Protein expression, crystallization and preliminary
X-ray crystallographic studies of LidA from
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LidA, a translocated substrate of the *Legionella pneumophila* Dot/Icm type IV secretion system, is associated with maintenance of bacterial integrity and interferes with the early secretory pathway. However, the precise mechanism of LidA in these processes remains elusive. To further investigate the structure and function of LidA, the full-length protein was successfully expressed in *Escherichia coli* and purified. LidA was crystallized using sitting-drop vapour diffusion and diffracted to a resolution of 2.75 Å. The crystal belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 57.5$, $b = 64.5$, $c = 167.3$ Å, $\alpha = \beta = \gamma = 90^\circ$. There is one molecule per asymmetric unit.

1. Introduction

Legionella pneumophila, the causative agent of legionnaires' disease, is an intracellular Gram-negative pathogen (Fraser *et al.*, 1977). After uptake into macrophages, *L. pneumophila* is internalized into a membrane-bound vacuole that bypasses the endocytic pathway and escapes fusion with the host-cell lysosomal network (Horwitz, 1983). The *L. pneumophila*-containing vacuole (LCV) recruits early secretory ER-derived vesicles and the mature LCV is also integrated into the early secretory pathway to facilitate this process. Within the specialized ER-like vacuole, *L. pneumophila* grows and replicates to a high density; the large numbers of bacteria then kill the host cell by lysing the host cell membrane and initiate a new round of infection.

The *L. pneumophila* Dot/Icm type IV secretion system is crucial for intracellular bacterial growth and replication-vacuole formation. This apparatus also translocates bacterial effector molecules into the host cell (Christie & Vogel, 2000). These translocated proteins are thought to allow establishment of the replication vacuole, bypass of entry into the endocytic pathway and targeting of *L. pneumophila* into an ER-bound compartment (Conover *et al.*, 2003), but their exact functions have mostly remained unknown.

LidA is one of the translocated substrates of the *L. pneumophila* type IV secretion system. Prior to uptake into macrophages, LidA protein is found in the bacterial cytoplasm and contacts the Dot/Icm translocator to intercept other translocated substrates. After uptake into macrophages, LidA is translocated immediately and is then localized on the cytoplasmic face of the phagosome (Conover *et al.*, 2003), which is necessary for LCV formation. In addition, LidA is a membrane-associated protein and associates with the cytoplasmic surface of the replicative vacuole, the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) and the Golgi apparatus to recruit early secretory vesicles for maintenance of the replication vacuole; therefore, LidA is necessary for intracellular growth during infection. The coiled-coil region of LidA targets the protein to the ERGIC and the Golgi. When LidA is overexpressed, it disrupts the ERGIC and the Golgi and interferes with the early secretory pathway (Derré & Isberg, 2005). LidA collaborates with another translocated substrate of the *L. pneumophila* type IV secretion system, SidM; the two proteins synergize to interact with Rab1, a small GTPase that regulates ER-to-Golgi traffic and collaborates in the recruitment of early secretory vesicles to the surface of LCV (Machner & Isberg, 2006). However, the precise mechanism of LidA in all of these pro-

cesses remains elusive. In this study, we have cloned, purified and crystallized the LidA protein and obtained preliminary X-ray crystallographic data for LidA.

2. Methods

2.1. Protein expression and purification

The *lidA* gene was amplified by PCR and cloned into pET28a expression vector to generate a recombinant protein that contained a 6×His tag at the C-terminus. The sequence was confirmed by DNA sequencing.

The recombinant protein was expressed in *Escherichia coli* strain BL21 (DE3). Cells were first cultured in 200 ml LB broth medium with 50 µg ml⁻¹ kanamycin overnight at 310 K and 210 rev min⁻¹ and were then transferred into 4 l LB medium and incubated until the OD₆₀₀ reached 0.6. 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce expression of LidA for 6 h at 303 K. The cells were collected by centrifugation at 5000 rev min⁻¹ for 15 min at 277 K and the pellet was suspended in binding buffer consisting of 50 mM HEPES pH 7.5, 500 mM NaCl and 5 mM imidazole. The cells were sonicated and the lysate was centrifuged at 38 900g for 30 min at 277 K. The supernatant was collected and filtered through a 0.22 µm filter. The LidA protein was purified by a two-step FPLC procedure consisting of Ni²⁺-affinity chromatography followed by gel-filtration chromatography (Superdex 200 size-exclusion column, GE Healthcare, Sweden) following a previously described procedure (Bai *et al.*, 2010). Selenium-labelled LidA protein was overexpressed in *E. coli* strain B834 (DE3) as described previously (Meng *et al.*, 2010) and purified using the procedure described above.

2.2. Protein crystallization

The purified LidA protein was concentrated to about 16 mg ml⁻¹ using a Centricon Plus-10 centrifugal filter device (Millipore, USA) and centrifuged at 17 968g for 15 min at 277 K prior to crystallization setup. Crystallization was performed at 293 K using the sitting-drop vapour-diffusion method.

Crystal Screen, Crystal Screen 2, Index, PEG/Ion and Natrix kits (Hampton Research, Riverside, California, USA) were used for

initial screening. Each drop consisted of 1 µl protein solution mixed with 1 µl reservoir solution and was equilibrated against 150 µl reservoir solution.

2.3. Diffraction data collection

For data collection, crystals were transferred into cryoprotectant solution consisting of 20% glycerol and the corresponding reservoir solution. The crystal was maintained at 100 K using nitrogen gas. X-ray diffraction data were collected on a MAR 225 image-plate detector on beamline BL17U1 at Shanghai Synchrotron Radiation Facility. The crystal-to-detector distance was 250 mm; a φ scan was performed with 1° oscillation angle per frame. 720 frames were collected; the exposure time per frame was 1 s. Data were processed with *HKL-2000* (Otwinowski & Minor, 1997) and *CCP4* (Winn *et al.*, 2011).

Figure 2
Crystals of full-length LidA obtained using 200 mM trimethylamine *N*-oxide pH 8.0, 20%(w/v) PEG 2000 and 1% glycerol.

Figure 1
SDS-PAGE analysis of purified LidA. Lane M, molecular-weight markers (kDa); lane 1, full-length purified LidA.

Figure 3
Diffraction pattern of the LidA crystal. The crystal diffracted to a resolution of 2.75 Å.

Table 1

Crystallographic parameters and data-collection statistics.

Values in parentheses are for the last resolution shell.

Wavelength (Å)	0.9791
Resolution (Å)	30–2.75 (2.84–2.75)
Completeness (%)	90.7 (58.6)
$R_{\text{merge}}^{\dagger}$ (%)	13.4 (59.4)
$\langle I/\sigma(I) \rangle$	24.7 (1.5)
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 57.5$, $b = 64.5$, $c = 168.3$
No. of observed reflections	15410
No. of unique reflections	1467
Molecules in asymmetric unit	1
V_M (Å ³ Da ^{−1})	1.95
Solvent content (%)	37

$$^{\dagger} R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}.$$

3. Results and discussion

The final yield of purified full-length LidA protein was about 2 mg per litre of culture, with a purity of about 90% (Fig. 1). SeMet LidA protein expressed from *E. coli* strain B834 (DE3) had a yield of only about 0.5 mg per litre of culture, with a purity of about 90%. The purified LidA protein was concentrated to about 16 mg ml^{−1} for crystallization.

LidA microcrystals were obtained in many initial conditions. Crystal optimization was performed by fine-tuning the pH, changing the protein concentration and the use of various additives [trimethylamine *N*-oxide (Sigma), MPD, glycerol and CaCl₂]. After optimization, the best crystal (with dimensions of 200 × 65 × 70 μm) was obtained using 200 mM trimethylamine *N*-oxide pH 8.0, 20% (w/v) PEG 2000 and 1% glycerol (Fig. 2). An SeMet-substituted protein crystal was obtained using 25% (w/v) PEG 2000 MME pH 8.0.

The LidA crystal diffracted to 2.75 Å resolution (Fig. 3) and belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 57.5$, $b = 64.5$, $c = 167.3$ Å, $\alpha = \beta = \gamma = 90^\circ$. There is only one molecule in each asymmetric unit, with a corresponding V_M value of 1.95 Å³ Da^{−1} and a solvent content of 37%. The crystallographic parameters and data-collection statistics are listed in Table 1. SeMet-labelled LidA was crystallized but did not diffract to sufficiently high resolution; crystal optimization of SeMet-labelled LidA is currently in progress. The structure of LidA will be reported in the future.

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References

- Bai, X., Meng, G., Li, G., Luo, M. & Zheng, X. (2010). *Acta Cryst.* **F66**, 73–75.
 Christie, P. J. & Vogel, J. P. (2000). *Trends Microbiol.* **8**, 354–360.
 Conover, G. M., Derré, I., Vogel, J. P. & Isberg, R. R. (2003). *Mol. Microbiol.* **48**, 305–321.
 Derré, I. & Isberg, R. R. (2005). *Infect. Immun.* **73**, 4370–4380.
 Fraser, D. W., Tsai, T. R., Orenstein, W., Parkin, W. E., Beecham, H. J., Sharrar, R. G., Harris, J., Mallison, G. F., Martin, S. M., McDade, J. E., Shepard, C. C. & Brachman, P. S. (1977). *N. Engl. J. Med.* **297**, 1189–1197.
 Horwitz, M. A. (1983). *J. Exp. Med.* **158**, 1319–1331.
 Machner, M. P. & Isberg, R. R. (2006). *Dev. Cell.* **11**, 47–56.
 Meng, G., Zhao, Y., Bai, X., Liu, Y., Green, T. J., Luo, M. & Zheng, X. (2010). *J. Biol. Chem.* **285**, 39898–39904.
 Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
 Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.