Crystal Structure of the Monomeric Porin OmpG

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The outer membrane (OM) of Gram-negative bacteria contains a large number of channel proteins that mediate the uptake of ions and nutrients necessary for growth and functioning of the cell. An important group of OM channel proteins are the porins, which mediate the non-specific, diffusion-based passage of small (<600 Da) polar molecules. All porins of Gram-negative bacteria that have been crystallized to date form stable trimers, with each monomer composed of a 16-stranded \( \beta \)-barrel with a relatively narrow central pore. In contrast, the OmpG porin is unique, as it appears to function as a monomer. We have determined the X-ray crystal structure of OmpG from \textit{Escherichia coli} to a resolution of 2.3 Å. The structure shows a 14-stranded \( \beta \)-barrel with a relatively simple architecture. Due to the absence of loops that fold back into the channel, OmpG has a large (~13 Å) central pore that is considerably wider than those of other \textit{E. coli} porins, and very similar in size to that of the toxin \( \alpha \)-hemolysin. The architecture of the channel, together with previous biochemical and other data, suggests that OmpG may form a non-specific channel for the transport of larger oligosaccharides. The structure of OmpG provides the starting point for engineering studies aiming to generate selective channels and for the development of biosensors.

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Keywords: OmpG; outer membrane; porin; crystal structure


table:

| Abbreviations used: OM, outer membrane; \( \alpha \)-HL, alpha hemolysin; LPS, lipopolysaccharide; LDAO, \( N,N \)-dimethyldodecylamine-N-oxide. |
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similar and show an arrangement of three individual 16-stranded β-barrels, with each barrel containing a central pore. In the case of the most extensively characterized member of the porins, E. coli OmpF, the pore has an approximate dimension of \( \sim 7 \text{ Å} \times 11 \text{ Å} \) at the narrowest point. This is much smaller than would be expected from a 16-stranded β-barrel, which has a diameter of 25–30 Å (when measured as the Cα – Cα distance). The reason that the actual pore is relatively small is because of the presence of a long extracellular loop (L3) that folds back into the barrel to form a narrow constriction or eyelet. Negatively charged residues in the L3 loop and opposing positively charged residues in the barrel wall create a substantial electric field across the constriction. This field is likely to orient the water molecules in a highly directional manner to favor the passage of polar rather than hydrophobic molecules.

The OmpG protein from E. coli K-12 has all the hallmarks of being a porin, but it is very unusual in several respects. First, OmpG most likely functions as a monomer, as based on the absence of intermolecule crosslinks, single-channel conductance data, as well as on a low-resolution projection structure that suggests a monomer. Second, based on liposome-swelling assays, OmpG appears to form a channel with an unusually large diameter (compared to other porins). The presence of a large channel in OmpG could be related to the apparent lack of the long L3 loop that forms the constriction in all other porins.

OmpG is not expressed, or expressed only at trace levels, in E. coli under all laboratory conditions investigated so far. Moreover, while E. coli OmpG does not show significant homology to other OM proteins on the basis of standard sequence alignments, antibody cross-reactivity experiments suggest that OmpG homologs may be constitutively expressed at low levels in Salmonella sp., Shigella sp. and Pseudomonas sp.

We have determined the X-ray crystal structure of E. coli OmpG to gain a more detailed understanding of the unusual properties of this porin. The structure, refined to a resolution of 2.3 Å, shows that the OmpG barrel consists of 14 β-strands with a large central pore. In addition to explaining a number of biochemical observations, the OmpG structure provides the starting point for transmembrane pore engineering studies and for the development of biosensors.

## Results

### Overview of the structure

OmpG was expressed with its native signal sequence in E. coli and isolated from the OM. During purification, OmpG eluted from gel-filtration chromatography columns at a position consistent with the protein being a monomer (data not shown). As expected, OmpG displays the characteristic heat modifiability observed with many OM proteins; before boiling, the protein migrates at an apparent molecular mass of \( \sim 29 \text{ kDa} \) on SDS-PAGE, whereas after boiling the apparent molecular mass shifts to \( \sim 34 \text{ kDa} \), due to unfolding of the protein.

The structure of OmpG was solved by single anomalous dispersion using a seleno-methionine derivatized crystal (Table 1). The anti-parallel packing of the OmpG monomers within the crystal lattice (Figure 1(a)) is clearly non-physiological and reinforces the current view that this OM protein functions as a monomer. The crystal packing density of OmpG is very high for a membrane protein, and is caused by the fact that crystal contacts are provided by polar interactions between loop residues as well as by extensive hydrophobic interactions between the apolar parts of the barrels (Figure 1(a)).

<table>
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<th>Table 1. Summary of data collection and refinement statistics</th>
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Values in parentheses are for the outermost shell.

\( R_{merge} = \frac{1}{l} \sum |<dl> - |l>| / \sum |l> \).

\( R_{merge} = \frac{\sum |F_{calc} - F_{obs}| / \sum |F_{calc}|}{\sum |F_{obs}|} \) for the 95% of reflection data used in refinement.

\( R_{merge} = \frac{1}{l} \sum |F_{calc} - F_{obs}| / \sum |F_{obs}| \) for the 5% of reflection data not used in refinement.

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Crystal Structure of the Monomeric Porin OmpG
Figure 1. Overview of the OmpG structure. (a) Arrangement of the crystal packing, showing that OmpG is a monomeric OM protein. (b) and (c) Backbone stereo representations of OmpG viewed (b) from the side in the plane of the membrane and (c) from the extracellular side, with strands colored green, extracellular loops orange, and periplasmic turns cyan. The locations of extracellular loops (L) and periplasmic turns (T) are indicated, as are the positions of the N and C terminus (colored pink). In (b), the aromatic girdle residues delineating the approximate position of the membrane interface are shown as stick models in grey. The approximate boundary of the membrane-spanning region is depicted. In (c), ionizable (red, negatively charged; blue, positively charged) and aromatic residues (grey) are shown. This and the following Figures, unless stated otherwise, were rendered with PyMol [http://www.pymol.org].
the residues of the mature protein; the residues 20–27 in loop L1, 59-60 in L2, 220–231 in L6 and 261–266 in L7 are not visible in the electron density maps, presumably because they are disordered. An overview of the OmpG structure is shown in Figure 1(b) and (c), and a comparison of the observed topology with two predictions is shown schematically in Figure 2.8,10 OmpG has 14 antiparallel β-strands \((n = 14, \text{where } n \text{ refers to the number of β-strands})\) that form a cylinder that is slightly flattened on one side, with a diameter between 24 Å and 26 Å (Cα – Cα distance). The shear number of the OmpG barrel is 18 \((n + 4)\), resulting in a tilt angle of 43° for the β-strands relative to the barrel axis.11 In this respect, the OmpG barrel is structurally similar to the larger barrels of OmpF and LamB, which also have shear numbers of \((n + 4)\). As is the case for all OM proteins, OmpG possesses the two girdles of aromatic residues that delineate the polar–apolar interfaces of the membrane (Figure 1(b)). The extracellular loops are interesting features of the OmpG structure, which, although being substantially longer than the periplasmic turns, are relatively short and do not fold back into the barrel as is the case for the general porins. As a result of this arrangement, OmpG has a large central channel.

The channel pore

The OmpG channel has its largest diameter (20–22 Å) at the periplasmic exit and tapers to a constriction located around the area of the upper aromatic girdle, close to the extracellular side. The constriction is formed solely by the side-chains of inward-pointing residues of the barrel wall, and not by surface loops folding inwards. This architecture gives rise to a relatively large central pore with a circular shape and a diameter of \(\sim 13\) Å. Interestingly, only three residue types, Tyr (50/135/172/253), Arg (68/92/111/194/211/235) and Glu (15/17/31/52/152/174/257), are part of the constriction (Figure 3(a)). The arginine and glutamic acid residues have a striking distribution, with clusters of the same residue types on opposite sides of the constriction (Figure 3(a)). By contrast, the tyrosine residues are distributed more evenly and occur on all sides of the constriction. The density for all residues in the constriction is very well defined, with generally low \(B\)-factors for the side-chains (data not shown). Within the acidic and basic clusters, the residue side-chains have an extended conformation and are often quite close together in space. This is most evident for the arginine residues. Since the acidic and basic residues will be ionized at the pH 5.5 used for crystallization, this observation indicates that there is likely to be a strong electric field across the constriction, keeping the charged residues close together in space. This is very similar to the situation in the classical porins; in these proteins, however, the clusters of positively and negatively charged residues are located on opposite sides of the constriction. Whereas both acidic and basic residues are present in the constriction zone of OmpG (Figure 3(a)), the surfaces of the channel leading into the pore are predominantly acidic on both the extracellular and periplasmic side of the pore constriction (Figure 3(b)). Another prominent feature of the channel on the extracellular side of the constriction is the presence of a large number of aromatic residues (Figure 1(c)).

Discussion

The OmpG structure represents the first structure of a monomeric porin in Gram-negative bacteria. As
can be seen from Figure 2, the observed locations of several of the 14 transmembrane β-strands (most notably S7, S10 and S11) differ substantially from their predicted positions. These discrepancies underscore the known difficulty in the prediction of β-barrels. The OmpG pore has an approximate circular shape and, as is clear from Figure 4(a) to (d), is considerably larger (~13 Å at the constriction zone) than the pores of other porins and substrate-specific transporters of Gram-negative bacteria, whose diameters at the narrowest point range from ~3 Å for the E. coli nucleoside channel Tsx12 to ~7–8 Å for OmpF/OmpC. The larger size of the OmpG channel is apparent in liposome-swelling experiments, in which OmpG was found to be much more efficient than OmpC in the transport of various sugars.10 By contrast, the single-channel conductance of OmpG (~0.8 nS in 1 M KCl)8 is substantially smaller than the single-channel conductance values of OmpF (~1.4 nS)13 and OmpC (1.0 nS)14 measured under similar conditions. Since the OmpG pore is considerably larger than those of OmpC/OmpF (Figure 4(c) and (d)), the ion conductance of OmpG can be regarded as relatively low. However, in addition to the size of the channel, the electrostatic potential at the pore constriction plays an important role in determining channel conductivity. In the case of OmpF, the isosteric D113N/E117Q mutant has a conductance of less than half that of the native protein. Likewise, a pentuple OmpF mutant with all five constriction charges removed, but with a much larger constriction cross-section, has a conductance lower than that of the native protein.15 Thus, in a pore with a

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**Figure 3.** Architecture of the OmpG pore. (a) Stereo backbone representation of OmpG in a direction perpendicular to the membrane and viewed from the extracellular side, showing the charged and aromatic residues in the pore constriction, with $2F_o - F_c$ electron density for the side-chains in blue, contoured at 1.2 $\sigma$. Residue numbers are indicated. (b) Surface representations of the OmpG channel viewed from the extracellular side (left, orientation similar to that in (a)) and the periplasmic side (right), showing the electrostatic surface potentials inside the channel. The surface is colored blue for potentials $> 15$ kT/e and red for potentials $< -15$ kT/e. The Figure was generated with GRASP.38
certain diameter, the conductivity values increase with increasing electrostatic potential at the pore constriction. In the case of OmpG, it is difficult to explain the lower conductance value relative to OmpF, since the two pores are very different: in addition to differences in pore diameter and the total number of charges in the constriction (five for OmpF and 15 for OmpG), the geometry of the charges (dipolar arrangement in OmpF, quadrupole arrangement in OmpG (Figure 3(a))) in the constriction also differs greatly between the two proteins.

An interesting comparison is that between OmpG and the cytotoxin α-hemolysin (α-HL) from *Staphylococcus aureus*. α-HL inserts into the plasma membranes of susceptible eukaryotic cells as a heptamer that assembles as a single transmembrane β-barrel, which, like OmpG, has 14 strands. Despite having different shear numbers (16 for α-HL and 18 for OmpG), which would normally give rise to different sized channels, the OmpG and α-HL barrels have very similar diameters due to the fact that the OmpG barrel is slightly flattened. In addition to having channel constrictions with similar diameters (Figure 4(e)), OmpG and α-HL also exhibit similar single channel conductance values. This is remarkable, since the architecture of the constriction is very different in both channels. In α-HL, the residues lining the constriction are Glu111, Lys147 and M113 from each monomer, resulting in a constriction that has 7-fold symmetry. In OmpG, the residues contributing to the restriction are Glu, Arg and Tyr, and they are arranged in a much less symmetrical fashion (Figure 3(a)). Apparently, the OmpG and α-HL pores are wide enough that ions can pass through them in hydrated form, without extensive interaction with the residues lining the channel, resulting in similar conductance values for the two different channels.

The large pore of OmpG could make expression of this channel to high levels toxic for the bacterial cell. This notion is borne out by data showing that cells expressing OmpG are more susceptible to hydrophilic antibiotics. In addition, although *E. coli* cells overexpressing OmpG show only limited growth defects in standard rich medium, we observed severe growth defects and cell lysis in the presence of high (>2 mM) concentrations of the bile salt cholic acid. By contrast, non-induced cells and cells over-expressing the small diameter nucleoside channel Tsx show much less pronounced growth defects under these conditions (data not available).
shown). Therefore, the toxicity of OmpG depends on the environment of the cells. In the normal *E. coli* habitat of the intestinal tract, the problem of avoiding entry of toxic molecules is acute, due to the high concentrations of bile acids. For this reason, OmpG expression may have evolved to be exquisitely regulated, with induction of this porin occurring only under very specific conditions. This would provide an explanation for the observation that induction of OmpG expression has not yet been observed in *E. coli* under any “regular” laboratory conditions.

What could be the physiological function of OmpG? The answer to this question is not yet clear. The α-hemolysin channel functions as a non-specific pore for polar, globular molecules of up to 2 kDa in size. The similarity of OmpG and α-HL therefore suggests that OmpG forms a non-specific pore for polar molecules as well. This notion is supported by proteoliposome-swelling experiments, in which hydrophobic antibiotics did not appear to pass readily through the pore. Moreover, no obvious substrate specificity of OmpG for maltose, lactose, sucrose, or an oligopeptide was detected in these experiments.

Whereas the large diameter of the pore makes it unlikely that OmpG has specificity for small molecules, there are several indications that this porin could be needed for the uptake of larger oligosaccharides. First, the *ompg* gene appears to be the last in a putative 11-gene operon that contains genes coding for oligosaccharide-transporting ABC transporters. Second, although the levels of sequence homology are very low, the OmpG family members appear to be distantly related to the cyclodextrin porin family and to the oligogalacturonate porin family. An involvement of OmpG in the transport of larger oligosaccharides is supported by the growth rates of *E. coli* strains. In a ΔLamB background, OmpG+ strains support growth on maltotetraose and maltopentaose, whereas OmpF/OmpC+ strains fail to grow on these substrates. In accordance with the hypothesis that OmpG forms a channel for oligosaccharides, the present structure shows the presence of a large number of both ionizable (glutamic acid, arginine) and aromatic residues in the channel (Figure 5), which is reminiscent of sugar channels like LamB. In particular, the aromatic residues Tyr98, Tyr96, Tyr94, Phe66 and Tyr50 form a patch on the extracellular side that leads to the constriction (Figure 5). This arrangement is similar to the “greasy slides” observed in sugar transporters, and which function in the binding of the hydrocarbon portions of the sugar substrates. Thus, the structure and other data are consistent with the assumption that OmpG could be needed for the transport of large oligosaccharides under very specific growth conditions.

The monomeric character of OmpG prompts the question of whether features (or the absence thereof) can be identified that cause this porin to function as a monomer. A consensus sequence for trimerization has not been identified in the

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**Figure 5.** Stereo backbone representation of *E. coli* OmpG from the extracellular side, showing the distribution of aromatic (grey) and charged residues (red, glutamic acid 15/17/31/52/152/174; blue, arginine 68/92/111/150) on the extracellular side of the constriction. The numbers of the aromatic residues (Y50, F66, Y94, Y96, Y98, F132, Y136 and Y146) that may provide binding sites for oligosaccharides are indicated. For clarity, the barrel has been tilted and only residues present on one side of the constriction are shown.
Crystal Structure of the Monomeric Porin OmpG

Materials and Methods

Cloning and purification

The gene for E. coli OmpG, including the signal sequence and with a hexa-histidine tag following the C-terminal phenylalanine residue, was amplified by PCR from genomic DNA, and cloned into the pB22 vector, which is under control of the arabinose promoter. OmpG was expressed in C43 (DE3) cells grown in 2×YT medium by induction with 0.2% (w/v) arabinose for 3 h at 37 °C. After harvesting by centrifugation, the cells were resuspended in TSB buffer (20 mM Tris–HCl pH 8.0, 300 mM NaCl, 10% (v/v) glycerol) and ruptured by two passes at 15,000x-hemolysin (α-HL 15,000–20,000 psi (1 psi ≈ 6.9 kPa) in a microfluidizer (Avestin Emulsiflex C-3). Total membranes were collected by centrifugation at 100,000 g and solubilized by homogenization in 1% (w/v) N,N-dimethylidodecylamine-N-oxide (LDAO) in TSB buffer, followed by stirring the suspension at 4 °C for 30 min. Following centrifugation at 100,000 g, the membrane extract derived from 12 l of cells (A600 ~ 1.0 absorbance unit) was loaded onto a 10 ml nickel affinity column (Chelating Sepharose; Amersham Biosciences) equilibrated with 0.2% LDAO in TSB. The nickel column was washed with 20 volumes of buffer containing 5 mM imidazole and eluted with 200 mM imidazole. OmpG was further purified by gel-filtration on a Superdex-200 26/60 chromatography column (Amersham Biosciences) in 10 mM sodium acetate (pH 5.5), 50 mM NaCl, 10% glycerol, 0.05% LDAO. This step was followed by anion-exchange chromatography on a Resource-Q column (Amersham Biosciences) equilibrated in 10 mM Tris (pH 8.0), 50 mM NaCl, 10% glycerol, 0.4% CaCl2 and using a linear NaCl gradient to a final concentration of 0.5 M. The pure protein (final yield ~ 4–5 mg from 12 l of cells) was concentrated to 5–10 mg/ml and flash-frozen in liquid nitrogen. SeMet-substituted protein was produced in wild-type C43 cells by inhibition of the methionine biosynthesis pathway, and purified as the native protein. No reducing agents or EDTA were added to the buffers.

Crystallization and data collection

Crystallization trials of native and SeMet-substituted OmpG were set up by mixing 0.8 μl of protein solution (4–7 mg/ml) with 0.8 μl of reservoir solutions from an in-house screen containing PEG 400 and PEG 4000 as the principal precipitants. Crystals for both wild-type and SeMet-substituted protein were readily obtained under several conditions. After optimization, one of these conditions (50 mM cacodylate (pH 5.5), 28–32% PEG 400, 1.0–1.5 M sodium formate) yielded very thin plates that grew to maximum dimensions of 30 μm × 500 μm × 5 μm in 10 days. These crystals belonged to space group P21 with a cell dimension of a = 122.5 Å, b = 120.5 Å, c = 96.4 Å and α = β = 90°, γ = 90°. Crystals contained one molecule in the asymmetric unit (Matthews coefficient, Vm = 2.97 Å3/Da, corresponding to 58% (v/v) solvent content). The crystals contained one molecule in the asymmetric unit (Matthews coefficient, Vm = 2.97 Å3/Da, corresponding to 58% (v/v) solvent content). The crystals were frozen directly from the mother liquor in liquid nitrogen for data collection.

A single anomalous dispersion (SAD) dataset at the selenium peak wavelength was collected for a SeMet-substituted OmpG crystal at the NSLS beamline X6A at Brookhaven National Laboratory, and processed with HKL2000. All the five selenium sites were identified classical porins. Instead, a large number of hydrophobic interactions between the exterior of the barrels, coupled with polar and charged interactions of extracellular loop L2 with the neighboring molecule, are likely to be responsible for trimerization. A negatively charged conserved residue in loop L2 is especially important, as mutation of this residue to a positively charged residue in PhoE results in stable monomers. Loop L2 interacts with a neighboring monomer by latching into a wedge in the barrel wall that exists due to the short length of β-strands S5 and S6. Interestingly, these short strands continue into the extracellular loop L3, which folds back into the barrel. We speculate that strands S5 and S6 of the classical porins are too short to completely cover the hydrophobic surface of the OM. This would create an energetically unfavorable situation that could be the driving force for the insertion of loop L2 from another monomer during biogenesis, shielding the short strands and the water-filled barrel interior from lipid. In this model, constriction of the channel by the inward folding of loop L3 would be coupled to trimerization. If this assumption is correct, OmpG forms stable monomers simply due to the absence of short β-strands in the barrel wall (Figure 1(b)).

The reason why porins like OmpF function as stable trimers is not known. The relatively narrow channel of a porin monomer is likely to hinder passage of toxic substances, but may not be very efficient in diffusion-based transport. The large extracellular funnel that is formed by the porin trimer may generate higher concentrations of solutes close to the pore constrictions. Trimerization may therefore be regarded as a mechanism that makes transport more efficient, without sacrificing the integrity of the OM. In the case of OmpG, we can speculate that its large pore is more efficient in diffusion-based transport, making a trimeric barrel arrangement not necessary. This hypothesis is supported by the fact that OmpG is more efficient than OmpF in the uptake of both monosaccharides and disaccharides, as measured by proteoliposome-exchange chromatography on a Resource-Q column (Amersham Biosciences) in 10 mM sodium acetate (pH 5.5), 50 mM NaCl, 10% glycerol, 0.05% LDAO. This step was followed by anion-exchange chromatography on a Resource-Q column (Amersham Biosciences) in 10 mM Tris (pH 8.0), 50 mM NaCl, 10% glycerol, 0.4% CaCl2 and employing a linear NaCl gradient to a final concentration of 0.5 M. The pure protein (final yield ~ 4–5 mg from 12 l of cells) was concentrated to 5–10 mg/ml and flash-frozen in liquid nitrogen. SeMet-substituted protein was produced in wild-type C43 cells by inhibition of the methionine biosynthesis pathway, and purified as the native protein. No reducing agents or EDTA were added to the buffers.

The structure of OmpG has provided clues that will form the basis for future biochemical and biophysical experiments, to elucidate the putative function of this porin in the transport of larger oligosaccharides. In addition, OmpG is regarded as a promising scaffold for the engineering of pores with a range of new functions, such as substrate-specific channels, and for the development of biosensors. Until now, the trimeric porins and α-hemolysin have been used for these studies. However, the multimeric character of these proteins makes them less than ideal for engineering purposes. Our structure has confirmed the monomeric character of OmpG, and has elucidated the relatively simple architecture of its channel at the atomic level, thereby paving the way for the use of this porin in channel engineering studies and in the development of biosensors.
using SOLVE. Phases were obtained by refinement of the heavy-atom sites within SHARP (Table 1). The resulting electron density map was of good quality and allowed 50–60% of the model to be built automatically with RESOLVE. Further model building was performed manually using Coot, followed by refinement in CNS. The data collection and refinement statistics are summarized in Table 1.

Protein Data Bank accession number

The refined coordinates and structure factors of OmpG have been deposited in the RCSB Protein Data Bank (PDB) with accession code 2F1C.

Acknowledgements

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References


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LETTERS

Wza the translocon for E. coli capsular polysaccharides defines a new class of membrane protein

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Many types of bacteria produce extracellular polysaccharides (EPSs). Some are secreted polymers and show only limited association with the cell surface, whereas others are firmly attached to the cell surface and form a discrete structural layer, the capsule, which envelopes the cell and allows the bacteria to evade or counteract the host immune system1. EPSs have critical roles in bacterial colonization of surfaces2, such as epithelia and medical implants; in addition some EPSs have important industrial and biomedical applications in their own right3. Here we describe the 2.26 Å resolution structure of the 340 kDa octamer of Wza, an integral outer membrane lipoprotein, which is essential for group 1 capsule export in Escherichia coli. The transmembrane region is a novel α-helical barrel. The bulk of the Wza structure is located in the periplasm and comprises three novel domains forming a large central cavity. Wza is open to the extracellular environment but closed to the periplasm. We propose a route and mechanism for translocation of the capsular polysaccharide. This work may provide insight into the export of other large polar molecules such as DNA and proteins.

There are two distinct capsule assembly pathways in E. coli1. The defining characteristic of the group 1 (Wzy-dependent) pathway is that individual lipid-linked polymer repeat units are synthesized and exported to the periplasm, where a putative polymerase (Wzy) assembles the full length polymer4. E. coli capsular serotype K30 is the prototype for the group 1 capsule assembly system and the working model of the pathway is shown in Fig. 1a. The K30 polysaccharide is assembled and exported by proteins encoded by a 12-gene operon5. The process requires a member of the OMA (outer membrane auxiliary) family for translocation of nascent polymer across the outer membrane6. Mature Wza, the best studied OMA member, is a 359-residue lipoprotein that forms SDS-stable octamers; it is synthesized as a precursor with a cleavable 20-residue amino-terminal signal sequence6. Cys 21 is modified by a thioether-linked diacylglycerol group and its amino group is acylated. Electron microscopy studies of two-dimensional crystals of Wza protein in lipid bilayers revealed octameric ring-like structures suggestive of a channel7. In negatively stained cryo-electron-microscopy (cryo-EM) images (15.5 Å resolution), Wza octamers form a mushroom-like structure with dimensions 90 Å × 90 Å × 100 Å (ref. 8) with a large central cavity, but these images provided no mechanism for translocation. Chromosomal ‘knockout’ wza mutants have no detectable capsule and produce no intracellular polymer6,2. This suggests a feedback process in which synthesis and export are coupled. Wza interacts with a tetrameric inner membrane tyrosine autokinase protein, Wzc7,9,10. Mutations that either eliminate Wzc, or compromise its phosphorylation, also turn off capsular polymer formation11,12. A non-acylated Wza mutant forms weakly stable oligomers, and polymer accumulates in the periplasm7. This may indicate that the non-acylated oligomer is not competent for export but maintains its interaction with Wzc, thus promoting polymer synthesis. Sequence relationships amongst OMA proteins are limited8, but family members contain the polysaccharide biosynthesis/export (PES) motif (Pfam02563) (Supplementary Figs 1, 2).

The 2.26 Å crystal structure of mature acylated Wza from E. coli K30 has eight monomers (Fig. 2a) in the asymmetric unit and exhibits eightfold rotational symmetry (Fig. 2b, c). We describe the octameric structure as having the shape of an ‘amphora’ without the handles (Fig. 2b). Wza has a large internal cavity that is open at a narrow ‘neck’ in the outer membrane and closed at its base. The long axis of the molecule is approximately 140 Å and the diameter at the widest point is 105 Å (Fig. 2b, c). Comparison of the crystal structure with that derived from negatively stained cryo-EM6 reveals that the ‘neck’ is missing in the cryo-EM reconstruction. This region may be unstructured in the conditions of the cryo-EM experiment. The dimensions of the remainder of the structure and the large central cavity match well. The cryo-EM structure has only fourfold rotational symmetry indicating conformational change may be induced by negative staining5; images of Wza in proteolipid samples showed eightfold symmetry2.

Domain 1 of Wza (residues 89–169) represents a novel fold; it comprises an anti-parallel β-sandwich with an α-helix at one edge. Domain 1 contains the conserved PES motif (Supplementary Figs 1b, 2). The eight copies of domain 1 combine to form a ring structure (ring 1) at the bottom of the Wza, with an eightfold axis through the centre (Fig. 2b, c). Ring 1 presents a concave surface at the base of the structure and the centre is filled by eight loops (residues 105 to 112 of each domain). Tyr 110 is located at the tip of each loop but is not clearly visible in the experimental map, suggesting that the side chain is flexible (Supplementary Fig. 3a). Inwards past Tyr 110, the ring has an internal diameter of over 25 Å (compared with 17 Å at the base of ring 1; Fig. 2b, c). Domain 2 (residues 68–84 and 175–252) has a novel structure, despite possessing similar dimensions to domain 1. This domain has a central five-stranded mixed β-sheet with three α-helices on one face. The eight copies of domain 2 form an eightfold symmetric ring structure (ring 2) with an inner diameter of 25 Å. Domain 3 (residues 46–64 and 255–344) is a structural duplication.

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of domain 2 (Supplementary Fig. 1c) and the eight copies come together to form a ring structure (ring 3; Fig. 2b) with a significantly larger external diameter (105 Å) than ring 2. The three rings sit one atop the other with a spacing of 10 Å between rings 2 and 3. A ribbon representation of the structure (Fig. 2b) gives the appearance of side holes being present, but these are filled by side chains (Fig. 3a, b).

Domain 4 comprises the carboxy terminus of the monomer (residues 345–376; Fig. 2a) and is an amphipathic helix. As a result of the rotational symmetry, the helices from the eight monomers create an α-helical barrel (Fig. 2b) at the ‘neck’ of the molecule. The barrel is tapered such that the end proximal to ring 3 has an internal diameter of approximately 30 Å. At the open end the internal diameter is 17 Å. The N terminus (residues 22–45) is wrapped around the top of ring 3 (Fig. 2a, b).

The central cavity of the protein has an internal volume of approximately 15,000 Å³ (Figs 2b, 3a), comparable to GroEL–GroES (84,000 Å³; ref. 13) and TolC (44,000 Å³; ref. 14). In Wza, the protein surface that encloses the central cavity is polar (Fig. 3a) and lined by residues with little sequence conservation. Those conserved residues that do exist are structural or located at the extensive subunit interfaces (Supplementary Information and Supplementary Fig. 2). The exterior surface of Wza is almost all polar (Fig. 3b). The exception is the helical barrel which has a markedly hydrophobic exterior (Fig. 3b) with bound lipid molecules and Trp 350 exposed (Fig. 3b and Supplementary Figs 3b, 4). The helical barrel is the transmembrane region; but the bulk of the structure is located in the periplasm. The C

![Figure 1](image1.png)

**Figure 1** Group 1 capsular polysaccharide export in Gram-negative bacteria. **a**, Model of the biosynthetic complex carrying out synthesis and export of serotype K30 group 1 capsule in *E. coli*. Repeat units of the polymer are assembled on a lipid (undecaprenol diphosphate; und-PP) acceptor, in the cytoplasm in a reaction initiated by the WbaP enzyme. The und-PP-linked repeat units are ‘flipped’ across the inner membrane by Wzx. Polymerization occurs at the periplasmic face and is dependent on another integral membrane protein, the putative polymerase, Wzy. Wzy-dependent polymerization requires the activity of the tetrameric Wzc protein10. Wzb is a protein tyrosine phosphatase enzyme responsible for dephosphorylating Wzc. The cycling phosphorylation of Wzc is crucial for export11,12. Wza and Wzc proteins interact to form a complex that spans the periplasm12. The precise role of Wzi, an integral membrane protein, is unclear. **b**, The chemical structure of the repeat unit has a width of 17 Å (assuming the most extended conformation).

![Figure 2](image2.png)

**Figure 2** The structure of Wza. **a**, Wza has four domains labelled as D1–4. Cys 21 (acylated N terminus) is shown as a blue ball and Arg 376 (last ordered residue) as a red ball. **b**, The Wza octamer is described as an amphora made of four rings (R1–4) with a large central cavity (orange space-filling shape). Ring 1 is formed by eight copies of domain 1, ring 2 by eight copies of domain 2, and so on. The helical barrel (R4) forms the ‘neck’ and ring 1 the ‘base’. The outer membrane (OM) is marked. The protein C terminus is outside the cell and rings 1, 2 and 3 inside the periplasm. **c**, View into the central cavity from outside the cell. **d**, View of the central cavity from the periplasm with Wza (surface render) coloured by electrostatic charge (blue, positive; red, negative). The concave surface of ring 1 is closed by each loop at Tyr 110 and has a band of negative charge.
terminus of each monomer is exposed on the cell surface (Fig. 2b), placing the acylated N terminus at the inner leaflet of the outer membrane, consistent with typical outer membrane lipoproteins. The orientation of Wza was established by showing that a fused C-terminal Flag epitope (Wza–Flag) is exposed on the cell surface (Fig. 3c and Supplementary Fig. 5). To date, all integral outer membrane proteins have contained a transmembrane β-barrel. Wza is the first example of a transmembrane α-helical barrel. Although transmembrane α-helices are well known in bacterial inner membrane proteins and eukaryotic membrane proteins, the integral membrane helical barrel arrangement seen in Wza has not been observed before. Channel-forming antimicrobial peptides have amphipathic helices and the C terminus of Wza may mimic antimicrobial peptide pore formation.

A simple model for translocation has the carbohydrate moving from the periplasm to the central cavity of Wza and exiting through the helical barrel. Assuming an extended conformation, the width of the oligosaccharide is approximately 17 Å (Fig. 1b), matching the internal diameter of the helical barrel (Figs 2c, 3a). Unlike β-barrel porin proteins that are involved in the uptake of small nutrients, Wza has no portal between the central cavity and periplasm (Fig. 2d and Supplementary Fig. 3c, d). Isolated Wza protein embedded in lipid bilayers shows no ion conductance (C. Whitfield and R. E. W. Hancock, unpublished data) consistent with a closed state. Regulation of the opening of the Wza central cavity would seem desirable in maintaining outer membrane barrier properties. We propose that the opening of the portal requires a substantial conformational change. Both WzaY110A and WzaY110W derivatives of Wza are fully competent for capsule export (Supplementary Fig. 6) indicating that gating is not controlled by a single residue. The trigger for such hypothetical conformation changes is unknown but the binding of Wzc (refs 7, 9) provides one logical candidate. The circle of negative charge and a concave surface at the base of the Wza oligomer, the conserved PES domain, offers a potential site for protein–protein interactions (Fig. 2d). The use of conformational changes to gate large channels was observed in FluA (ref. 19). TolC has a barrel closed at one end that opens in response to periplasmic binding events. Wza may represent a model of some of the multimeric ‘secretin’ protein complexes found in type II, III and IV secretion systems, most notably the single-stranded DNA export through type IV systems. Electron microscopy structures of several secretins show these contain ring-like structures capable of accommodating large polar substrates.

The potassium transporter and the conceptually similar ammonium transporter use side chains inside the channel to bind substrate and thus avoid the desolvation penalty. Carbohydrates contain many polar atoms whose desolvation en masse would be unfavourable. Lactose permease binds the two carbohydrate rings inside a central cavity satisfying the hydrogen bonds. The protein then undergoes a conformational change, releasing the sugar on the opposite side of the membrane. A similar model has been proposed for the lipopolysaccharide lipid A flipase, MsbA. Extended carbohydrate binding sites in proteins are well known, but are characterized by exquisite specificity. Using protein side chains to recognize a transient EPS chain with hundreds of carbohydrate rings, would seem a formidable task. We propose that Wza, by permitting both water molecules and carbohydrate to be present simultaneously in its large polar cavity, uses water molecules to ‘plug’ gaps. This ensures that the polar protein side chains and sugar hydroxyl groups make hydrogen bonds. This ‘lubrication’ of export negates the need for specific recognition between protein and carbohydrate. In support of this model, we note that an essentially identical Wza is employed by E. coli and Klebsiella pneumoniae despite them having chemically distinct EPSs. Moreover, Wza homologues that export different EPSs can complement Wza-deficient E. coli K30 (refs 6, 9).

**METHODS**

Full details of the purification and crystallization of the native protein have been published. The 2.26 Å data set was collected on ID29 of the European Synchrotron Radiation Facility (ESRF) on a single selenomethionine crystal protected with 25% (v/v) glycerol in the mother liquid and cooled to 100 K. Experimental phases were calculated by locating Se atoms. A full description of the structural analysis is given in Supplementary Information.

Plasmid pWQ126 (ref. 6) provided the template for mutagenesis using the QuikChange Site-Directed Mutagenesis Kit (Stratagene). Y110A, Y110W and Δ108–112 (deletion) mutants were constructed. The phenotypes of the mutants were determined after transforming E. coli CWG281 (wza::aacC1, waz2, zac1, adA) into E. coli K30 (wza::aacC1, waz2, zac1, adA; Gm, Sp)". The gene encoding the Flag-epitope at the C-terminus of the marker protein fusion (Wza–Flag) was constructed by PCR. Cells were grown and imaged as described in Supplementary Information.

**FIGURE 3** The surfaces of Wza. **a**, The central cavity of Wza is large and polar (oxygen, red; nitrogen, blue; carbon, selenium and sulphur, white). **b**, Lipid molecules are shown as black spheres on the exterior surface. The helical barrel is clearly non-polar. A band of tryptophan residues is exposed on the helical barrel (Supplementary Fig. 4b). **c**, For both the control sample (E. coli LE392) and E. coli LE392 expressing Wza–Flag (Wza–Flag), a differential interference contrast image is shown in the upper frame and the corresponding fluorescence image in the lower. The Flag-tag is clearly exposed on the cell surface.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.
Structure of the monomeric outer-membrane porin OmpG in the open and closed conformation

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OmpG, a monomeric pore-forming protein from Escherichia coli outer membranes, was refolded from inclusion bodies and crystallized in two different conformations. The OmpG channel is a 14-stranded β-barrel, with short periplasmic turns and seven extracellular loops. Crystals grown at neutral pH show the channel in the open state at 2.3 Å resolution. In the 2.7 Å structure of crystals grown at pH 5.6, the pore is blocked by loop 6, which folds across the channel. The rearrangement of loop 6 appears to be triggered by a pair of histidine residues, which repel one another at acidic pH, resulting in the breakage of neighbouring H-bonds and a lengthening of loop 6 from 10 to 17 residues. A total of 151 ordered LDAO detergent molecules were found in the 2.3 Å structure, mostly on the hydrophobic outer surface of OmpG, mimicking the outer membrane lipid bilayer, with three LDAO molecules in the open pore. In the 2.7 Å structure, OmpG binds one OG and one glucose molecule as sugar substrates in the closed pore.

Introduction

The outer membrane of Gram-negative bacteria acts as a selective permeability barrier and prevents uncontrolled exchange of solutes and nutrients such as sugars, nucleotides, amino acids and ions (Benz et al., 1978; Nikaido, 2003). The uptake of these substances from the medium to the periplasm occurs through channel-forming integral membrane proteins known as outer-membrane porins, which function as molecular filters. Outer-membrane porins were among the first membrane proteins to be crystallized (Garavito and Rosenbusch, 1980), and high-resolution structures of the pore-forming proteins OmpF (Cowan et al., 1992), PhoE (Cowan et al., 1992), LamB (Schirmer et al., 1995), ScrY (Forst et al., 1998) and others (Weiss et al., 1991) have been determined. So far, all outer-membrane porins involved in solute uptake are membrane-spanning β-barrels of 16 or 18 strands surrounding an aqueous pore. They conform to a common pattern whereby three monomers associate into trimers. This is their oligomeric state in 2D (Jap, 1989; Sass et al., 1989) and 3D crystals, and also their functional state in lipid bilayers (Benz et al., 1978).

Other β-barrel outer-membrane proteins do not conform to this pattern. Txs, a distorted 12-stranded barrel (Ye and Van den Berg, 2004) and FadL, a 14-stranded barrel (van den Berg et al., 2004) form narrow channels specific for nucleosides or long fatty acids, respectively. Small 8-stranded β-barrels seem to serve primarily as membrane anchors (OmpA), or promote bacterial adhesion to mammalian cells (OmpX), and may be promising candidates for vaccine development (NspA). Others are membrane-bound enzymes, such as the protease OmpT or the phospholipase OmpLA. Yet others are large barrels with a plug domain, and are specifically adapted to the sequestering of essential compounds such as siderophores for iron uptake (e.g. FhuA; Ferguson et al., 1998), or vitamin B₁₂ (BtuB; Kurisu et al., 2003), demonstrating the great versatility of β-barrel outer-membrane proteins. Similar β-barrel-forming membrane proteins are thought to participate in solute exchange and protein translocation in the outer membranes of chloroplasts (Schleiff et al., 2003) and mitochondria (Pfanner and Wiedemann, 2002).

In Escherichia coli the main porins for sugar uptake are LamB (Szmelcman et al., 1976) and ScrY (Schmid et al., 1991). In mutants where LamB is either nonfunctional or deleted, the uptake of sugars is facilitated by OmpG (Misra and Benson, 1989). The gene for OmpG encodes a polypeptide with 301 amino acids, and the mature OmpG protein shows all features of an outer-membrane porin: a signal sequence of 21 amino acids at its N-terminus, which is cleaved during export, absence of long hydrophobic stretches, lack of cystein residues (Fajardo et al., 1998) and a C-terminal phenylalanine, which is important for membrane insertion (Struyve et al., 1991). Circular dichroism spectroscopy of detergent-solubilized OmpG indicated that, like other outer-membrane porins, it consists largely of β-sheet with little, if any, α-helix content. Proteoliposome swelling assays have shown that OmpG is a nonelectrophoretic and two-dimensional electrophoresis suggested that OmpG is a monomer (Fajardo et al., 1998), unlike the classical outer-membrane porins which are trimers (Nikaido, 2003). Projection maps at 6 Å resolution obtained by electron cryo-microscopy of two different two-dimensional (2D) crystal forms revealed a ring-shaped density indicative of a monomeric β-barrel, with no evidence of oligomer formation. Initial secondary structure predictions of OmpG...
suggested 16 β-strands (Fajardo et al., 1998), but more recent estimates (Conlan et al., 2000; Behlau et al., 2001) put this number at 14, which agrees better with the diameter of the β-barrel in the projection maps.

To investigate OmpG and its role in nutrient uptake in detail, we expressed OmpG at high level in E. coli, refolded it from inclusion bodies, crystallized the refolded protein and determined its structure in two different states. OmpG is indeed a 14-stranded β-barrel and a pore-forming monomer. A long extracellular loop assumes two distinct, well-defined conformations, apparently in response to the pH of the medium. At neutral pH this loop projects into the extracellular medium, leaving the pore wide open, whereas at low pH it folds across the pore channel and blocks it, suggesting a direct role in pH-dependent pore gating.

Results and discussion

**Pore architecture**

OmpG forms a β-barrel of 14 antiparallel β-strands (S1–S14, Figure 1). The orientation of OmpG in the membrane is indicated by the location of the N and C termini next to one another, which are on the periplasmic side in all outer-membrane porins. The β-strands are connected by six short turns (T1–T6) of three to five residues on the periplasmic side, and by 7 longer loops (L1–L7) on the extracellular side. L4 contains one full turn of an α-helix, whereas the short helices found so far in loops and turns of other outer-membrane porins are of the 3_10 variety (Cowan et al., 1992, 1995; Dutzler et al., 1999). The average angle between the β-strands and the membrane plane is ~60°. Except for the first four N-terminal residues, the main chain was traced completely both in the 2.3 Å map derived from triclinic crystals and in the 2.7 Å map of the orthorhombic crystals (Table I). All side chain densities were well-defined except for the loop residues 24, 26, 58–62 and 224–227, indicating a degree of flexibility in L1, L2 and L6.

The β-strands and turns are indistinguishable in both crystal forms (Figure 1A and C), with an r.m.s.d. of 0.74 Å. The OmpG pore is elliptical in cross-section, with one side slightly flattened at strands S6–S10 (Figure 1B and D). In the centre of the membrane, the pore diameter measured between Cx atoms of opposite β-strands is 29 by 25, and 15 × 12 Å when taking sidechains into account. The OmpG channel is lined predominantly by charged and polar residues, mainly Glu and Arg, arranged in clusters, as well as strings of aromatic residues (Figure 2A). Interestingly, clusters of the same charge are positioned opposite one another in the barrel (Figure 2B and C). The purpose of this clustering is not clear, but it might create an electrostatic barrier that prevents ions and charged compounds from entering the channel.

**Open and closed conformation**

Major differences were found in the extracellular loops in the two crystal forms. In the triclinic crystals, the loops constrict the outer barrel entrance slightly to an effective diameter of 8 × 12 Å, but otherwise they extend into the medium, leaving the pore wide open. In the orthorhombic form, the pore is obstructed on the extracellular side, mainly as a result of a substantial conformational change of L6, which folds across and into the barrel (Figure 1D). This is achieved by an increase in length from 10 to 17 residues (Figure 2D), due to the unzipping of hydrogen bonds between β-strands leading up to L6 and L7. As a result of the same effect, L7 doubles in length from four residues in the open state to eight residues in the closed conformation. L7 and the adjacent L1 move outward to make room for the reoriented L6. To some extent, the conformational changes propagate to L2, while L3, L4 and L5 remain unaffected.

Comparison of projection maps calculated from the atomic coordinates of the open and closed form (Figure 3A and B) with those obtained from 2D crystals of native (Behlau et al., 2001) and refolded OmpG (Figure 3C and D) suggested that OmpG in the 2D crystals was closed. Moreover, the structures of the native (Figure 3C) and refolded protein (Figure 3D) are indistinguishable at 6 Å resolution in projection.

**pH gating**

The change in conformation appears to be induced by pH, as the triclinic crystals were grown at pH 7.5, while the orthorhombic crystals formed at pH 5.6. Looking for a pH-sensitive switch that might trigger this rearrangement, we found a pair of solvent-exposed histidine residues (His231 and His261) in adjacent β-strands S12 and S13 (Figure 2D), at the C-terminal end of L6 and the N-terminal end of L7, respectively. At pH 7.5 the centre-to-centre distance of the imidazole rings is 4.9 Å, whereas at pH 5.6, this distance increases to 13.7 Å, consistent with the protonated histidines repelling one another at acidic pH. This, and the refolding of L6 into a more compact conformation, might provide the energy for the unzipping of neighbouring H-bonds. At neutral pH, the two histidines would be uncharged, allowing the H-bonds to reform, and L6 to assume its extended conformation projecting into the extracellular space.

Our findings explain the pH-dependent channel gating that was observed in electrophysiological measurements with recombinant, refolded OmpG reconstituted into lipid bilayers (Conlan and Bayley, 2003). At neutral and basic pH the OmpG channel was open, but it was closed at acidic pH below pH 5. At pH 6, OmpG fluctuated between the open and closed state. These observations are entirely consistent with our two structures, which show the open conformation of OmpG at pH 7.5, and the closed conformation at pH 5.6.

In the triclinic crystals at neutral pH, OmpG binds a number of Ca²⁺ ions from the crystallization buffer in its hydrophilic surface regions. On the periplasmic side there are one or two per monomer at T6, and 5–8 per monomer in the extracellular loops (not shown). Most likely Gd³⁺, which closes the pore (Conlan et al., 2000), binds to one of the loop sites, perhaps exerting an effect similar to that of low pH on L6.

There are no reports on the potential physiological role of pH-dependent channel closure of OmpG in the literature. OmpG may be a ‘rescue porin’ that is expressed under special circumstances if other outer-membrane porins are insufficient for nutrient uptake, for example, of larger oligosaccharides (Fajardo et al., 1998), or are otherwise unavailable. It is reasonable to suggest that the large, comparatively non-specific pore of OmpG should need to be closed at ~pH 6 or below, to prevent a massive influx of protons into the periplasm in an acidic environment, which may be particularly undesirable under conditions of nutrient stress. Previous
claims that the size of the channel in other, trimeric porins is reduced at acidic pH have not been substantiated (Saint et al., 1996), and X-ray crystallography of OmpF at 3.2 Å resolution failed to show a corresponding conformational change (Saint et al., 1996). So far, OmpG is thus the only outer-membrane porin for which pH-dependent gating has been demonstrated (Conlan and Bayley, 2003) and validated by X-ray crystallography of the open and closed states.

OmpG is a monomeric outer-membrane porin

Unlike the classical trimeric porins, OmpG seems to function as a monomer (Conlan et al., 2000), and there is no evidence to suggest a physiological oligomer. Although hydrophobic interaction between OmpG monomers gives rise to apparent dimers in the 3D crystal lattice, these are clearly nonphysiological, as indicated by the up/down orientation of the two monomers (Figure 3E–H). The c12 symmetry of one 2D...
crystal form (Behlau et al., 2001) stipulates an alternating up and down orientation of adjacent molecules, so that OmpG is monomeric also in the lipid environment of membrane crystals. The interactions that give rise to the c12 crystal form seem to be the same as the crystal contacts found in the orthorhombic 3D crystals (Figure 3F and H). Otherwise the hydrophobic interactions between OmpG monomers in all six crystal forms we obtained appear to be different, which in itself is a strong argument against an oligomer. Nor is there any need for OmpG to oligomerize in order to form a functional pore, because its β-strands are all of similar length, and the wall of OmpG is equally high all the way round. This is not the case in the classical outer-membrane porins, which can achieve a pore wall of uniform height only by forming trimers. The monomeric structure is fully consistent with electrophysiological measurements, which indicate individual, single channels in OmpG reconstituted into lipid bilayers (Conlan et al., 2000), while the trimeric outer-membrane porins show three connected channels (Benz et al., 1978). The pores of these 16- or 18-stranded porins are restricted by loops, which do not undergo large conformational changes (Nikaido, 2003), and are stably integrated into the larger barrels.

**Detergent structure mimics lipid bilayer**

In addition to the protein, we found a total of 151 elongated densities of the shape and size of detergent molecules in the triclinic unit cell, which were fitted with LDAO (Figure 4A). Another ~20 densities that resembled partly disordered detergent molecules were left empty. Well-ordered LDAO molecules were distributed more or less evenly around the hydrophobic outside of the OmpG barrel (Figure 5A–C), except for an ~250 Å² area of direct hydrophobic contact between adjacent OmpG molecules (Figure 5B). Each of the four different OmpG molecules in the AU had between 27 and 44 LDAO molecules associated with it. Of these, 15 were found in the same positions in all four OmpGs, while another eight were common to two or three of them. The hydrocarbon chains of nearly all LDAO molecules around the perimeter are in van der Waals contact with the hydrophobic outer surface of the OmpG barrel over most of their length (Figure 5A and B). Some sit in distinct grooves that would be filled by lipid fatty acid chains in the native membrane. Note that OmpG was refolded without any lipid, so that the detergent can occupy all potential lipid-binding sites on the protein surface. As an ensemble, the LDAO molecules thus mimic the membrane lipid in the immediate surroundings of OmpG, and provide a striking example of an almost complete, ordered bilayer around a membrane protein. With one exception, the polar detergent head groups of all LDAO molecules are arranged in a ring around the periplasmic and extracellular side of OmpG that would coincide with the periplasmic and exterior membrane surface. One LDAO molecule is sandwiched between two Trp118 of adjacent OmpG molecules (Figure 4B), which, together with the OH groups, forms a hydrophobic contact. The detergent structure is thus fully consistent with electrophysiological measurements, which indicate individual, single channels in OmpG reconstituted into lipid bilayers (Conlan et al., 2000), while the trimeric outer-membrane porins show three connected channels (Benz et al., 1978). The pores of these 16- or 18-stranded porins are restricted by loops, which do not undergo large conformational changes (Nikaido, 2003), and are stably integrated into the larger barrels.

### Table 1 Data collection and refinement statistics

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**Data collection**

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<td>Completeness (%)</td>
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<td>Redundancy</td>
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**Refinement**

| Resolution (Å) | 15–2.3 |
| No. of unique reflections | 79,984 |
| Rwork/Rfree | 22.37/26.94 |
| No. of atoms | 12,129 |
| Protein | 9,924 |
| B-factors | 56.6 |
| R.m.s deviations | Bond lengths (Å) | 0.02 |
| Bond angles (deg) | 2.5 |

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Figure 2 Stereo views of OmpG. (A) Lines of aromatic residues (‘aromatic slides’) leading from the extracellular channel entrance to the pore interior, perhaps serving as guide rails for substrate molecules. Top (B) and side view (C) of charged sidechains in the pore, with clusters of positively (mostly Arg, blue) and negatively charged residues (mostly Glu, red) on opposite sides of the barrel wall. (D) Loops L6 and L7 in the open (green) and closed (purple) conformation. His231 in S12 and His261 in S13 together act as a pH-sensitive switch, with the protonated sidechains repelling one another at low pH. In the open conformation, L6 is stabilized by hydrogen bonds from His231 to Ser218 and His 261.
groups of Thr155, evidently create a sufficiently polar local environment to accommodate the amine oxide head group.

The hydrophobic region between the detergent head groups forms a continuous band around the outer perimeter of OmpG that is on average 26 Å wide. This correlates closely with the average distance between the belts of aromatic side chains along the periplasmic and extracellular membrane surface (Figure 6). Similar aromatic belts in other membrane proteins demarcate the hydrophobic surface immersed in the lipid bilayer. However, while this zone measures 30–35 Å in α-helical inner membrane proteins (e.g. in the bacterial reaction centre; Deisenhofer and Michel, 1989), its narrower dimension in OmpG and the other outer-membrane porins (Cowan et al., 1992; Forst et al., 1993) indicates that the hydrophobic bilayer core of the outer E. coli membrane is significantly thinner than that of the inner membrane. While inner membrane lipids typically have fatty acid chains of 16 or 18 carbon atoms, the hydrocarbon chains in outer-membrane lipids are on average four carbon atoms shorter. Indeed, five of the six hydrophobic chains of lipid A, the main component of the E. coli outer membrane outer leaflet, are in effect 12 carbon atoms long (Nikaido, 2003), the remaining one having 14 carbon atoms. With its 13 Å chain of 12 carbon atoms, LDAO is thus ideally suited to replace lipids in outer-membrane porins, which may account for the exquisite order in the first detergent shell around OmpG.

By contrast, the detergent surrounding OmpG in the orthorhombic crystals is less well ordered. A total of 13 OG molecules were assigned to characteristically shaped densities (not shown), while several other densities probably correspond to partly ordered OG. The binding sites of all 13 ordered OGs coincide completely or partially with those of LDAO molecules in the other crystal form. The comparatively poor order of OG molecules in the first detergent shell may be related to their shorter carbon chain, which fits the hydrophobic surface of OmpG less well than LDAO.

**Crystal packing**

The detergent micelles surrounding OmpG clearly extend beyond this shell of frozen LDAO molecules. A rough estimate of the micelle dimensions is obtained from the distance

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**Figure 3** Projection maps of OmpG monomers calculated from atomic coordinates of the open (A) and closed conformation (B), and determined by electron crystallography of 2D crystals of the native OmpG isolated from E. coli outer membranes (Behlau et al., 2001) (C), or of 2D crystals of refolded recombinant OmpG (D), all at 6 Å resolution. Top (E, F) and side views (G, H) of the noncrystallographic OmpG dimer (E, G) in the triclinic, pH 7.5 crystal form, and the crystallographic dimer (F, H) in the orthorhombic, pH 5.6 crystal form, with the position of a potential lipid bilayer indicated in (G, H).
between OmpG molecules in the crystal lattice. Assuming a toroid micelle, and estimating the volume of one LDAO molecule from the ordered first shell as \(460 \text{ Å}^3\), one obtains a total of 70–100 LDAO per OmpG, in good agreement with the aggregation number. The majority of LDAO molecules in the micelle thus do not occupy defined positions. The triclinic OmpG lattice is characterized by layers of virtually continuous tubes of OmpG cylinders arranged head to tail (Figure 7), held together by extensive hydrophobic protein–protein contacts (Figure 5B), with alternating tubes running in opposite directions. Evidently the LDAO micelles are solid enough to ensure long range order and tight stacking of the layers, even though they are separated by \(\sim 18 \text{ Å}\) wide bands of apparently featureless space, containing the bulk of the detergent, plus some solvent. In the resulting, highly unusual crystal packing, there are no protein–protein contacts.

Figure 4 Stereo views of structural details with electron density. (A) Five LDAO molecules (green) in the detergent layer on the outer surface of OmpG in triclinic crystals (pH 7.5) at 2.3 Å resolution. (B) Trp118 and Thr155 creating a local polar environment at the hydrophobic interface between adjacent OmpG monomers in the triclinic crystal form, accommodating an LDAO molecule with buried head group. (C) OG and glucose (yellow) with neighbouring residues in the interior of the closed pore in the orthorhombic crystals (pH 5.6) at 2.7 Å resolution (B).
between layers of OmpG tubes, accounting perhaps for the extreme fragility and poor reproducibility of these crystals.

**Substrate binding**

The open pore of each of the four OmpG molecules in the AU of the pH 7.5 crystals contains three well-ordered LDAO molecules in identical positions (Figure 5C), suggesting that they are firmly bound. The OmpG pore is thus surprisingly amphipathic. In the pH 5.6 crystals grown in the presence of OG, we found two distinct densities in the closed OmpG pore (Figures 4C and 5D), one of which fitted one complete OG molecule. The other density lacked a clear hydrocarbon tail and was fitted with a glucose molecule that either represents the head group of a second, partially disordered detergent molecule or was picked up from the cryoprotectant. The sugar head group of the complete OG molecule is coordinated by hydrogen bonds to residues Arg168, Tyr196, Glu154, Glu253 and Trp131, while the adjacent glucose molecule forms hydrogen bonds with Trp131, Tyr135 and Glu152. Both binding sites coincide with those of LDAO molecules in the other crystal form. The glucose molecule and the OG glucoside head group most likely indicate the positions of sugar binding sites in the OmpG pore, and the side chains involved in sugar binding have the same conformation in both structures.

Nonspecific uptake of oligosaccharides is one of the main roles of OmpG in *E. coli* (Fajardo et al., 1998). In the classical sugar-uptake porins LamB (Dutzler et al., 1996), ScrY (Forst et al., 1998) and maltoporin from *Salmonella typhimurium* (Meyer et al., 1997), the entry of disaccharides into the channel is thought to be facilitated by a line of aromatic residues roughly parallel to the barrel axis, sometimes referred to as the ‘greasy slide’. In OmpG we found a strikingly similar arrangement of aromatic residues Tyr98, Tyr96, Tyr94,
Phe66 and Tyr50 (Figure 2A), which we prefer to call an ‘aromatic slide’, as it is less conspicuous for its hydrophobicity than for its line of tyrosine OH groups. The similarity even extends to Tyr146 in L4, which corresponds exactly to Tyr118 in the restriction loop 3 of LamB, with its hydroxyl group pointing towards the aromatic slide. This similarity is all the more remarkable since it applies to a structural motif that is discontinuous in terms of polypeptide sequence, with contributions from completely different strands and loops in the two porin classes. Its presence in both OmpG and the trimer-forming sugar-uptake porins (Schirmer et al, 1995) suggests that it is an essential feature for solute uptake or pore stability that was either conserved from a most ancient precursor protein, or represents an extraordinary example of convergent evolution.

In OmpG the aromatic slide divides into two branches at Tyr96 and Tyr94. The second branch has no correspondence in the trimeric porins and continues to Phe137, Tyr135 and Trp131, with Tyr146 in L4 pointing towards F137. Interestingly, Trp131 at the end of this second branch participates in coordinating the glucose molecule, suggesting that the OH groups in the aromatic slide may indeed function as a guide rail for incoming substrate. Possibly, the presence of this extra branch makes OmpG, which may be a ‘rescue porin’, more effective in the uptake of oligosaccharides.

**Biotechnological potential**

Because of their ruggedness, pore-forming β-barrel proteins have considerable potential in biotechnology. One candidate is the bacterial toxin α-haemolysin, a heptamer of seven identical 33 kDa monomers, which inserts into the plasma membrane of target cells, forming large (>20 Å) nonspecific and unregulated pores of 14 β-strands (Song and Gouaux, 1998). As a monomer, OmpG is clearly preferable over α-haemolysin or the trimer-forming porins in this regard. OmpG forms large, nonspecific pores, can be produced easily in large quantities, is efficiently reconstituted into artificial lipid bilayers and even forms extensive 2D lattices quite readily. Another attractive feature is its pH-dependent channel gating. Compared to other pore-forming proteins, OmpG thus seems to be not only the most efficient and economical in terms of polypeptide use, but also the most potentially versatile for biosensor development and other biotechnological applications.
Conclusion
We determined the structure of OmpG, an unusual outer-membrane porin from E. coli. Unlike the classical outer-membrane porins, which are trimers, OmpG is a structural and functional monomer. Its β-barrel has only 14 strands, whereas those of the classical outer membrane porins have 16 or 18. As another unique feature, OmpG is gated by pH, whereby its uncommonly large channel is open at neutral pH, and closed at acidic pH below 6, presumably to protect the cell against acidification of the periplasm if the protein is expressed under conditions of nutrient stress. By using crystals grown at pH 7.5 or at pH 5.6, we were able to determine the structure of OmpG both in the open and in the closed state at 2.3 and 2.7 Å resolution, respectively. The structures show that pore closure results from the rearrangement of an extracellular loop, L6, which is triggered by the repulsion of two histidine residues in neighbouring β-strands that would be protonated at acidic pH, forming a simple pH-sensitive switch. Like other porins involved in oligosaccharide uptake, OmpG has lines of aromatic residues on its inner pore surface, which may guide incoming sugar molecules into the periplasm. The remarkable correspondence of this discontinuous motif in two classes of porins that share no recognizable sequence homology appears to be a rare example of convergent evolution. OmpG was refolded from inclusion bodies in the absence of membrane lipids, which in the crystals are replaced by tightly bound detergent molecules. An unprecedented feature of the higher-resolution, open structure is an almost complete double ring of well-resolved detergent molecules, representing the first shell of the detergent micelle immobilized by tight contact with the hydrophobic outer surface of the OmpG cylinder. These detergent molecules effectively mimic the lipid bilayer of the outer membrane, which is thus seen to be only 26 Å thick. As a particularly rugged, monomeric membrane porin that is easily refolded from inclusion bodies, OmpG is predestined for biotechnological applications and biosensor development.

Materials and methods
Protein expression and purification
For 3D crystallization, the OmpG gene fragment without its signal sequence (amino acids 22–301) was cloned into the pET26b plasmid vector, resulting in an additional methionine at the N-terminus, and expressed in E. coli strain BL21(DE3)-C41 grown in TB medium. The protein formed inclusion bodies, which were collected by low-speed centrifugation after breaking the cells in a cell disruptor (Constant Systems). The pellet was washed in buffer (25 mM Tris–HCl, pH 8) containing 1 M urea and 1% Triton-X 100, and the inclusion bodies were dissolved in 8 M urea in the same buffer. The solubilized protein was loaded onto an anion exchange chromatography column, and unfolded OmpG was eluted by a NaCl step gradient. Refolding of OmpG was achieved by dilution in 1% (wt/vol) n-octyl-β-D-glucopyranoside (OG), at a final urea concentration of 3 M. Refolding was monitored by SDS–PAGE, taking advantage of the final yield was 20–30 mg of refolded OmpG per litre of culture. Protein purity was greater than 95%, as estimated by SDS–PAGE and Coomassie staining.

Selenomethionine-substituted OmpG was expressed similarly in BL21(DE3)-C41 cells in M9 minimal medium, suppressing methionine biosynthesis by a five-fold increase in the concentration of the amino acids Leu, Ile, Lys, Phe, Thr and Val. Precultures were grown overnight in LB medium, desired signal protein by gentle centrifugation prior to inoculation. Expression and purification was performed as described for the underivatized protein.

2D crystallization
2D crystals of refolded OmpG were obtained by detergent dialysis (Kühbrandt, 1992) in the presence of E. coli polar lipids (Avanti) essentially as described (Belau et al, 2001; Hiller et al, 2005). At lipid/protein ratios (LPR) between 0.25 to 1.5 (wt/wt), reconstituted OmpG formed 2D lattices in tubular vesicles that were ~150 nm wide and up to 1 μm long. At lower LPR 2D crystals were extensive membrane sheets measuring several μm across. Electron micrographs of tubular 2D crystals washed with 4% unbuffered trehalose as a cryo-protectant were recorded at a specimen temperature of ~4 K in a JEOL 3000 SFF electron microscope. Crystallographic image processing (Crowther et al, 1996) yielded projection amplitudes and phases to ~8 Å resolution, from which projection maps were calculated.

3D crystallization
OmpG was dialyzed against buffers with different detergents including OG, n-decyl-β-D-maltopyranoside (DM), lauryldimethylamino-N-oxide (LDAO), polyoxyethylene (C18E8) and mixtures of these at final protein concentrations of 2.5, 5, 7.5, 10 and 15 mg/ml. Initial crystallisation conditions were found in Greiner 96 three-well sitting-drop plates using commercial crystallization reagents and KCl, NaCl, Na2CO3 or NaOH in 25 mM Tris at pH 8.0 with 5 mM LDAO mixed with 1 μl of 100 mM HEPES pH 7.5, 30% PEG 4000 and 200 mM CaCl2 at 18°C. The characteristic habit of these very fragile crystals resembled a flattened rice grain of dimensions 700 × 400 × 40 μm². They diffracted anisotropically to 1.9 Å, and belonged to the trigonal space group P1 (a = 71 Å, b = 107 Å, c = 127 Å, α = 120 °, β = 89.3 °, γ = 89.3 °), with 66% solvent content and four molecules in the asymmetric unit (A U). Another crystal form grew in sitting drops for 4–5 days at 18 °C in 0.4 μl of 7.5 mg OmpG in 25 mM Tris at pH 8.0 and 20 mM OG mixed with 0.4 μl of 100 mM Na-Citrate at pH 5.6, 150 mM NaCl and 12% PEG 3350. These low-pH crystals were bipymidal and belonged to the orthorhombic space group P2₁2₁2₁, (a = 70.4 Å, b = 71.1 Å, c = 191.61 Å, α = β = γ = 90 °), with two molecules in the A U. Soaks with various heavy-metal compounds were not successful, but crystallization in the presence of GdCl3, an inhibitor of channel activity (Conlan et al, 2000), improved the low-pH crystals slightly. However, the reproducibility was low and SAD and MAD data collected on the Gd edge were not usable for phasing.

Crystals of selenomethionine-derivatized OmpG were obtained in hanging drops, by mixing 1 μl of 9 mg/ml protein in 25 mM Tris–HCl buffer at pH 8 and 20 mM OG with 1 μl of reservoir solution containing 2.1 M ammonium sulfate and 4% ethylene glycol in 100 mM Tris–HCl pH 7.5. This yielded elongated, rod-shaped orthorhombic crystals containing one molecule in the A U and 66% solvent (space group P2₁2₁2₁, a = 56.2 Å, b = 71.2 Å, c = 122.5 Å, α = β = γ = 90 °).

Data collection, structure determination and refinement
For data collection the crystals were transferred to cryo-protectant solutions and flash-frozen in liquid nitrogen. Best results for the tricorn crystals were obtained by increasing the PEG 3350 concentration to 20–30% by gradual addition of concentrated PEG solution. As the low-pH crystals of underivatized OmpG were stable only in their mother liquor, equilibrated drops with ~25% glucose were added to the crystals, which were then also flash-frozen in liquid nitrogen. Crystals of selenomethionine-derivatized OmpG were more robust and could be frozen in different cryoprotectants. Best results were obtained with a mixture of paraffin oil and Panatone-N.
SeMet-MAD data to 2.9 Å resolution were collected at three wavelengths from a single crystal at 100 K on beamline 1911-3 at the Swedish synchrotron radiation facility MAX-lab at Lund University (Table I). The data sets were processed with XDS (Kabsch, 1993). Four of the expected six selenium sites in the AU were found and used to calculate MAD phases at 3.1 Å with the program SOLVE (Terwilliger, 2004), followed by automatic model building. The resulting electron density map was used to build an initial model, which was corrected manually and supplemented with COOT (Emsley and Cowtan, 2004).

Native data from both crystal forms were collected at beamline PXI and PXII at the Swiss Light Source (SLS) and processed with XDS. The initial model obtained from the SeMet-MAD data was used as a search model for the orthorhombic data set in PHASER (McCoy et al., 2005) and was rebuilt and completed in the loop regions. The partially refined model, excluding parts of L1 and L6, then served as a search model for the triclinic data set to find the four molecules in the AU. The model was subjected to iterative rounds of rebuilding into 2c and 2c–Fo–Fc electron density maps, and refined using COOT and REFMAC (Murshudov et al., 1997). The two molecules in the orthorhombic unit cell and the four molecules in the triclinic unit cell were averaged by applying noncrystallographic symmetry (NCS) either in REFMAC or in COOT. For structure validation we used COOT and PROCHECK in the CCP4 suite (Collaborative Computational Project, 1994).

Figures were generated using the programs POVSRIPT (Fenn et al., 2003), PyMOL (Delano, 2004) and POV-Ray (http://www.povray.org). Superpositions were carried out with the SSM. Superposition routine (Krissinel and Henrick, 2004) within COOT. For electrostatic surface potential calculation APBS (Baker et al., 2001) and PyMOL were used.

Coordinates of the open (2iwv) and closed form (2iww) of OmpH have been deposited in the pdb.

Acknowledgements

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