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Expression, crystallization and preliminary X-ray crystallographic studies of the outer membrane protein OmpW from *Escherichia coli*

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OmpW is an eight-stranded 21 kDa molecular-weight β-barrel protein from the outer membrane of Gram-negative bacteria. It is a major antigen in bacterial infections and has implications in antibiotic resistance and in the oxidative degradation of organic compounds. OmpW from *Escherichia coli* was cloned and the protein was expressed in inclusion bodies. A method for refolding and purification was developed which yields properly folded protein according to circular-dichroism measurements. The protein has been crystallized and crystals were obtained that diffracted to a resolution limit of 3.5 Å. The crystals belong to space group $P422$, with unit-cell parameters a = 122.5, c = 105.7 Å. A homology model of OmpW is presented based on known structures of eight-stranded β-barrels, intended for use in molecular-replacement trials.

1. Introduction

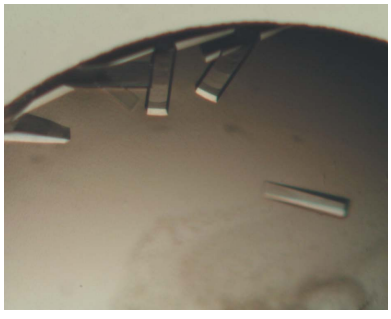
OmpW is a β-barrel outer membrane protein in Gram-negative bacteria. The *Escherichia coli* protein is a receptor for colicin S4 (Pilsl *et al.*, 1999); it has a cleavable signal peptide and a molecular weight of 21 kDa (192 amino acids) in its mature form. It is present in secreted vesicles (Gophna *et al.*, 2004; Horstman & Kuehn, 2000) and minicells and is mainly localized at the cell poles (Lai *et al.*, 2004).

In *Vibrio cholerae* the homologue is highly immunogenic (Jalajakumari & Manning, 1990) and its gene is used in PCR-based strain-identification methods (Nandi *et al.*, 2000). OmpW homologues form a family within the superfamily of eight-stranded porins (Baldermann *et al.*, 1998), which also includes three proteins of known structure: OmpA (PDB code 1qjp), NspA (1p4t) and OmpX (1qj8).

Expression of OmpW and its homologues is regulated by different environmental conditions. Iron availability influences OmpW expression *via* the transcriptional regulator Fur, but while OmpW is upregulated by iron in *E. coli* and *Pasteurella multicoda* (McHugh *et al.*, 2003; Paustian *et al.*, 2001), it is downregulated in the environmental organism *Shewanella oneidensis* (Thompson *et al.*, 2002). Moreover, osmotic stress has a strong influence on OmpW expression in several *Vibrio* species (Xu *et al.*, 2004, 2005; Nandi *et al.*, 2005). Interestingly, close homologues of OmpW are located in operons responsible for oxidative degradation of organic compounds, *e.g.* in *Comamonas testosteroni* (Kim *et al.*, 2004) and *Pseudomonas oleovorans* (van Beilen *et al.*, 1992). It has also been shown that Omp21 from *C. acidovorans* is upregulated under oxygen-limiting conditions (Baldermann *et al.*, 1998).

Apart from its immunogenic effects during *V. cholerae* infection, few reports exist on the medical relevance of OmpW. In *Salmonella typhimurium*, expression seems to be influenced by the BaeR two-component system and increased OmpW levels correlate with an increased resistance to the cephalosporin antibiotic ceftriaxone (Hu *et al.*, 2005). During *E. coli* infection of mice, OmpW expression increases (Motley *et al.*, 2004) and OmpW-containing outer membrane vesicles induce a weak pro-inflammatory response in A498 cell cultures (Soderblom *et al.*, 2005).

In this study, we report the purification and crystallization of His-tagged OmpW from *E. coli*.



2. Materials and methods

2.1. Chemicals

Buffers, salts and DNAse I were from AppliChem, Darmstadt, Germany. LDAO was purchased from Fluka (Buchs, Switzerland). PCR reagents and restriction enzymes were supplied by MBI Fermentas, St Leon, Germany.

2.2. Cloning, expression, refolding and purification

The OmpW gene without the signal peptide sequence was amplified from *E. coli* W3110 genomic DNA using the primers Fwd, 5′-GGAATTCCATATGGCGCATGAAGCAGGCG-3′, and Rev, 5′-CCGGTTACTCGAGTTAAAAACGATATCCTGCTGAGAAC-ATAAAC-3′.

The PCR product was cut with *Nde*I and *Xho*I and was cloned into the vector pET28b (Merck Biosciences, Bad Soden, Germany), producing pET28-His-OmpW, or into pET30b for production of the untagged protein.

pET28-His-OmpW or pET30-OmpW was transformed into *E. coli* BL21Omp8 cells (Prilipov *et al.*, 1998). Cells were grown in LB medium containing 100 mg l^{-1} ampicillin. Induction with 1 m*M* IPTG was performed in the logarithmic growth phase at an OD_{600} of 0.7 and cells were harvested by centrifugation after 4 h.

E. coli cells resuspended in PBS buffer containing 1 m*M* $MgCl_2$ and a small amount of DNAse I were lysed using a French press. Inclusion bodies were harvested by centrifugation at 3000*g* for 10 min. The pellet was resuspended in PBS containing 1%(*w*/*v*) Triton X-100, centrifuged again and washed several times with water to remove the detergent.

Inclusion bodies of His-tagged OmpW were solubilized in 6 *M* guanidinium chloride buffer containing 500 m*M* NaCl, 10%(*w*/*v*) glycerol and 50 m*M* Tris–HCl pH 8. Ni–NTA chromatography was performed on an ÄKTA purifier system (GE Healthcare) using an XK26 column with 30 ml Ni Sepharose HP (GE Healthcare). A linear gradient of 0–500 m*M* imidazole in solubilization buffer was used to elute the protein. Pooled fractions typically contained 10–20 mg ml^{-1} His-OmpW and 150–200 m*M* imidazole. For the untagged protein, inclusion bodies were solubilized in 8 *M* urea containing 1 m*M* EDTA and 20 m*M* Tris–HCl pH 8. Purification was performed using a 20 ml Mono-Q column with a linear gradient of 0–1 *M* NaCl in 6 *M* urea, 1 m*M* EDTA, 20 m*M* Tris–HCl pH 8.

Purified and denatured OmpW or His-OmpW was adjusted to 10 mg ml^{-1} in solubilization buffer and quickly diluted 1:20 into a buffer containing 1% LDAO, 1 m*M* EDTA and 20 m*M* Tris–HCl pH 8 on ice. The resulting 100 ml of dilute protein solution was dialyzed overnight against 5 l of 20 m*M* Tris buffer containing 0.1% LDAO and 1 m*M* EDTA. The refolded protein (0.5 mg ml^{-1}) showed no signs of precipitation after 12 h.

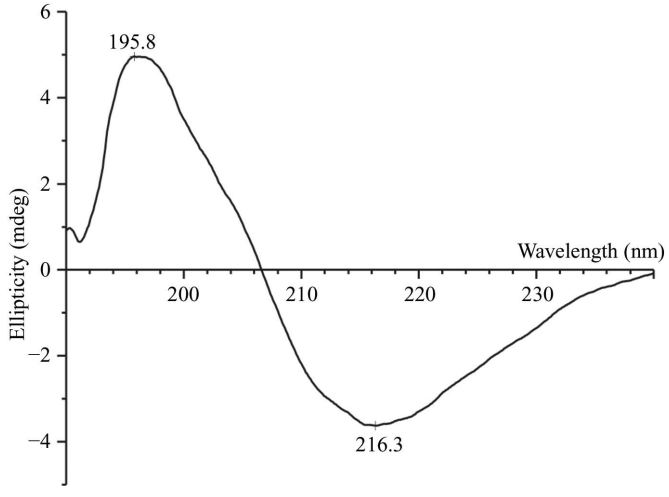


Figure 1
CD spectrum of His-tagged OmpW after refolding. The maximum and minimum peak positions and the point where the spectrum cuts the wavelength axis correspond almost ideally to the values for pure β-sheet spectra (maximum peak 195 nm, minimum peak 216 nm and $y = 0$ at 207 nm).

2.3. Secondary-structure determination by circular-dichroism spectroscopy

Circular-dichroism (CD) spectra were recorded on a Jasco J-810 spectrophotometer in a 0.1 mm quartz cuvette. Ten spectra were accumulated per measurement, using a data pitch of 0.1 nm, a scan speed of 20 nm s^{-1}, 1 nm slit width and a response time of 2 s. 0.5 mg ml^{-1} His-OmpW in 0.1% LDAO, 1 m*M* EDTA and 10 m*M* Tris–HCl pH 8 was used in all measurements.

2.4. Modelling and channel radius calculation

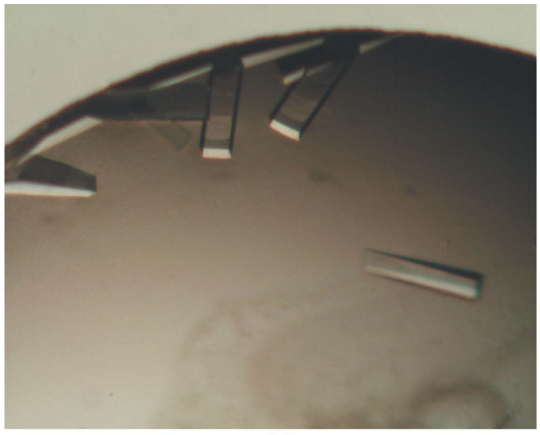
We searched the SCOP database (Murzin *et al.*, 1995) with the *HHpred* remote homology-detection server (Söding *et al.*, 2005) and found three eight-stranded outer membrane proteins with *E* values below 1×10^{-20} that we selected for modelling in *HHpred*: OmpA (PDB code 1qjp), NspA (1p4t) and OmpX (1qj8). *HHpred* automatically constructed a structural alignment of the selected templates with *STAMP* (Russell & Barton, 1992) and merged the template alignments into a single super-HMM. This super-HMM was aligned with the query HMM (OmpW) to give a multiple alignment of the query with the templates. We edited this alignment by hand and shifted the inserts to the appropriate positions in the outer loops. The alignment within the loop regions is therefore tentative, whereas the alignment of the core, *i.e.* the eight β-strands, is quite reliable. We used the homology-modeling software *MODELLER* (Sali & Overington, 1994) to construct a three-dimensional model from the alignment and *VERIFY3D* (Luthy *et al.*, 1992) to assess the model quality and improve the placement of the inserts.

The calculations of the channel radii in the homology model were performed with the program *HOLE* (Smart *et al.*, 1993) through the *iMolTalk* server (Diemand & Scheib, 2004). *HOLE* could not find a channel through OmpW until Trp176 was replaced by glycine in the model. For visualization with *RASMOL* (Sayle & Milner-White, 1995), the *HOLE* coordinates for the channel without Trp176 were joined with the Trp176 coordinates from the homology model.

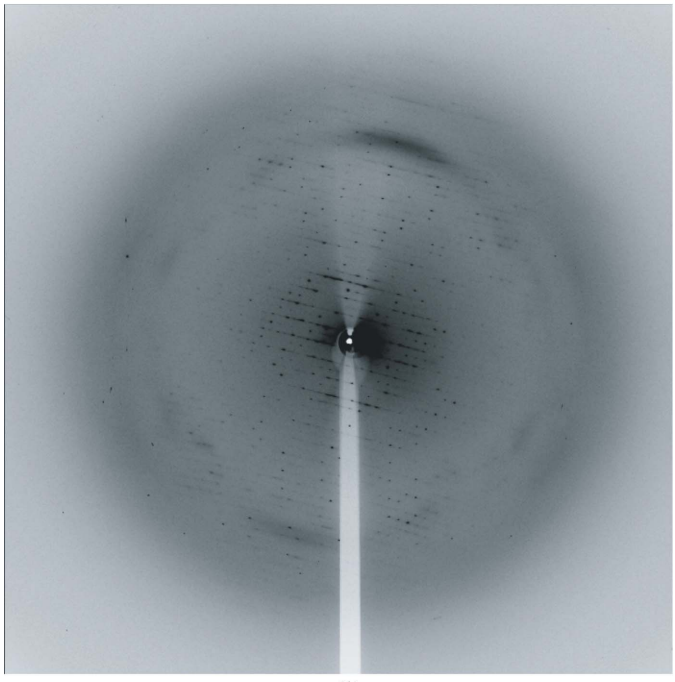
2.5. Crystallization and X-ray crystallographic analysis

Crystallization trials of purified His-OmpW concentrated to 9 mg ml^{-1} in 20 m*M* Tris pH 8, 150 m*M* NaCl, 1 m*M* EDTA, 0.02% NaN_3 containing 0.1% LDAO were performed using Crystal Screens I and II, Index and PEG/Ion Screens (Hampton Research) using 1 + 1 µl hanging drops. Needle-shaped crystals appeared under several conditions; the best results were obtained from condition No. 69 of Index Screen. Crystal size and mechanical stability could be improved by performing the crystallization at 277 K. Optimized crystals were obtained with 17%(*w*/*v*) PEG 3350, 0.3 *M* $(NH_4)_2SO_4$, 0.1 *M* bis-tris propane pH 9. Prior to data collection, His-OmpW crystals were soaked for a few seconds in a mother-liquor reservoir solution containing 20%(*v*/*v*) PEG 400 and cryocooled in liquid nitrogen. Data were collected at beamline PX10 of the synchrotron-radiation

source SLS (Swiss Light Source, Villigen, Switzerland). Data were collected at 100 K and a wavelength of 0.9543 Å. Crystals were rotated by 0.5° during data collection and the diffraction patterns were recorded on a 225 mm MAR CCD. All data were indexed, integrated and scaled using the programs *XDS* and *XSCALE* (Kabsch, 1993). Molecular-replacement trials were performed using the program *MOLREP* (Vagin & Teplyakov, 1997).



(*a*)



(*b*)

Figure 2
(*a*) Needle-shaped crystals of His-OmpW from *E. coli* with dimensions of 0.3 × 0.05 × 0.05 mm crystallized in space group *P*422. (*b*) Diffraction pattern of His-OmpW crystals recorded at beamline PX10, SLS.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

Beamline	PX10, SLS
Detector	MAR CCD 225 mm
Crystal-to-detector distance (mm)	296
Resolution (Å)	40–3.46 (3.6–3.46)
Wavelength (Å)	0.9543
Oscillation angle (°)	0.5
Unique reflections	19774
Completeness (%)	98.4 (97.6)
Redundancy	5.9
Average $I/\sigma(I)$	9.4 (3.2)
Unit-cell parameters (Å)	$a = b = 122.5$, $c = 105.7$
$R_{\rm merge}$†	0.13 (0.47)
Mosaicity (°)	0.47
No. of subunits per a.u.and solvent content	2 monomers/70%, 3 monomers/55%

† $R_{\rm merge} = \sum_{\rm unique\ reflections}(\sum_{i=1}^{N}|I_i - \bar{I}|)/\sum_{\rm unique\ reflections}(\sum_{i=1}^{N} I_i)$, where N represents the number of equivalent reflections and I the measured intensity.

3. Results and discussion

We have cloned the outer membrane protein OmpW from *E. coli* with an N-terminal His tag for purification. The protein was expressed without its native signal peptide, which resulted in inclusion-body formation. We purified the protein in its denatured form using a guanidine buffer system and an Ni–NTA column. Refolding was performed by fast dilution into a detergent buffer containing 1% LDAO. After dialysis to remove residual guanidine and imidazole, no precipitation occurred and the protein contained no visible impurities in SDS–PAGE. As expected for a β-barrel protein, CD spectra (Fig. 1) show a high β-sheet content and match the CD spectra of OmpW from *V. cholerae* (Nandi *et al.*, 2005).

The non-His-tagged version of the protein, purified *via* anion exchange and refolded in the prescence of LDAO, yielded thin needle-like crystals which were mechanically unstable and limited to a resolution of 10 Å. The His-tagged version, refolded under the same conditions, was significantly purer and formed crystals under condition No. 69 of Index Screen at 277 K which were mechanically stable, reproducible and diffracted to a resolution of 3.5 Å (Fig. 2). The crystals were formed in the tetragonal space group *P*422, with unit-cell parameters $a = 122.5$, $c = 105.7$ Å, an overall $R_{\rm merge}$ of 13% and a mean $I/\sigma(I)$ of 9.4. We cannot estimate precisely at present whether there are two or three molecules in the asymmetric unit,

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OmpW   21 AHEAGEFFMRAGSATV-RPTEGAGGTLGSLGGFSVTNNTQLGLTFTYMA----T-DNIGVELLAATPFRH
OmpA   22 APKDNTWYTGAKLGWSQYHDTGLINNNGPT----HENKLGAGAFGGYQV----N-PYVGFEMGYDWLGRM
NspA   20 -EGASGFYVQADAAHA-KASSS----------LGSAKGFSPRISAGYRI----N--DLRFAVDYTRYKN-
OmpX   24 --ATSTVTGGYAQSD-A----------------QGQMNKMGGFNLKYRYEEDNSPLGVIGSFTYTEKSRT

OmpW   85 KIGTRATGDIATVHHLPPTLMAQWYFGDASSKFRPYVGAGINYTTFFDNGFNDHGKEAGLSDLSL-KDSW
OmpA   83 PYKGSVE--NGAYKAQGVQLTAKLGY-PITDDLDIYTRLGGMVWRADTYSN--------VYGKNH-DTGV
NspA   81 YKAP-----STDFKLYSIGASAIYDF-DTQSPVKPYLGARLSLNRASVDL---------GGSDSFSQTSI
OmpX   75 ASS------GDYNKNQYYGITAGPAY-RINDWASIYGVVGVGYGKFQTTEY--------PTYKN-DTSDY

OmpW  154 GAAGQVGVDYLINRDWLVNMSVWYMDIDTTANYKLGGAQQHDSVRLDPWVFMFSAGYRF
OmpA  141 SPVFAGGVEYAITPEIATRLEYQWTNNIGDAHTI-------GTRPDN-GMLSLGVSYRF
NspA  126 GLGVLTGVSYAVTPNVDLDAGYRYNYIGKVN----------TVKNVRSGELSAGVRVKF
OmpX  129 GFSYGAGLQFNPMENVALDFSYEQSRI----------------RSVDVGTWI-AGVGYR
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Figure 3
Alignment of the sequences used in homology modelling. All sequences are without signal peptides. The tryptophan that blocks the channel in the model is highlighted in red. β-Sheet predictions (underlined) are from *HHpred*.

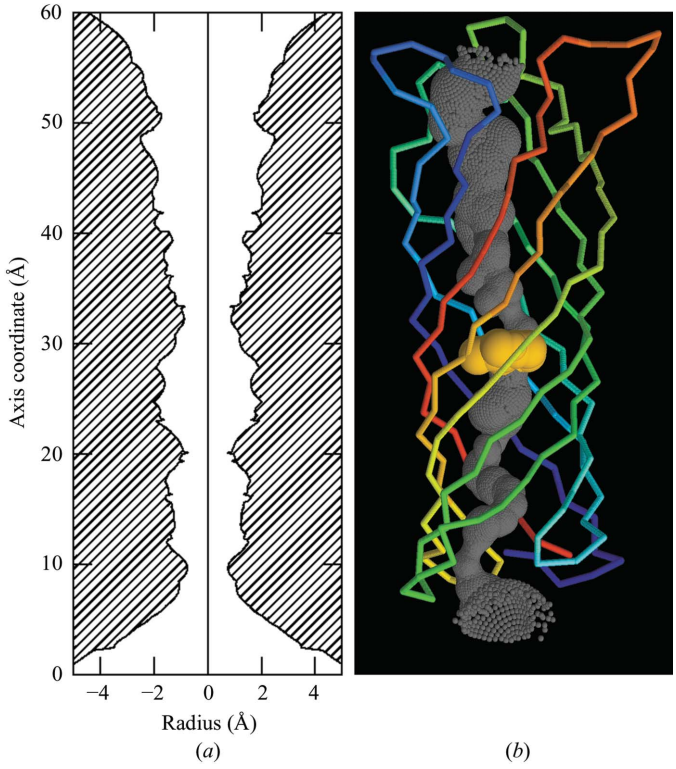

Figure 4
Homology model of OmpW. OmpW forms a narrow hydrophobic channel that is blocked by a tryptophan residue. (*a*) Calculated channel radius without the central tryptophan residue. The program *HOLE* could not find a channel until Trp176 was replaced by a glycine residue in the model. (*b*) Visualization of the channel containing the tryptophan residue.

which would result in a solvent content of 70 or 55%, respectively. However, from the self-rotation function it seems that there are only two molecules in the asymmetric unit, which would fit well with the typically high solvent content in outer membrane protein structures. Data-collection statistics are summarized in Table 1.

To solve the phase problem we expressed SeMet-labelled protein, but the samples were prone to proteolysis and degraded before crystals were formed. At present, we are screening for derivatives using heavy-atom soaking of the crystals.

In parallel, we have built a homology model for molecular replacement. Despite their clear homology, OmpW, OmpA, OmpX and NspA have only 15–20% pairwise sequence identity (Fig. 3). Hence, the quality of the model can be expected to be of the same order as the structural similarity between the templates, which is around 1–2 Å r.m.s.d. for the ∼130 residues of the β-barrel. This should in principle be sufficient for molecular replacement (MR). In initial trials using this homology model, the crystal packing of some MR solutions looks useful but needs to be refined further. An interesting feature of the homology model is Trp176, which is located in the center of the pore and blocks it at its narrowest point (Fig. 4). Since this is the only residue closing the pore, it might form part of a gating mechanism. A high-resolution structure will shed more light on this question.

Note added in proof. While this manuscript was under review, another paper describing the crystal structure of OmpW from *E. coli* was published (Hong *et al.*, 2006).

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