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Crystallization and preliminary X-ray diffraction studies of the C-terminal domain of *Chlamydia trachomatis* CdsD

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The inner membrane ring of the bacterial type III secretion system (TTSS) is composed of two proteins. In *Chlamydia trachomatis* this ring is formed by CdsD (gene name *CT_664*) and CdsJ (gene name *CTA_0609*). CdsD consists of 829 amino acids. The last 400 amino acids at its C-terminal end relate it to the type III secretion system YscD/HrpQ protein family. The C-terminal domain, consisting of amino acids 558–771, of *C. trachomatis* CdsD was overexpressed in *Escherichia coli* and purified using immobilized metal-affinity chromatography (IMAC) and size-exclusion chromatography. The protein was crystallized using the vapour-diffusion method. A data set was collected to 2.26 Å resolution. The crystals have the symmetry of space group *C*2, with unit-cell parameters $a = 106.60$, $b = 23.91$, $c = 118.65$ Å, $\beta = 104.95^\circ$. According to the data analysis there is expected to be one molecule in the asymmetric unit, with a Matthews coefficient of 3.0 Å^3 Da^{-1}.

1. Introduction

Chlamydia trachomatis is a Gram-negative obligate intracellular bacterium and is the most common sexually transmitted pathogen. *C. trachomatis* infection can cause, for example, blindness and urogenital tract infections. Prolonged uterine infections can lead to ectopic pregnancies and infertility (reviewed by Bébéar & de Barbeyrac, 2009). Like many other pathogenic Gram-negative bacteria, *C. trachomatis* also utilizes the type III secretion system (Peters *et al.*, 2007) to deliver its virulence proteins directly into the host cells.

C. trachomatis CdsD is a structural protein of the inner membrane ring of the type III secretion system (TTSS). It is an 89 kDa protein with one predicted transmembrane domain corresponding to amino acids 530–552. It also has two forkhead-associated domains (FHA domains; residues 24–75 and 405–457) and a C-terminal BON domain (residues 691–754) (Fig. 1). CdsD forms higher oligomers which are mediated by disulfide bridges and the appearance of these complexes is strongly linked to the development cycle of the bacterium (Betts-Hampikian & Fields, 2011). CdsD is a putative substrate for the *C. trachomatis* protein kinase PknD (Johnson & Mahony, 2007).

The structure of one of the FHA domains of CdsD (residues 380–485) has previously been solved (PDB entry 3gqs; Midwest Center for Structural Genomics, unpublished work). In this study, we have crystallized the C-terminal fragment consisting of amino acids 558–771. This fragment shares comparatively low sequence identity with the previously described structures of the C-terminal domains of YscD of *Yersinia enterocolitica* (PDB entry 4alz) and PrgH of *Salmonella enterica* (PDB entry 4g1i) (Kudryashev *et al.*, 2013; Spreter *et al.*, 2009), with 18 and 8% sequence identity, respectively. The fragment that we have crystallized includes the BON domain (residues 691–754; Fig. 1), which is a putative membrane-binding domain (Yeats & Bateman, 2003). The location of the crystallized fragment in the inner membrane ring is expected to be in the periplasmic space.

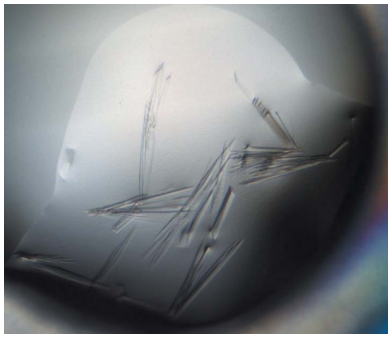

Table 1
Production information for CdsD(558–771).

Source organism	*C. trachomatis*
DNA source	Synthetic gene (GenScript)
Expression vector	pET-28a (Novagen)
Cloning site	*Nde*I–*Bam*HI
Additional residues	N-terminal His tag, thrombin cleavage site
Expression host	*E. coli* C41(DE3)
UniProtKB entry	O84671
Residues included in the construct	558–771

2. Materials and methods

2.1. Macromolecule production

The synthetic codon-optimized gene encoding amino acids 558–771 of CdsD of *C. trachomatis*, cloned into the expression vector pET-28a (Novagen), was obtained from GenScript (Table 1). The proper length of the construct with minimal degradation was defined experimentally, initially using a longer construct which was found to be proteolytically degraded into a smaller fragment that was characterized by mass spectrometry (data not shown). For protein expression the plasmid was transformed into *E. coli* strain C41(DE3). An initial culture of 20 ml LB broth supplemented with 60 µg ml^{-1} kanamycin and 1%(*w*/*v*) glucose was started up with inoculation of one colony. This culture was grown overnight at 37°C for 16 h with shaking at 200 rev min^{-1}. The next morning this culture was transferred into 4 l M9ZB medium (Studier *et al.*, 1990) supplemented with 60 µg ml^{-1} kanamycin. Cells were grown at 37°C and 200 rev min^{-1} until the OD_{600} reached 0.6 and were subsequently induced with 400 µ*M* isopropyl *β*-D-1-thiogalactopyranoside for 3 h at 37°C and 200 rev min^{-1}. The cells were collected and suspended in 200 ml lysis buffer [50 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 5%(*v*/*v*) glycerol] and frozen at −20°C.

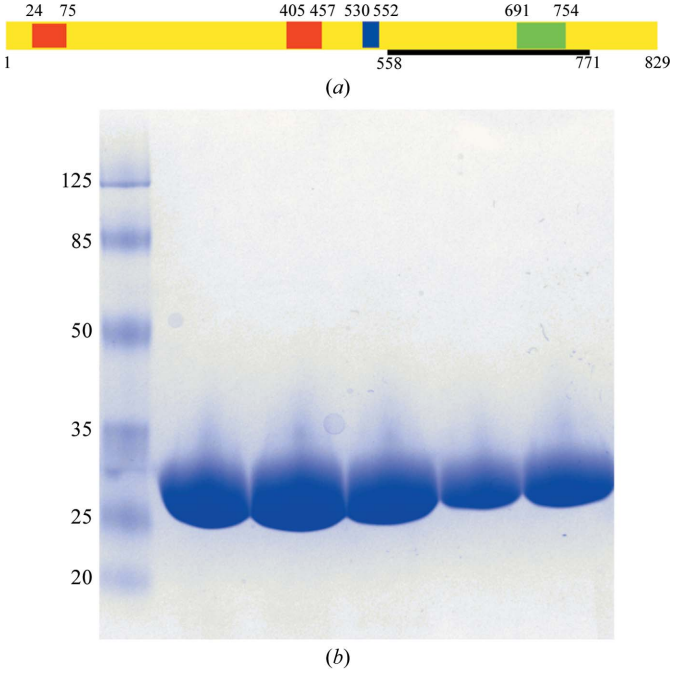

Figure 1
The CdsD(558–771) construct. (*a*) A schematic presentation of the domain structure of full-length CdsD. The two FHA domains are coloured red, the predicted transmembrane domain is shown in blue and the BON domain is shown in green. The black bar visualizes the domain that was crystallized. Residue numbers are presented at the beginning and the end of the corresponding sequences. (*b*) A Coomassie Blue-stained 4–20% Mini-Protean TGX Gel (Bio-Rad) of the purified CdsD(558–771) (24 kDa). The protein moves slightly more slowly than expected from its molecular weight compared with the molecular-weight standard (labelled in kDa).

Table 2
Crystallization.

Method	Sitting-drop vapour diffusion
Plate type	Corning 3556
Temperature (K)	295
Protein concentration (mg ml^{-1})	4.2
Buffer composition of protein solution	10 m*M* Tris–HCl pH 7.5
Composition of reservoir solution	50 m*M* MES pH 6.0, 3% PEG 4000, 15% 2-propanol, 50 m*M* $MgCl_2$
Volume and ratio of drop	750 nl, 2:1 protein:well solution
Volume of reservoir (µl)	80

The cell suspension was thawed at room temperature and lysed by sonication. Cell remnants were removed by centrifugation and the protein was purified using a 2 ml Ni–NTA (Qiagen) column. The N-terminal His tag was removed using the Thrombin CleanCleave kit (Sigma–Aldrich). Purification was finalized by size-exclusion chromatography using a HiLoad 16/600 Superdex 75 PG column (GE Healthcare) using 10 m*M* Tris–HCl pH 8.0, 250 m*M* NaCl, 1 m*M* DTT as a buffer. The sample quality was assessed by SDS–PAGE analysis (Fig. 1).

2.2. Crystallization

The protein buffer was changed to 10 m*M* Tris–HCl pH 7.5 prior to crystallization and the protein was concentrated to 4.2 mg ml^{-1} as determined by measuring the absorbance at 280 nm. Initial crystallization conditions were screened using the ProPlex screen (Molecular Dimensions). Crystallizations were performed in 96-well plates using the sitting-drop vapour-diffusion technique. As the screen suggested many possible good crystallization conditions, several rounds of optimization were performed. The optimizations were performed by mixing 500 nl protein solution with 250 nl well solution and incubation at 295 K over 80 µl well solution consisting of 100 m*M* MES pH 6.0, 3% PEG 4000, 15% 2-propanol, 50 m*M* $MgCl_2$ (Table 2).

2.3. Data collection and processing

Crystals were flash-cooled in liquid nitrogen using mother liquor supplemented with 10% 2-methyl-2,4-pentanediol (MPD) as a cryo-

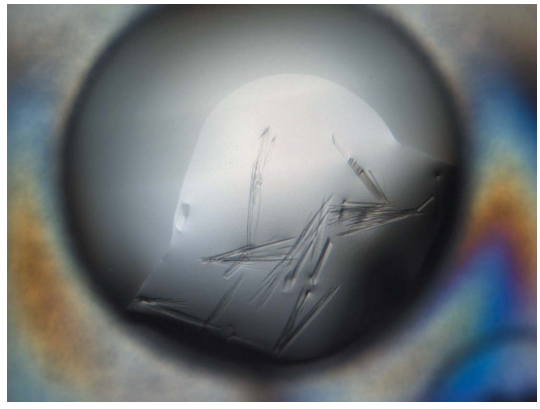

Figure 2
Crystallization of CdsD(558–771). The image was taken by a Rock Imager 54 (Formulatrix) 12 h after setting up the drop and no remarkable change was subsequently observed. The typical crystal size was about 400 × 10 × 5 µm. Owing to the high percentage of 2-propanol the crystals are rather fragile and difficult to handle. The data were collected from a fragment of a crystal similar to that shown here.

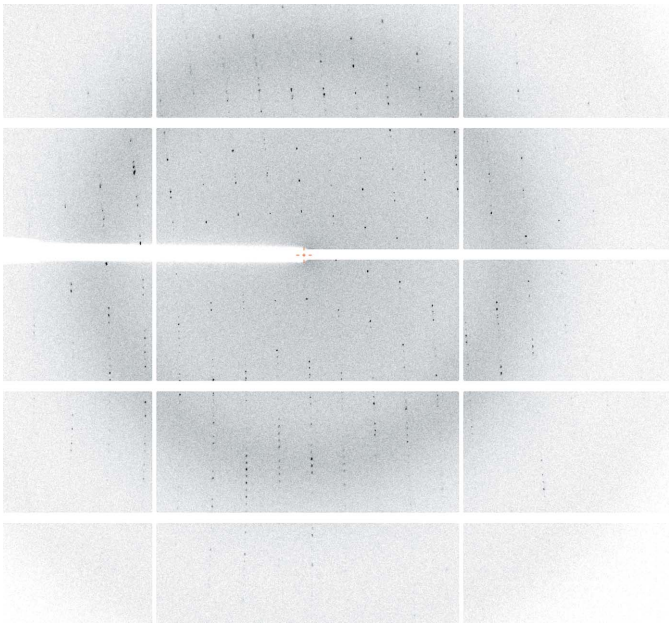

Figure 3
X-ray diffraction pattern measured from a *C. trachomatis* CdsD(558–771) crystal. The oscillation angle was 0.5°. Data were collected on beamline I04-1 at Diamond Light Source using a PILATUS detector. The reflections at the outer edge of the detector correspond to 2.26 Å resolution.

protectant. Data were collected at 100 K on beamline I04-1 at Diamond Light Source, UK. Data were processed, integrated and scaled using the *XDS* package (Kabsch, 2010; Table 3).

3. Results and discussion

The CdsD domain (residues 558–771) was purified as described in §2 (Fig. 1). Initial crystallization screens using the ProPlex screen from Molecular Dimensions gave several good conditions which were further optimized until the optimal crystallization condition was found. The initial small crystals appeared in less than 30 min and stopped growing after 12–24 h (Fig. 2).

For cryoprotection, the crystal was transferred to a new drop consisting of well solution supplemented with 10% MPD. After a 2 min soak, the protein was flash-cooled in liquid nitrogen. A 360° data set with 0.5° oscillation was collected from a single crystal on beamline I04-1 at Diamond Light Source (Fig. 3). The crystal diffracted to 2.26 Å resolution and the data were processed with *XDS* (Kabsch, 2010). The crystal belonged to space group *C*2, with unit-cell parameters a = 106.60, b = 23.91, c = 118.65 Å, β = 104.95°. The calculated Matthews coefficient V_M (Matthews, 1968) was found to be 3.0 Å^3 Da^{-1}, corresponding to a solvent content of 59.6% for one monomer per asymmetric unit.

Table 3
Data-collection and processing statistics.

Values in parentheses are for the outer shell.

Diffraction source	I04-1, Diamond Light Source
Wavelength (Å)	0.9
Temperature (K)	100
Detector	PILATUS
Crystal-to-detector distance (mm)	221.15
Rotation range per image (°)	0.5
Total rotation range (°)	360
Space group	*C*2
a, b, c (Å)	106.60, 23.91, 118.65
α, β, γ (°)	90.00, 104.95, 90.00
Mosaicity (°)	0.4
Resolution range (Å)	28.66–2.26 (2.36–2.26)
Total No. of reflections	91714 (10339)
No. of unique reflections	13969 (1589)
Completeness (%)	97.6 (92.2)
Multiplicity	6.6 (6.5)
$\langle I/\sigma(I)\rangle$	11.8 (2.5)
R_{meas} (%)	13.7 (89.1)
Overall B factor from Wilson plot (Å^2)	40.4

Currently, there are no suitable models that could be used to solve the structure by the molecular-replacement method. We are aiming to solve the structure using the single-wavelength anomalous dispersion (SAD) method. Initial phases have been obtained from a lead derivative.

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