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Crystallization and preliminary X-ray diffraction analysis of P30, the transmembrane domain of pertactin, an autotransporter from *Bordetella pertussis*

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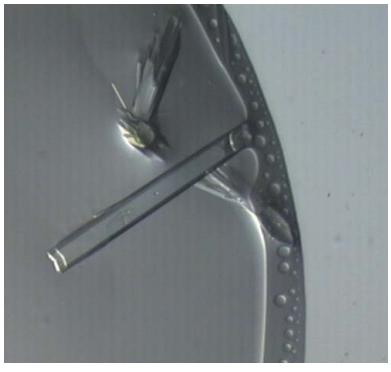
P30, the 32 kDa transmembrane C-terminal domain of pertactin from *Bordetella pertussis*, is supposed to form a β-barrel inserted into the outer membrane for the translocation of the passenger domain. P30 was cloned and expressed in inclusion bodies in *Escherichia coli*. After refolding and purification, the protein was crystallized using the sitting-drop vapour-diffusion method at 292 K. The crystals diffract to a resolution limit of 3.5 Å using synchrotron radiation and belong to the hexagonal space group $P6_122$, with unit-cell parameters $a = b = 123.27$, $c = 134.43$ Å.

1. Introduction

Proteins secreted by Gram-negative bacteria must cross the lipid bilayers of the inner and outer membranes as well as the periplasmic space containing the peptidoglycan layer. The autotransporter family of proteins is the largest family of Gram-negative bacterial virulence proteins, with functions including adherence, invasion, cytotoxicity, serum resistance, cell-to-cell spread and proteolysis (Pholner *et al.*, 1987; Henderson & Nataro, 2001; Desvaux *et al.*, 2004; Henderson *et al.*, 2004). All autotransporters are multidomain proteins consisting of an N-terminal signal peptide, an internal passenger domain that is delivered to the extracellular environment and a C-terminal translocator domain, also called the β-domain, that remains embedded in the outer membrane. The process of autotransporter secretion begins with export across the inner membrane *via* a Sec-dependent process initiated by the signal peptide. Subsequently, the translocator domain inserts into the outer membrane and is believed to adopt a β-barrel conformation, forming a pore through which the passenger domain is transported to the cell surface (Pholner *et al.*, 1987; Desvaux *et al.*, 2004; Henderson *et al.*, 2004).

In the conventional autotransporter secretion system, the C-terminal translocator domain is monomeric and relatively uniform in size, containing approximately 300 amino acids. Over the past few years, studies on the structures of the translocator domain of two prototypical autotransporters have yielded conflicting results. Veiga *et al.* (2002) carried out biochemical and electron-microscopy studies on the solubilized translocator domain of the IgA1 protease and observed a multimeric ring-like structure with a central cavity ~2 nm in diameter, suggesting that the translocation of the passenger domain takes place through the common channel. In contrast, Oomen *et al.* (2004) determined the crystal structure of the *in vitro* folded translocator domain of NalP protease and found a monomeric β-barrel consisting of 12 transmembrane β-strands and a hydrophilic central pore containing a transmembrane N-terminal α-helix. To gain insight into the molecular mechanism of autotransporter secretion, we are studying several autotransporter proteins from *Bordetella pertussis*, a strictly human pathogen responsible for whooping cough, an acute respiratory disease, particularly in young children (Locht, 1999; Parton & Roberts, 2001). *B. pertussis* produces a number of toxins and adhesins involved in its pathogenicity. One such virulence factor is pertactin, an outer membrane protein with an apparent molecular weight of 69 kDa. The structural gene, *prn*, encodes a protein of 94 kDa, which is transported into the periplasm by a classical signal peptide-dependent secretion mechanism. The



C-terminal ~32 kDa domain, P30, is essential for surface expression of pertactin and is supposed to form a β-barrel that is inserted into the outer membrane for pertactin translocation. We have determined the crystal structure of the extracellular domain of pertactin at 2.5 Å resolution and shown that it adopts a β-helix fold (Emsley *et al.*, 1996). Here, we report the purification and crystallization of P30, the translocator domain of pertactin.

2. Materials and results

2.1. Protein expression and purification

A gene with 26 residues (MGSSHHHHHHSSGLVPRGSRRAS-VHM) at the N-terminus of P30 was transformed into *Escherichia coli* pET BL21 cells. This recombinant *E. coli* was grown in Luria broth medium (Sigma) containing 30 mg l^{-1} kanamycin for 3 h. After addition of 1 m*M* isopropyl β-D-thiogalactopyranoside, the culture was grown for a further 6 h to induce the expression of His_6-tagged protein. The cells were harvested by centrifugation, resuspended in buffer *A* (50 m*M* Tris–HCl pH 7.6, 300 m*M* NaCl, 5 m*M* benzamidine) and lysed by sonication. After centrifugation, the pellet was resuspended in buffer *A* containing 1% Triton X-100, centrifuged again and washed with water to remove the detergent.

Inclusion bodies of His-tagged P30 were solubilized in buffer *A* containing 6 *M* urea. Refolding was initiated by rapid tenfold dilution of the protein from stock solution in urea into buffer *B* (50 m*M* HEPES pH 7.8, 500 m*M* sodium chloride, 1.0% LDAO). Refolding was carried out for 72 h at 277 K. The protein was subsequently dialysed against buffer *C* (50 m*M* Tris–HCl pH 7.8, 300 m*M* NaCl, 0.1% LDAO). Ni–NTA chromatography was performed on an AKTA purifier system (GE Healthcare). A linear gradient of 0–300 m*M* imidazole was used to elute the protein. P30 was further purified by gel filtration on a Superdex-200 26/60 column (Amersham Biosciences) in 50 m*M* Tris–HCl, 300 m*M* NaCl, 0.1% LDAO pH 7.8. This step was followed by cation exchange on a Mono S column (Amersham Biosciences) equilibrated in 20 m*M* HEPES, 50 m*M* NaCl, 0.1% LDAO pH 7.5, employing a linear gradient of NaCl to 0.5 *M*. In addition to LDAO, P30 was also purified using different detergents (0.6% C_8E_4, 1% β-octylglucoside and heptylglucoside) in the final cation-exhange step. The purified protein was concentrated to 40 mg ml^{-1} and flash-frozen in liquid nitrogen.

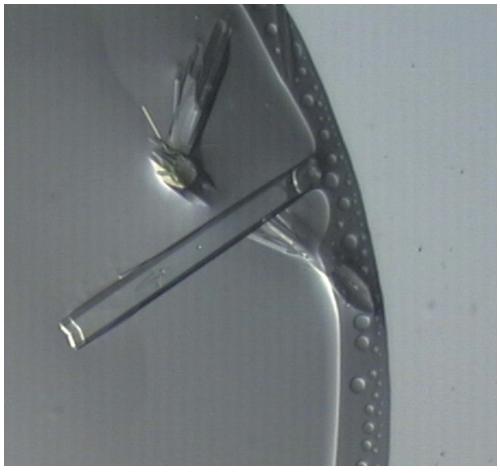


Figure 1
A P30 crystal. The longest dimension is 0.4 mm.

Table 1
Data-collection statistics for P30.

Values in parentheses are for the highest resolution shell.

Beamline	ID 14.1, ESRF
Wavelength (Å)	0.934
Resolution range (Å)	42.2–3.5 (3.63–3.50)
Crystal-to-detector distance (mm)	323.5
Space group	$P6_122$
Unit-cell parameters	$a = b = 123.27$, $c = 134.43$
Measured reflections	124268 (6124)
Unique reflections	14106 (1268)
Multiplicity	8.81 (4.83)
Completeness (%)	93.1 (78.5)
R_{merge}† (%)	13.9 (46.6)
$\langle I/\sigma(I)\rangle$	6.9 (1.8)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I_i(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$.

2.2. Crystallization

Crystallization trials of P30 purified in each of the detergents (LDAO, β-octylglucoside, heptylglucoside and C_8E_4) were set up at 292 K by mixing 1 µl protein solution and 1 µl reservoir solution from commercially available crystallization screens (Crystal Screens I and II and MemFac from Hampton Research and MemStart and Memsys from Molecular Dimensions). All crystallization experiments used the sitting-drop vapour-diffusion method in 24-well CrysChem plates (Hampton Research). Whereas crystals proved difficult to obtain with P30 purified in LDAO, β-octylglucoside and heptylglucoside, P30 purified in C_8E_4 crystallized readily under various conditions. After optimization, the best results were obtained with 25% PEG 2000 MME (in 100 m*M* MOPS pH 6.5) as precipitant (Fig. 1).

2.3. X-ray analysis

A P30 crystal picked up from a droplet was transferred to reservoir solution containing 26% PEG 2000 MME as a cryoprotectant, mounted on a LithoLoop (Molecular Dimensions) and placed directly in a cold nitrogen-gas stream at 100 K. X-ray diffraction images were collected using a Quantum CCD detector and

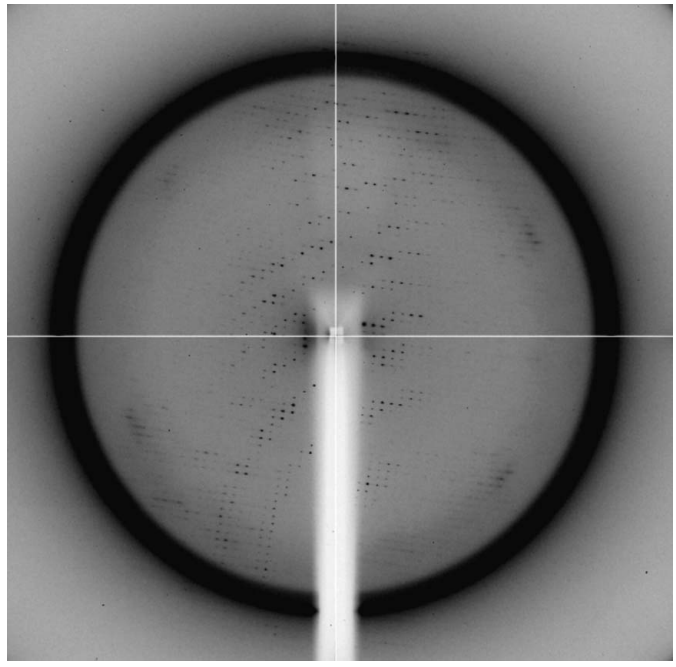


Figure 2
X-ray diffraction pattern recorded at station ID 14.1 at ESRF.

synchrotron radiation of wavelength 0.934 Å at station ID 14.1 of ESRF (Grenoble, France; Fig. 2). The crystal-to-detector distance was set to 323.5 mm and 1° oscillation images were recorded with 30 s exposure. All data were indexed, integrated and scaled using the program *d*TREK* (Pflugrath, 1999). The space group was determined to be $P6_122$ (hexagonal), with unit-cell parameters $a = b = 123.27$, $c = 134.43$ Å. The V_M value (crystal volume per unit protein molecular weight; Matthews, 1968) was calculated to be 4.4 Å^3 Da^{-1}, assuming the presence of one molecule in the asymmetric unit, with a solvent content of 72.27%, which corresponds to the typically high solvent content in outer membrane protein structures. Data-collection statistics are summarized in Table 1.

Attempts to phase the diffraction data by molecular replacement using the program *Phaser* (McCoy *et al.*, 2005) and the structure of NalP (Oomen *et al.*, 2004) with deleted loops at both ends as a search model found a solution with acceptable packing in the unit cell. However, the crystals show high anisotropy and have a high mosaic spread. Work is in progress to improve the crystal quality.

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