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Crystallization and preliminary crystallographic studies of MOMP (major outer membrane protein) from *Campylobacter jejuni*

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Campylobacter jejuni is the leading bacterial cause of human enteritis linked to ingestion of contaminated food or water. MOMP, the major outer membrane protein from these Gram-negative bacteria, belongs to the porin family. In order to determine the three-dimensional structure of this protein and to elucidate the underlying molecular mechanisms, the MOMP from *C. jejuni* strain 85H has been purified and crystallized by vapour diffusion. Two crystal forms were characterized for this membrane protein. X-ray diffraction data were collected to a resolution of 3.1 Å using a synchrotron-radiation source from the orthorhombic crystal form, which belonged to space group $P2_12_12$ with unit-cell parameters $a = 170.1$, $b = 101.9$, $c = 104.9$ Å. With a trimer in the asymmetric unit, the solvent content is 64% (V_M = 3.4 Å Da^{-1}). The other form exhibits trigonal symmetry (space group $R3$) with hexagonal unit-cell parameters $a = b = 94.2$, $c = 161.2$ Å, but diffracts X-rays poorly to about 4 Å with significant anisotropy.

1. Introduction

Campylobacter jejuni is a major cause of bacterial gastroenteritis throughout the world (Friedman *et al.*, 2000). This Gram-negative bacterium has a worldwide distribution and a broad host range. The complete genomic sequence of this enteric pathogen has been elucidated (Parkhill *et al.*, 2000) and the proteomic characterization of *C. jejuni* is currently being performed (Parrish *et al.*, 2004). The membrane surface of *Campylobacter* is organized in a regular hexagonal lattice mainly constituted of a protein named MOMP (major outer membrane protein; Amako *et al.*, 1996) associated with peptidoglycan. This trimeric integral membrane protein (Bolla *et al.*, 1995; Zhuang *et al.*, 1997) not only functions as a porin permitting the diffusion of a wide variety of compounds (Dé *et al.*, 2000), but is also involved in the structural organization and stabilization of the outer membrane (Amako *et al.*, 1997) and may mediate adhesion to cultured cells (Schröder & Moser, 1997). Moreover, the development of antimicrobial resistance in Gram-negative bacteria related to decreased permeability underlines the importance of characterizing these pore-forming molecules (Nikaido, 2003).

The functional superfamily of bacterial porins usually consists of homotrimeric proteins with subunits that are 300–420 residues in length (Schirmer, 1998). Each protomer forms a hydrophilic channel through the outer membrane with variable diffusion properties and selectivities. High-resolution crystal structures of several of these proteins are known (Schulz, 2002). They consist of 16 or 18 transmembrane β-strands organized in an antiparallel β-barrel perpendicular to the plane of the membrane. These structures show that the structural motifs are conserved among different bacterial species, even in those with a relative low level of primary sequence homology. Here, the MOMP from *Campylobacter* 85H strain, which is constituted of 405 residues (sequence accession No. AJ841785) and is predicted to have an 18 β-stranded β-barrel topology (Zhang *et al.*, 2000; Labesse *et al.*, 2001), shows only 15% sequence identity with the sucrose-specific porin (PDB code 1a0t). On this basis, MOMP would appear to be a specific rather than a general porin. Significant differences can be expected between the structure of MOMP and the other 18 β-stranded porins, in particular (i) differences in the lengths and orientations of surface-exposed loops, (ii) variability in the protomer interfaces and (iii) a specific arrangement of the pore-lining residues. Thus, crystallographic studies are required to establish the structural basis of the trimer stability, the pore selectivity and diffusion properties of this protein. The analysis of MOMP from different *C. jejuni* strains shows that they share 80% or higher sequence identity and have the same overall fold. Most amino-acid substitutions occurred in the putative external loops,

while the predicted β-strands were formed of conserved sequences (Zhang *et al.*, 2000; Labesse *et al.*, 2001).

Here, we report the crystallization of MOMP of *C. jejuni* strain 85H and describe the preliminary X-ray data collected from two crystalline forms of this protein. The three-dimensional structure of this porin from a pathogenic bacteria will be helpful in clarifying its functional properties as well as in making a structural comparison with other porins from Gram-negative bacteria. It will also be useful in further understanding of the mechanisms of antibiotic resistance in some clinical strains (Dé *et al.*, 2001).

2. Materials and methods

2.1. Purification of MOMP from *C. jejuni* 85H

The isolation and purification of the MOMP protein were performed as previously described (Dé *et al.*, 2000). Briefly, *C. jejuni* 85H strain was grown on Columbia Agar (Biomérieux, France) at 315 K in a microaerophilic environment. MOMP proteins were extracted and resuspended in 1%(*w*/*v*) octyl-POE (*n*-octyl-polyoxyethylene; Bachem, Switzerland). Proteins in octyl-POE micelles were recovered by ultracentrifugation (100 000*g*, 1 h, 277 K). The purification was performed with ion-exchange chromatography on a Mono-Q column. Purified MOMP was dialysed against 15 m*M* sodium phosphate buffer at pH 7.4 containing 30 m*M* NaCl and 0.3%(*w*/*v*) octyl-POE. SDS–PAGE was used to determine the purity of the samples and the absence of lipopolysaccharide (LPS) molecules in the purified MOMP fractions was checked by performing a specific silver staining of polyacrylamide gels (Tsai & Frash, 1982). The protein solution was finally concentrated to about 20 mg ml^{-1} and stored at 253 K. A typical purification procedure yielded 15 mg of pure protein from 2.8 g wet cell pellets. The protein concentration was estimated by measuring the absorbance at 278 nm, employing the calculated extinction coefficient of 1.435 mg ml^{-1} cm^{-1}.

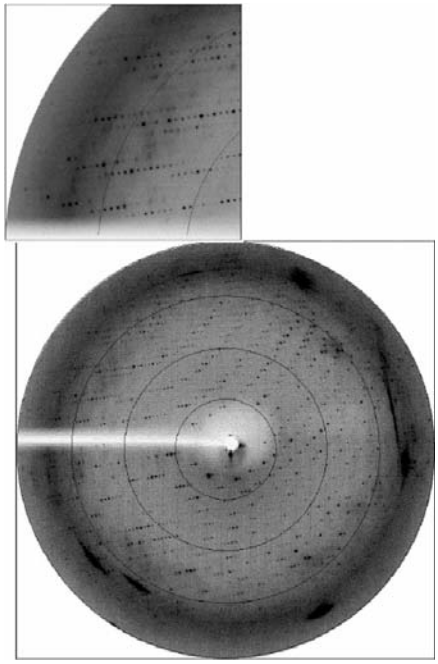

Figure 1
A typical diffraction pattern obtained at ESRF from a crystal of *C. jejuni* 85H MOMP. The 0.5° oscillation frame was exposed for 40 s on a MAR CCD detector. The diffraction limit is 3.1 Å.

2.2. Crystallization

Initial crystals of MOMP were obtained by the vapour-diffusion method in hanging drops at 290 K. The procedure used a variety of screening kits (Hampton Research, USA; Molecular Dimensions Ltd, UK). All drops were set up by mixing 1 µl MOMP stock solution and 1 µl screen solution and were equilibrated against 1 ml reservoir precipitant solution. Initial crystallization conditions were identified when 0.8–0.9%(*w*/*v*) β-D-octylglucoside (β-OG; Sigma, USA) was added to the protein stock. After optimization, form I crystals grew within 1–2 weeks to sizes of around 0.3 × 0.3 × 0.2 mm with reservoir solution containing 20%(*w*/*v*) PEG 4000 (Fluka, Germany), 150 m*M* potassium/sodium tartrate, 50 m*M* ADA buffer pH 6.7. However, these crystals were characterized by frequent twinning, high mosaic spread and weak and anisotropic diffraction. Further variation of the nature of the detergent and the addition of amphiphilic molecules or diverse additives (Ostermeier & Michel, 1997) failed to improve the crystal quality. Form II crystals were obtained after a partial exchange of the residual octyl-POE detergent using an additional dialysis of the protein stock in 50 µl microdialysis buttons against a solution composed of 10 m*M* PIPES buffer pH 6.9, 20 m*M* NaCl and 0.9%(*w*/*v*) β-OG. The crystallization conditions were 18–22%(*w*/*v*) PEG 2000, 0.2 *M* ammonium phosphate and 50 m*M* PIPES buffer pH 6.9. Single crystals appeared after a week and continued to grow to maximum dimensions of around 0.2 × 0.2 × 0.1 mm over the next 3–5 weeks.

Table 1
Data-collection and processing statistics for *C. jejuni* 85H MOMP crystals.

Values in parentheses refer to the highest resolution shell.

Space group	$P2_12_12$	$R3$
Unit-cell parameters	a = 170.1, b = 101.9, c = 104.9	$a = b$ = 94.2, c = 161.2
Wavelength (Å)	0.928	0.928
Resolution (Å)	30–3.1	25–3.9
Mosaicity (°)	0.7	1.5
Observed reflections	89601	9987
Unique reflections	21078	3661
Completeness	91.3 (84.4)	77.8 (74.9)
R_{merge} (%)	8.0 (35.0)	10.0 (34.0)
Average $I/\sigma(I)$	9.8 (2.9)	10.8 (2.2)

2.3. Data collection

The crystals were transferred to reservoir solution supplemented with 0.3%(*w*/*v*) octyl-POE detergent, 0.8%(*w*/*v*) β-OG and 10–13%(*w*/*v*) (2*R*,3*R*)-2,3-butanediol (Aldrich, Germany) as a cryoprotectant and flash-frozen in liquid nitrogen. X-ray data from single crystals of forms I and II were collected on a MAR Research CCD detector at the ESRF BM30A beamline (Roth *et al.*, 2002). The X-ray wavelength was 0.928 Å, the oscillation range was 0.5° and the crystal-to-detector distance was set to 250 mm. Diffraction images were integrated and scaled using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

Protein crystals of *C. jejuni* 85H MOMP suitable for diffraction studies were obtained and characterized. Crystal form II belongs to an orthorhombic space group and diffracts to 3.1 Å resolution (Fig. 1). Examination of the systematic absences along the three crystallographic axis established the space group to be $P2_12_12$. The unit-cell parameters are a = 170.1, b = 101.9, c = 104.9 Å. A packing analysis based on a molecular weight of 44 kDa indicates the presence of a trimer in the asymmetric unit of the orthorhombic form. This corresponds to a typical Matthews coefficient of 3.4 Å^3 Da^{-1} (Matthews, 1968) and a solvent (plus detergent) content of 64%. Crystal parameters and data-collection statistics are summarized in Table 1. The self-rotation function computed with *MOLREP* (Vagin & Teplyakov, 1997) is also consistent with a trimeric quaternary structure of this porin, as it shows one peak at κ = 120° with a relative height of 3.5σ, while the most intense twofold crystallographic peaks are 18σ.

The form I crystals of *C. jejuni* 85H MOMP diffract X-rays poorly. They were typically less ordered and frequently twinned. They diffract X-rays to about 4 Å resolution at ESRF with a strongly anisotropic pattern. A preliminary data set was collected (Table 1). Autoindexing is consistent with the trigonal space group *R*3 with hexagonal unit-cell parameters $a = b = 94.2$, $c = 161.2$ Å. Assuming the presence of one monomer per asymmetric unit, the solvent content of the crystals was 60%, corresponding to a packing parameter V_M of 3.1 Å^3 Da^{-1}. In addition, these *R*3 unit-cell parameters are similar to the two-dimensional hexagonal lattice constants (∼100 Å) found in native *C. jejuni* outer membranes (Amako *et al.*, 1996, 1997), suggesting a similar packing of MOMP into layers.

Initial calculations to solve the phase problem by molecular-replacement methods using various models of the maltoporin-like family are in progress. Because of the low sequence homology of the model structures, a search for suitable heavy-atom derivatives of this protein has also been initiated in parallel.

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