult to conceive in the case ferrichrome-dependent activity, since the ferrichrome binding site is located much deeper into the channel mouth, in an extracellularly facing binding pocket at the interface of the plug and the FhuA barrel (Locher et al., 1998). The idea that channel gating is triggered by different mechanisms in the case of ferrichrome or T5 is substantiated by the fact that T5 opens the channel in the absence of TonB, but the TonB-ExbB-ExbD complex is absolutely required for ferrichrome-dependent FhuA activity. Recent in vitro experiments have also shown that the interaction between TonB and FhuA and the resulting formation of a complex is enhanced by the presence of the ligand (Moeck and Letellier, 2001).

It is unlikely that channel gating merely involves the removal of the plug, because of the extensive salt bridging and H-bonding interactions between the plug and the barrel. Evidence that conformational changes occur in response to ligand binding to FepA has been demonstrated, but the exact nature of these changes is still poorly understood. A powerful approach that has recently emerged for the characterization of conformational changes in proteins is site-directed spin labeling (Feix and Klug, 1998). The technique is based on the dependence of the electron spin resonance (ESR) spectrum of a specific spin-labeled residue on the immediate proteinic environment, and takes advantage of this sensitivity to report on changes in tertiary contacts or accessibility to aqueous or lipidic phase at this site. Cysteine residues are typically chosen for derivitization with spin labels. By applying site-directed mutagenesis to introduce single cysteines at specific locations, one can follow the structural alterations of a region or a stretch of amino acids in response to a particular perturbation or stimulus, such as ligand binding for example. This strategy was used recently to demonstrate the time-dependent motion of the ligand-binding surface loop PL5 in FepA after siderophore binding (Jiang et al., 1997). The loop was shown to move away from the protein, and the displacement was TonB- and energy-dependent. Its kinetics correlated well with FepA turnover rates, and thus this loop motion appears to coincide with channel opening during ferric enterobactin transport. Presumably, the ESR experiments captured the displacement of the loop as it was releasing the bound ligand into the FepA channel. The application of ESR spectroscopy to the study of porin function is still a relatively new field, and these experiments so far are only giving a glimpse of the molecular motions involved, but this approach promises to give a wealth of information on the dynamics of ligand-gated, and other, porins.

**Single Barrels Assembled from Multiple Subunits**

All the above examples are monomeric or trimeric proteins where each β-barrel is formed from a single β-stranded polypeptide that wraps around a central axis. There are also other cases where the β-barrel is...
itself a multimeric assembly, where each subunit contributes a few \( \beta \)-strands. Two particularly striking examples are the trimeric TolC protein from \( E. \) \( \text{coli} \), involved in multidrug efflux and protein secretion (Koronakis et al., 2000), and the heptameric staphylococcal hemolysin toxin (Figure 1), that punctures host cell membranes (Song et al., 1996).

**Multidrug Efflux and Protein Secretion**

TolC mutations are known for their pleiotropic effects. The protein is indeed involved in a large number of processes, all having in common the passage of chemicals directly from the cytoplasm to the outside, without an intermediate periplasmic step (Zgurskaya and Nikaido, 2000). A classic example is the excretion of hemolysin through a tripartite complex bridging the inner and the outer membranes, and consisting of the cytoplasmic membrane protein HlyB, the periplasmic protein HlyD and the outer membrane protein TolC. HlyB and HlyD form a stable complex, and interactions with the unfolded substrate (HlyA) appear to recruit TolC for a functional – but transient – transport machinery (Thanabal et al., 1998). Presumably, the unfolded HlyA threads through a continuous channel formed across the two membranes and the periplasmic space. There is biophysical and structural evidence that TolC forms a membrane pore. Reconstituted TolC supports ionic currents in BLM, and forms a channel sensitive to blockage by tripeptides (Benz et al., 1993). Recently, the detergent solubilized TolC X-ray structure was solved, and disclosed a very peculiar architecture of a \( \beta \)-barrel extended into an \( \alpha \)-helical barrel of coiled-coil helices (Koronakis et al., 2000). Each monomer contributes 4 \( \beta \)-strands and 4 \( \alpha \)-helices, making the assembled \( \beta \)-barrel domain 12-stranded. The \( \beta \)-barrel is 40 Å in height, presumably spanning the outer membrane, and is indeed very similar to a porin structure. The height of the \( \alpha \)-helical domain is 100 Å, making this domain sufficiently long to span the entire periplasm. Thus, the whole trim or resembles a “cannon”, with a large solvent-filled internal cavity which could accommodate a variety of solutes. The proximal end of the \( \alpha \)-barrel is tapered, however, and conformational changes – possibly uncoiling of the helices – must occur for the exported protein to gain access to the channel lumen.

It is possible that the molecular mechanism for TolC function is somewhat different in drug efflux systems. The substrates are more lipophilic in nature, and there is evidence that they are delivered to the export machinery from the lipid phase (Zgurskaya and Nikaido, 2000). In the multidrug AcrAB-TolC transporter system of \( E. \) \( \text{coli} \), the periplasmic AcrA appears to bring the outer and inner membrane in close apposition, and possibly promote membrane fusion (Zgurskaya and Nikaido, 1999). The role of TolC in such systems is more elusive. Interestingly, AcrA also has a high content of coiled-coil structure, as found in the TolC \( \alpha \)-barrel domain.

The outer membrane protein OprM of \( Pseudomonas \) \( \text{aeruginosa} \) is also believed to function as a conduit for drug efflux in the MexAB-OprM multidrug efflux complex. Recent BLM experiments have shown that OprM forms channels with conductance values similar to TolC (Wong and Hancock, 2000). A predicted topology depicts OprM as 16-stranded \( \beta \)-barrel with short periplasmic turns, a structure more closely similar to pores than TolC. It will be interesting to determine how this architectural difference in structure correlates with the nature of interactions between the different components of the HlyBD, AcrAB and MexAB systems.

**Bacterial Toxins**

\( \alpha \)-Hemolysin is considered the major virulence factor of \( Staphylococcus \) \( \text{aureus} \), and is secreted as a water soluble monomer. The assembly into a transmembrane pore is proposed to include three major steps: the binding of the monomers to the target membrane, the oligomerization of the monomers into a heptameric prepore, and the final insertion of the prepore into the membrane to form a functional channel. Each monomer comprises a amino latch, and three domains rich in \( \beta \)-structure: a \( \beta \)-sandwich domain, a rim domain, and a stem domain (Song et al., 1996). The stem domain is composed of two long glycine-rich \( \beta \)-strands that, once inserted into the membrane, will constitute the wall of the \( \beta \)-barrel pore. Since there is little change in secondary structure upon oligomerization, it is believed that the monomers have a similar tertiary structure in the water-soluble and prepore-forms. In order to prevent assembly in aqueous solution, the stem \( \beta \) strands and the amino latch may be in close contact in the water-soluble form. Such an arrangement has been demonstrated in the case of the staphylococcal leukocidin LulF, a toxin homologous to hemolysin for which the crystal structure of the water soluble monomer has recently been solved (Olson et al., 1999). Once bound to the membrane, oligomerization results in a heptameric prepore, whose axis is already oriented perpendicular to the membrane. This configuration has recently been visualized by atomic force microscopy in a mutant protein that stalls at the prepore stage (Malghani et al., 1999). Heptamer stability is insured by interactions of the amino latch with the inner wall of the \( \beta \)-sandwich domain. The \( \beta \)-sandwich and rim domains protrude outside of the membrane, forming a mushroom-like cap positioned over the \( \beta \)-barrel stem (Figure 1). Within the cap, the pore diameter reaches 46 Å, while within the stem, the diameter ranges from 14 to 24 Å. These large pore dimensions are in line with the toxin’s ability to pass molecules of molecular weight up to ~ 2000 Da. The heptamer structure was obtained from detergent-solubilized proteins, but biochemical studies performed on toxin inserted into target cell membrane confirmed this topology, at least for the membrane spanning domain (Valeva et al., 1997).

The biophysical properties of the hemolysin pore have been known for many years from the reconstitution of purified proteins into BLM’s (Menestrina, 1986). The heptamer forms a highly conductive water-filled pore that is slightly anion-selective. The protein has
been amenable to a large variety of genetic and biochemical manipulations, which have engineered a pore with “built-in triggers and switches” (see Bayley, 1999, for a review). For example, binding sites for various blockers have been introduced (Kasianowicz et al., 1999), and recently, the selectivity of the pore could be manipulated by lodging various types of cyclodextrin “adapters” within the hemolysin pore itself (Gu et al., 2000). Besides causing changes in membrane conductance, hemolysin toxins induce a plethora of responses in the eukaryotic host cells (Dinges et al., 2000). Interestingly, some host cells are able to repair the damage cause by the toxin, not by degradation or extrusion of the toxin out of the membrane, but by forcing closure of the pore through an unknown mechanism that involves lipid-mediated membrane remodeling (Valeva et al., 2000).

Many other pore-forming toxins secreted by bacteria appear to adopt a similar β-barrel configuration once they oligomerize in the membrane of target cells. The X-ray structures of the water soluble monomer of aerolysin, leukocidin, and the cholesterol-binding toxin perfringolysin O are known, and models of β-barrel oligomeric states have been presented (Parker et al., 1994; Rossjohn et al., 1997, 1998; Olson et al., 1999; van der Goot, 2000). A common feature of all these proteins is their ability to transition from a water-soluble form to a membrane-spanning channel-forming β-barrel, by burying β-hairpins within amphipathic helices and exposing them only once membrane contact has occurred, together with changes in secondary structure in some cases (van der Goot, 2000).

Conclusion

As our discovery of β-barrel structures continues to expand, we find that this motif is used with amazing consistency for the assembly of membrane proteins that undergo a transitional step in a hydrophilic environment, be it the periplasmic space for outer membrane proteins or the external medium for bacterial toxins. The somewhat modular aspect of the β-hairpins and β-barrels allows for a multiplicity of final structures, from the single OmpA monomer to the large multimeric perfringolysin complex. Associated with these structures is a surprising plasticity in functional properties. As work continues on the functional aspects of β-barrels, we can anticipate the elucidation of an increasing number of mechanisms for the dynamic and regulated control of activity of these proteins.

References


