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Crystallization and preliminary crystallographic studies of the C-terminal domain of outer membrane protein A from enterohaemorrhagic *Escherichia coli*

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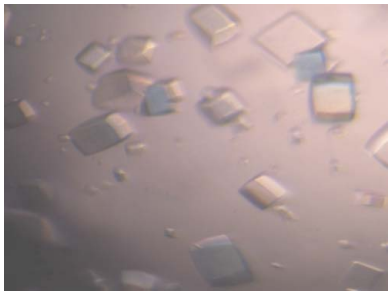
Outer membrane protein A (OmpA) of enterohaemorrhagic *Escherichia coli* (EHEC) plays multiple roles in bacterial physiology and pathogenesis, such as mediation of bacterial conjunction, maintenance of cell shape, induction of adhesion of EHEC to host cells *etc.* Better understanding of the functions of OmpA will help in the control of EHEC infections. OmpA is composed of two domains: the N-terminal domain and the C-terminal domain. The N-terminal domain is a β-barrel structure and embeds in the outer membrane of the bacterium. The structure and function of the C-terminal domain of OmpA (OmpAC) remain elusive. In this study, recombinant OmpAC from EHEC was purified and crystallized and a diffraction data set was collected to 2.7 Å resolution. The crystals belonged to space group $I4_132$, with unit-cell parameter a = 158.99 Å. The Matthews coefficient and solvent content were calculated to be 2.55 Å^3 Da^{-1} and 51.77%, respectively, for two molecules in the asymmetric unit.

1. Introduction

On colonizing the large intestine, the Gram-negative bacillus enterohaemorrhagic *Escherichia coli* (EHEC) can cause diarrhoea, haemorrhagic enteritis and even the life-threatening haemolytic uraemic syndrome (HUS) in humans and animals (Karch *et al.*, 2005). Outbreaks of EHEC infection have been reported worldwide since the pathogen was first identified in the 1980s (Riley *et al.*, 1983). The main features of EHEC that are involved in pathogenesis include the production of shiga toxin (Stx), intimate adhesion, destructive inflammation and the induction of attaching and effacing lesions (Tarr *et al.*, 2005). In contrast to other bacterial infections, the use of conventional antibiotics can enhance pathogenesis, which makes the prevention and treatment of EHEC infections problematic (Wong *et al.*, 2000).

Outer membrane protein A (OmpA) is an abundant surface protein that is highly conserved in Gram-negative bacteria (Delcour, 2002). It is located in the outer membrane of the bacterial cell and is able to mediate F-factor-dependent conjunction (Schweizer & Henning, 1977). OmpA also plays an important role in maintaining the integrity of the outer membrane and the normal shape of the bacterium (Sonntag *et al.*, 1978). In addition to the physiological functions mentioned above, OmpA from EHEC also acts as an active pathogenic component. It has been demonstrated that OmpA is able to mediate the initial adhesion of EHEC to host cells (Torres & Kaper, 2003). This protein has also been reported to be capable of stimulating the host immune response by recruiting dendritic cells to the sites at which the bacteria attach and inducing the production of proinflammatory cytokines (Torres & Kaper, 2003). Therefore, better understanding of the pathogenesis of OmpA at the molecular level will help in the control of EHEC infections.

OmpA from EHEC EDL933 contains 346 amino acids and is composed of two domains. The N-terminal domain (Met1–Pro198) has a structure consisting of an eight-stranded all-antiparallel β-barrel with long flexible loops at the external end, as determined by both X-ray diffraction (Pautsch & Schulz, 2000) and NMR (Cierpicki



et al., 2006). It embeds in the outer membrane of *E. coli* and acts as the transmembrane part of OmpA. The C-terminal domain of OmpA (OmpAC) extends from Val199 to Ala346 and is thought to interact with peptidoglycan in the periplasm to provide outer membrane stability and cell integrity.

To shed light on the structural basis of the multiple functions of OmpA, it is necessary to obtain a high-resolution atomic structure of the C-terminal domain of this bacterial protein. Here, we report the crystallization, diffraction data collection and preliminary crystallographic studies of EHEC OmpAC.

2. Materials and methods

2.1. Cloning, expression and purification

The gene encoding the OmpAC fragment (Glu209–Ala346; accession No. NP_286832) was amplified from the EHEC EDL933 genome using primers P_F (5′-CATATGGAAGTACAGACCAAGCAC-3′) and P_R (5′-CTCGAGAGCTTGCGGCTGAGTTAC-3′). It was then cloned into pET-21a(+) at *Nde*I and *Xho*I restriction sites, generating pET21a-OmpAC. A six-histidine tag was introduced into the C-terminus of the protein.

After verification by DNA sequencing, pET21a-OmpAC was transformed into *E. coli* BL21 (DE3) and transformants were selected on LB agar plates containing 100 µg ml^{-1} ampicillin. The cells were grown in LB medium at 310 K until the optical density at 600 nm (OD_{600}) reached 0.6; isopropyl β-D-1-thiogalactopyranoside (IPTG) was then added to a final concentration of 1 m*M* to induce expression of OmpAC. After 5 h induction, the cells were harvested and resuspended in buffer *A* (10 m*M* Tris, 25 m*M* NaCl, 5% glycerol pH 8.0) and homogenized by sonication. The lysates were centrifuged at 18 000*g* for 30 min at 277 K and the supernatant was loaded onto an Ni^{2+}–NTA column (Qiagen). After washing to remove contaminant proteins, the protein was eluted with buffer *A* containing 50 m*M* imidazole. For further purification, the protein was loaded onto a Mono Q (GE Healthcare) column in buffer *A* and eluted with a 1–100% gradient of buffer *B* (10 m*M* Tris, 1 *M* NaCl, 5% glycerol pH 8.0) in 120 min. A Superdex 75 10/300 (GE Healthcare) column was used for final purification of the protein in buffer *A* as described above. The purity of the protein was estimated using the *Quantity One* software based on the band density of the proteins on SDS–PAGE.

2.2. Crystallization

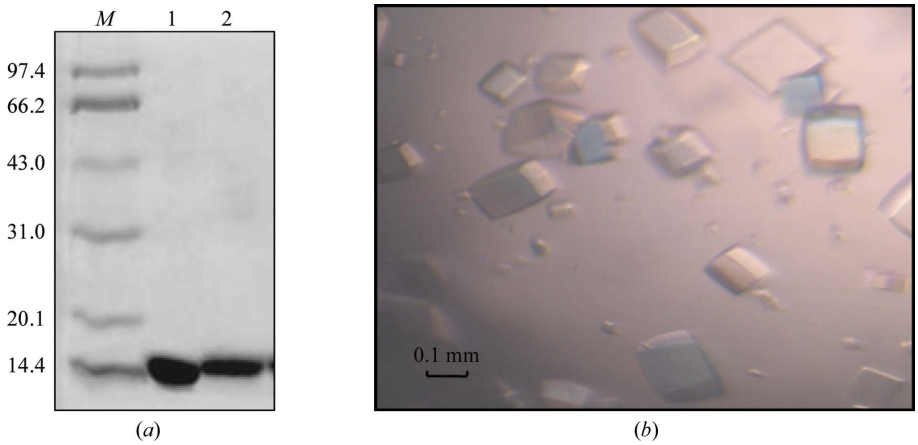
The purified protein was concentrated to approximately 10–20 mg ml^{-1} in buffer *A* as determined from the absorbance at 280 nm. Crystallization conditions were screened using Crystal Screen, Crystal Screen 2, Index Screen, PEG/Ion Screen and SaltRx from Hampton Research by the hanging-drop vapour-diffusion method. Each drop was formed by mixing equal volumes (1.5 µl) of protein solution and reservoir solution and was equilibrated against 200 µl reservoir solution at 291 K. Positive hits were then optimized by varying the type and the concentration of the precipitant, salts, buffers and organic compounds and the pH and by the use of additives.

2.3. Data collection and processing

Crystals were soaked for a few seconds in a mixture containing 70% reservoir solution and 15% glycerol, which serves as a cryoprotectant. The crystal was then mounted in a nylon loop and flash-cooled in a cold nitrogen-gas stream at 100 K using an Oxford Cryostream. Data collection was performed by the rotation method using a MAR CCD detector with on beamline BL17A at Shanghai Synchrotron Radiation Facility (SSRF). The crystal-to-detector distance and beam width were set to 230 and 25 mm, respectively. A total of 360 frames of data were collected with an oscillation angle of 1.0° and an exposure time of 4 s for each image. The data were indexed, integrated and scaled using *DENZO* and *SCALEPACK* as implemented in the *HKL*-2000 program suite (Otwinowski & Minor, 1997). The Matthews coefficient and solvent content were calculated using the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

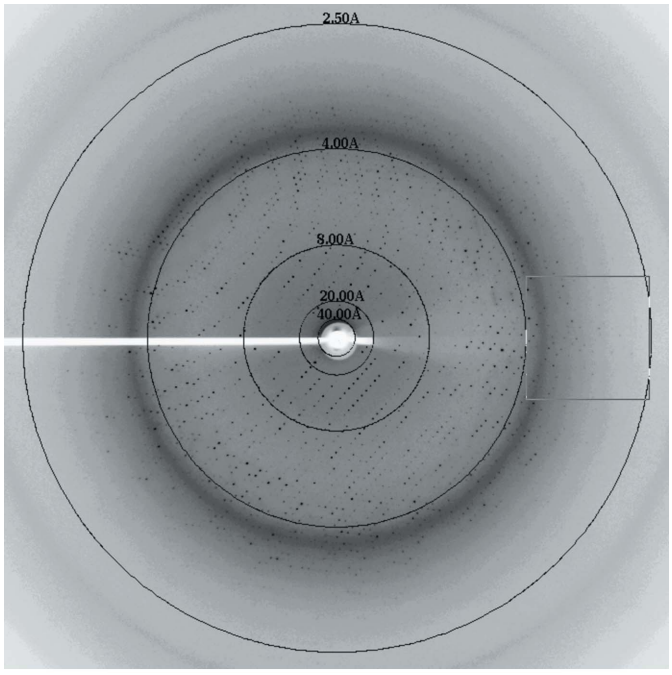
3. Results

After purification by affinity chromatography, anion-exchange chromatography and size-exclusion chromatography, the purity of OmpAC was about 95% as determined by SDS–PAGE (Fig. 1*a*). Crystallization of the protein proved to be easy: protein crystals could be obtained using several different conditions, including Crystal Screen 2 condition No. 41 (0.01 *M* $NiCl_2$, 0.1 *M* Tris pH 8.5, 1.0 *M* $LiSO_4$), PEG/Ion Screen condition No. 39 (0.2 *M* NaH_2PO_4, 20% PEG 3350 pH 4.7), PEG/Ion Screen condition No. 41 (0.2 *M* KH_2PO_4, 20% PEG 3350 pH 4.7) and Crystal Screen condition No. 20 (0.2 *M* ammonum sulfate, 25% PEG 4000, 0.1 *M* sodium acetate pH

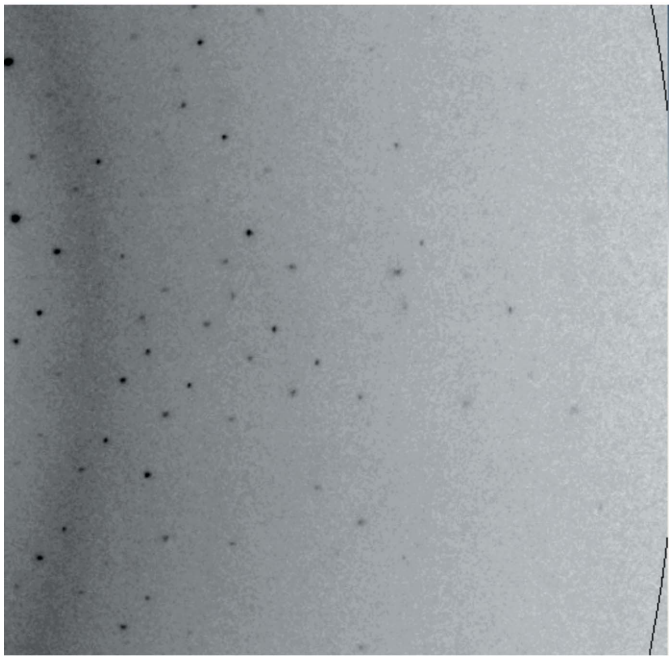


(*a*) (*b*)

Figure 1
SDS–PAGE analysis and crystallization of OmpAC. (*a*) The purified OmpAC was separated on a 12% polyacrylamide gel and stained with Coomassie blue. Lane *M*, protein molecular-weight markers (kDa); lanes 1 and 2, purified OmpAC. The purity of OmpAC was about 95% as determined using *Quantity One* software. (*b*) OmpAC crystals grown by the hanging-drop method in 0.2 *M* KH_2PO_4, 20% PEG 3350 pH 4.7, 0.01 *M* β-nicotinamide adenine dinucleotide hydrate. Typical crystal dimensions are about 0.1 × 0.1 × 0.2 mm..



(*a*)



(*b*)

Figure 2
(*a*) A typical X-ray diffraction pattern from a crystal of OmpAC. The diffraction image was collected on a MAR image-plate detector. The oscillation range is 1°. (*b*) An enlarged image of the area indicated in (*a*).

Table 1
Data-collection and processing statistics for OmpAC.

Space group	$I4_132$
Unit-cell parameters (Å, °)	$a = b = c = 158.99$, $\alpha = \beta = \gamma = 90.00$
Resolution (Å)	2.70
Wavelength (Å)	0.97924
Observed reflections	283679
Unique reflections	9710
Completeness (%)	100.0 (100.0)
$I/\sigma(I)$	52.4 (11.0)
$R_{\rm merge}$† (%)	8.5 (43.0)
Multiplicity	32.4 (31.4)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean of the observations $I_i(hkl)$ of reflection hkl.

4.6). After optimization, high-quality crystals were obtained using 0.2 *M* KH_2PO_4, 20% PEG 3350 pH 4.7, 0.01 *M* β-nicotinamide adenine dinucleotide hydrate. As shown in Fig. 1(*b*), the typical crystal dimensions are about 0.1 × 0.1 × 0.2 mm. Diffraction data were collected to 2.7 Å resolution from a single crystal (Fig. 2). The crystals belonged to space group $I4_132$, with unit-cell parameter $a = 158.99$ Å. The data-collection statistics are summarized in Table 1. Based on the molecular weight of the protein and the unit-cell volume as determined from the unit-cell dimensions, the Matthews coefficient and solvent content were calculated to be 2.55 $Å^3\ Da^{-1}$ and 51.77%, respectively, for two molecules in the asymmetric unit. Research is in progress to determine the three-dimensional structure of EHEC OmpAC.

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