

Membrane proteins: A tale of barrels and corks

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Crystal structures have been solved for two bacterial outer membrane proteins, FhuA and FepA, which mediate active transport of chelated iron. Analysis of ligand-induced changes in the structure of FhuA has provided our first structural insights into an active transport mechanism for a complex solute.

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To enter a Gram-negative bacterial cell, a solute must cross two membranes: it must first be transported across the outer membrane into the periplasmic space, and then has to cross the inner membrane to enter the cytoplasm. Proteins of the outer membrane are particularly amenable to structure determination by X-ray crystallography. *Escherichia coli* cells accumulate ferric (Fe^{3+}) ions by transport of a siderophore — a small cyclic peptide or related molecule which chelates the ferric ion and retains it in solution. The structures of two related outer membrane proteins, FhuA and FepA, which transport different siderophore–iron complexes — ferrichrome–iron and enterobactin–iron, respectively — have recently been solved. Indeed, three structures have appeared almost simultaneously — two independent structures for FhuA [1,2] and one for FepA [3]. The structure of FhuA was determined by both groups with and without bound ferrichrome–iron, while the FepA crystal included incompletely bound enterobactin–iron. These five structures provide sufficient information to enable formulation of more detailed models of transport mechanisms than was previously possible. They also extend our vocabulary of possible membrane protein structures.

Bacterial iron import

The import of siderophore–iron complexes into *E. coli* cells is an example of TonB-mediated transport. The siderophore receptor, either FhuA or FepA, spans the outer membrane and interacts with TonB, a protein that traverses the ‘periplasmic’ space between the outer and inner membrane. In combination with two inner membrane proteins, ExbB and ExbD, TonB couples transport across the outer membrane to the electrochemical gradient across the inner membrane. TonB thus renders transport by FhuA and FepA an active process — that is, the accumulation of siderophore–iron occurs against a concentration gradient.

After its release into the periplasmic space, the siderophore–iron complex interacts with a soluble periplasmic binding protein, and is subsequently transported across the inner membrane by an ‘ABC’ transporter protein (a bacterial member of the transporter family that includes eukaryotic proteins such as the P-glycoprotein that makes cells multidrug-resistant).

FhuA has long been thought to be a β -barrel membrane protein. But unlike the bacterial porins [4] and glycoporins [5,6] — well-characterized β -barrel membrane proteins which are passive transporters — when FhuA is incorporated into lipid bilayers it does not form channels permeable to ions and other small solutes. Deletion of a surface loop of FhuA converts it to a channel-forming molecule [7]. This led to the suggestion that FhuA might form a 32-stranded β barrel, with an outer surface loop — lost in the deletion mutant — that folds back into the barrel so as to close the pore. But nature still has some structural surprises in store for us: the FhuA and FepA structures show that this model is wrong and that, in reality, the β barrel is smaller than was imagined and the pore constriction is nothing to do with a surface loop.

Interestingly, FhuA is not only responsible for ferrichrome–iron transport across the outer membrane of *E. coli* cells. It can also mediate the transport of certain antibiotics and of much larger molecules. The latter include colicins — bacterial protein toxins which form pores in the inner membrane of *E. coli* — and the DNA of several bacteriophages. So the characterisation of siderophore–iron transport may also further our understanding of how a variety of somewhat larger molecules enter bacterial cells.

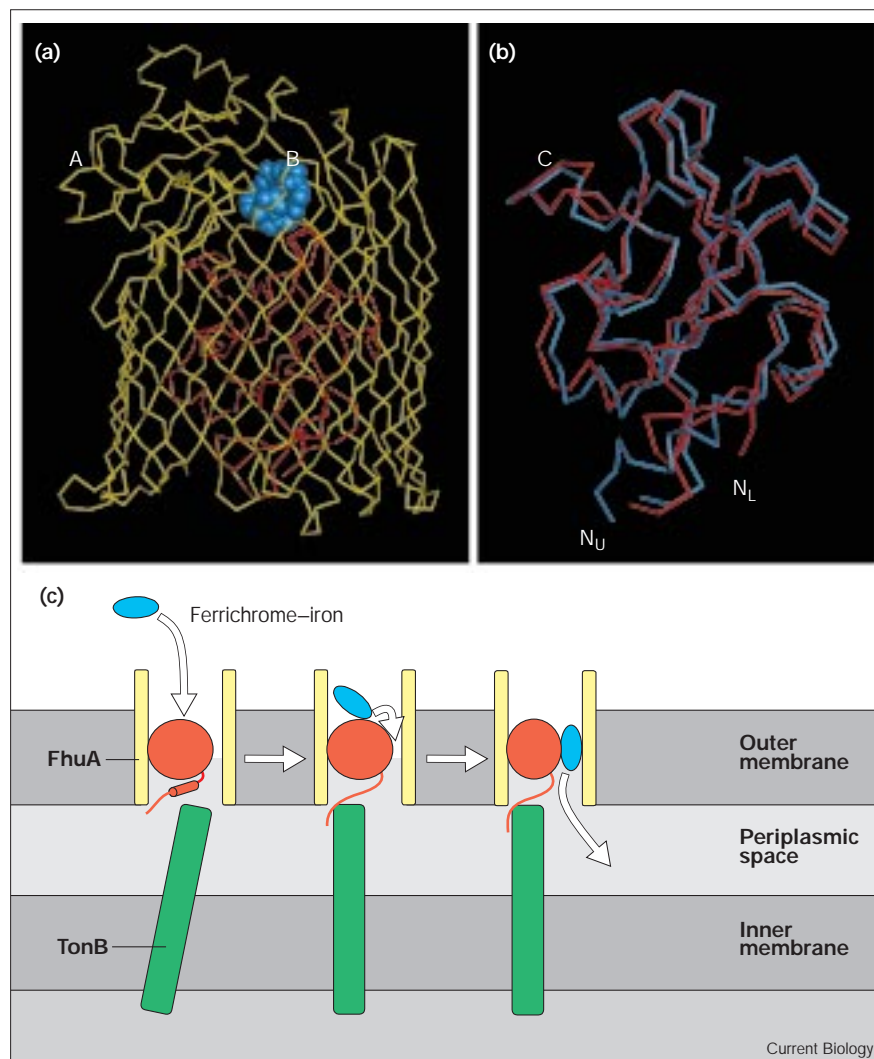
Structure of FhuA

The overall structure of FhuA [1,2] — and of the closely related protein FepA [3] — is a 22-stranded anti-parallel β barrel. The barrel is made up of residues 161–723 of the FhuA polypeptide; the 160 amino-terminal residues form a globular domain, known as the ‘cork’, which is seen in the crystal structure to reside inside the β barrel (Figure 1a). Interestingly, the 18 most amino-terminal residues of the cork — which include the TonB-binding sequence known as the ‘TonB box’ — is disordered in all four FhuA structures, whereas part of the TonB box is ordered in the FepA crystal.

The β barrel of FhuA resembles that of a porin, with the 22 β strands running sequentially anti-parallel to one another, tilted at an angle of about 45° to a line perpendicular to the plane of the bilayer. There are, however, significant differences between FhuA and the porins. A first

Figure 1

(a) The overall crystal structure of FhuA: a C α trace, showing the 22-strand β barrel in yellow, the amino-terminal cork domain in red and the ferrichrome-iron complex in blue. (b) A comparison of the C α traces of the cork domain before (blue) and after (red) ligand binding. Note the change in location of the amino terminus between the unliganded (N_U) and liganded (N_L) forms of FhuA. (c) A proposed scheme for the early stages of transport of ferrichrome-iron by FhuA, showing the ligand-induced loss of the amino-terminal α helix of the cork domain and the subsequent change in interaction with TonB and transport of ferrichrome-iron across the outer membrane.



point to note is that FhuA is monomeric, whereas the porins are trimeric. The FhuA barrel is laterally compressed, such that it is approximately elliptical in cross section. The periplasmic turns (T1 to T10) are short, as they are in the porins; the outer loops (L1 to L11), however, are longer than those in the porins and, rather than folding back into the pore, project away from the membrane surface. Combined with somewhat longer β strands than in the porins, this means that the outer mouth of FhuA is about 35 Å from the bilayer surface — more distant than is the case for the porins.

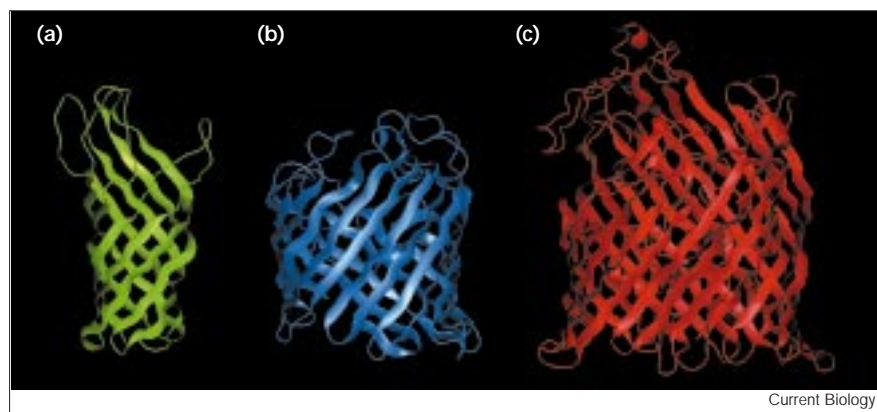
The amino-terminal cork domain fits snugly inside the β barrel, dividing the barrel into two ‘pockets’ — one at the periplasmic mouth and a larger one at the external mouth. The external pocket forms the ferrichrome-iron binding site: the ferrichrome interacts with several aromatic residues

in the pocket lining and binds in such a way that its iron-containing moiety points downwards into the pocket, interacting with the uppermost loops of the cork domain. The cork domain itself has a novel fold, with a central five-stranded β sheet, four small flanking helices and relatively long loops. The cork effectively occludes the barrel (see below), and there are numerous hydrogen bonds between the cork and the barrel wall. Such extensive contacts make a mechanism in which the cork is ‘pulled out’ of the barrel somewhat unlikely, as such a process would be expected to have an insurmountably large activation energy. Instead, some sort of local distortion of the cork and/or β barrel, so as to open up a channel, seems more likely.

A possible transport mechanism

What clues do the FhuA [1,2] and FepA [3] crystal structures provide as to the mechanism of TonB-assisted

Figure 2



A comparison of the folds of three well-studied β -barrel membrane proteins. (a) OmpA, with an 8-stranded β barrel. (b) OmpF, with a 16-stranded β barrel. (c) FhuA, with a 22-stranded β barrel plus plug domain. In each case, the molecule is oriented such that its periplasmic face points downwards.

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transport? Comparison of the structures of unliganded FhuA with those of the protein's complexes with ferrichrome-iron has shed light on the first stage of the process. During ligand binding, the barrel domain remains almost unchanged but two of the loops of the cork domain are pulled up by about 2 Å (Figure 1b); these loops form hydrogen bonds with the ferrichrome-iron ligand. This conformational change is propagated so as to generate an allosteric change at the periplasmic face of the cork domain, where residues 19–31 switch from an α -helical to an extended conformation. This region is immediately adjacent to the (disordered) TonB box, suggesting that the change in conformation might signal to TonB that FhuA has bound a ligand. Intriguingly, the TonB box can be seen to adopt an extended conformation in the partially liganded FepA structure, so one can imagine a cycle of changes in TonB–TonB-box interactions that is coupled to a cycle of ligand occupancy and transport.

Careful examination of the FhuA structure also suggests the possible existence of a 'proto-channel', through which the ferrichrome-iron might pass in a subsequent stage of the transport process. The region between barrel strands β 7, β 9 and β 10 and the cork domain forms a narrow, water-filled channel. It is suggested [2] that small conformational changes in some of the loops of the cork domain might open up this channel, allowing the ligand to pass.

An overall scheme for the FhuA–TonB transport mechanism is summarised in Figure 1c. According to this scheme, binding of ferrichrome-iron results in a conformational change at the TonB box. This alters the interactions between TonB and FhuA, leading in turn to a further conformational change in FhuA that opens the proto-channel. The ligand then passes through the channel. What remains unclear is how TonB couples this cycle of conformational transitions to the electrochemical gradient across the inner membrane. Furthermore, it has been suggested [3] that the extended loops at the outer mouth

of the β barrel might change their conformation so as to close the outer mouth of the pore while the ligand crosses the membrane. Certainly these loops are disordered in the FepA structure [3] and molecular dynamics simulations of FhuA show that these are the most mobile regions of the structure (my unpublished results).

As is often the case, the crystal structures do not reveal the mechanism of FhuA, but rather they allow design of more subtle experiments. The idea that a channel opens between the cork domain and the β barrel could be tested by introduction of disulphide bridges to prevent such opening [1]. Simulations may be used to look at dynamic fluctuations in the dimensions of the proto-channel [8]. Finally, the crystal structure of a FhuA–TonB complex might provide clues as to the energy transduction mechanism [1].

Relevance to membrane proteins

FhuA and FepA also contribute to our understanding of membrane proteins in general. It is thought that the majority of membrane proteins are made up of α -helix bundles [9], and that β -barrel topologies are restricted to bacterial outer membranes. However, it is evident that β barrels are found in a range of functionally distinct membrane proteins. For example, β -barrel structures (Figure 2) have now been identified as membrane anchors such as OmpA [10], passive pores such as the porins [4] and glycoporins [6], and components of active transport systems, as in the cases of FhuA and FepA discussed in detail here. Perhaps it is time to re-examine eukaryotic genomes to determine whether β -barrel membrane proteins are more widespread than has been thought?

Finally, the results of Ferguson *et al.* [2] provide an additional bonus for those interested in how membrane proteins interact with their lipid bilayer environment. Their crystal structure contains a single molecule of outer membrane lipopolysaccharide, non-covalently bound to the surface of FhuA. Thus, a single crystal structure accommo-

dates the three main components of membranes — protein, lipid and oligosaccharide. An achievement indeed.

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