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Crystallization and preliminary crystallographic study of the outer-membrane lipoprotein receptor LolB, a member of the lipoprotein localization factors

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The Lol system mediates the translocation of the water-insoluble outer-membrane lipoprotein across the periplasm of Gram-negative bacteria depending on the sorting signal. The outer-membrane lipoprotein receptor LolB (21.2 kDa) is a member of the Lol system. A soluble mutant of LolB (mLolB) from *Escherichia coli* was crystallized in two forms. Monoclinic crystals diffract X-rays to 1.9 Å resolution and belong to space group $P2_1$, with unit-cell parameters $a = 37.2$, $b = 112.4$, $c = 47.8$ Å, $\beta = 111.4^\circ$. The V_M value is most likely to be 2.2 Å^3 Da^{-1}, assuming the presence of two molecules in the asymmetric unit. Hexagonal crystals diffract X-rays to 2.2 Å resolution and belong to space group $P6_322$, with unit-cell parameters $a = b = 71.4$, $c = 133.9$ Å. The V_M value is determined as 2.3 Å^3 Da^{-1}, assuming a single molecule in the asymmetric unit. A four-wavelength data set was collected from a monoclinic crystal of selenomethionylated mLolB in order to perform MAD phasing. The quality of the initial electron-density map was sufficient to build a molecular model.

1. Introduction

Escherichia coli has more than 90 species of lipoproteins that have covalently bound lipids (Masuda *et al.*, 2002; Terada *et al.*, 2001). These lipoproteins are each synthesized as a precursor with a signal peptide sequence at the N-terminus. The lipoproteins become a mature form on the periplasmic side of the inner membrane. The signal peptide is cleaved and the resultant N-terminal cysteine residue is modified by lipids (Sankaran & Wu, 1994). Two acyl chains are connected to the side chain of the N-terminal cysteine *via* glycerol and one is connected to the amide of the cysteine (Sankaran & Wu, 1994). Localization of the lipoprotein is determined by the residue next to the N-terminal cysteine. Lipoproteins having aspartic acid next to the N-terminal position are localized in the inner membrane; all others are localized in the outer membrane (Yamaguchi *et al.*, 1988).

The Lol system mediates the translocation of the insoluble outer-membrane lipoproteins. The Lol system is composed of a periplasmic chaperone LolA, an outer-membrane receptor LolB and the LolCDE complex, which belongs to an ATP-binding cassette (ABC) transporter family (Yakushi *et al.*, 2000). The LolCDE complex recognizes the sorting signal and releases the outer-membrane lipoprotein from the outside of the inner membrane using energy from ATP hydrolysis (Yakushi *et al.*, 2000). The released outer-membrane lipoprotein forms a 1:1 complex with LolA (Matsuyama *et al.*, 1995). The outer-membrane lipoprotein is accepted by LolB and is subsequently incorporated into the periplasmic side of the outer membrane (Matsuyama *et al.*, 1997). Depletion of LolB factors is lethal to *E. coli* (Tanaka *et al.*, 2001). Although homologues of Lol factors have been widely discovered in Gram-negative bacteria, no structural information is yet available.

We report here the crystallization and preliminary X-ray crystallographic analysis of LolB from *E. coli*.

2. Expression and purification of a water-soluble mutant of LolB

Because the outer-membrane receptor LolB is an insoluble lipoprotein, a soluble mutant LolB (mLolB; Cys1→Ala) was used for crystallization. The N-terminus of the mutant is not modified by hydrophobic acyl chains. mLolB was overexpressed and purified as described previously (Matsuyama *et al.*, 1997). The plasmid (pYKT102) encoding mLolB with its signal sequence was transformed in *E. coli* strain MC4100. The cells were grown in LB medium at 310 K. Expression of the gene was induced with 1 m*M* isopropyl-D-thiogalactoside. Harvested cells were converted to spheroplasts by treatment with lysozyme and EDTA (Osborn & Munson, 1974). The spheroplasts were removed by centrifugation at 10 000*g* for 10 min. The resulting supernatant was further centrifuged at 100 000*g* for

30 min to remove insoluble materials and concentrated (Centriprep YM-10, Millipore) and then dialyzed against 25 m*M* sodium acetate pH 5.0. The fraction was applied to a MonoS 10/10 column (Amersham Pharmacia Biotech) which had been equilibrated with 25 m*M* sodium acetate pH 5.0. The column was eluted at a flow rate of 4 ml min^{-1} with a linear gradient of sodium chloride (0–300 m*M*). mLolB was further purified using a MonoQ 10/10 column (Amersham Pharmacia Biotech) that had been equilibrated with 25 m*M* Tris–HCl pH 8.0 and developed with a linear gradient of NaCl (0–300 m*M*). The eluted mLolB was stored in a freezer at 193 K. Stored mLolB was reapplied to the MonoS 10/10 column just prior to crystallization. The column was equilibrated with 25 m*M* sodium acetate pH 5.0 and eluted at a flow rate of 1.0 ml min^{-1} with a linear gradient of sodium chloride. The purified protein was desalted and concentrated (with Centricon YM-10, Millipore) to ∼20 mg ml^{-1} in 10 m*M* Tris–HCl buffer pH 8.0.

Selenomethionyl mLolB was overexpressed in *E. coli* strain P4X8 (Hfr P4X λ$^-$ *met*B*1*). Cells were grown in minimal medium containing selenomethionine at 310 K. Purification was performed using the same method as that for non-selenomethionylated mLolB. The substitution ratio of the five methionine residues was determined by matrix-assisted laser desorption ionization mass spectrometry (MALDI–MS; PerSeptive Biosystems, Voyager-DE RP). Solutions of selenomethionylated and non-selenomethionylated mLolB were desalted using the Ziptip C4 (Millipore) for MALDI-MS experiments. 1.0 µl of protein solution was mixed with 4.0 µl of a solution containing 10 mg ml^{-1} sinapinic acid, 0.1% trifluoroacetic acid and 30% acetonitrile. The mixed solutions were dried on a gold-coated sample plate. 128 scans were averaged for each sample. The MALDI–MS experiments showed that on average 85% of the methionine residues in mLolB were substituted with selenomethionine.

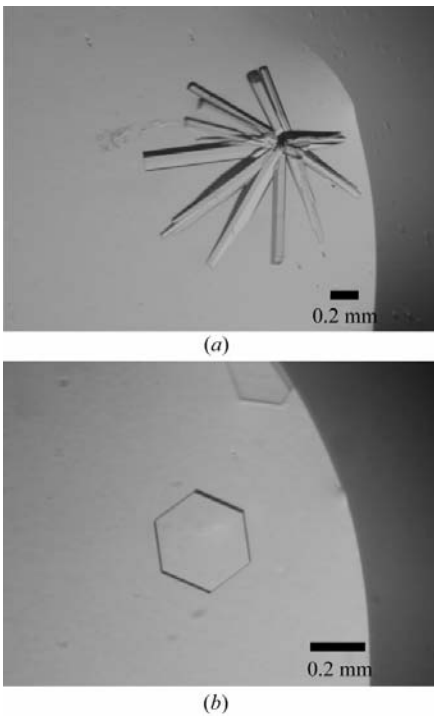

Figure 1
Crystals of mLolB. The scale bar in the photographs indicates 0.2 mm. (*a*) Monoclinic ($P2_1$) form. (*b*) Hexagonal ($P6_322$) form.

3. Crystallization

Crystallization was carried out by the sitting-drop vapour-diffusion method using 24-well culture plates. The initial crystallization trials were performed using sparse-matrix kits (Crystal Screens I, II and Cryo, Hampton Research). 1.0 µl protein solution was mixed with equal volumes of reservoir solution on a microbridge (Hampton Research). The drop was equilibrated against 200 µl reservoir solution. Rod-shaped monoclinic crystals with average dimensions of 0.5 × 0.1 × 0.1 mm were obtained in 3 d using Crystal Screen II solution No. 13 containing 30% polyethylene glycol 2000 monomethyl ether (PEG MME 2000), 0.1 m*M* sodium acetate pH 4.6 and 200 m*M* ammonium sulfate (Fig. 1*a*). Another crystal form was obtained by adding 150 m*M* sodium iodide to the above crystallization condition. Thin hexagonal crystals with dimensions of 0.02 × 0.2 × 0.2 mm grew in one week (Fig. 1*b*). Monoclinic crystals of selenomethionylated protein were obtained in 30% PEG MME 2000, 0.1 m*M* sodium acetate pH 4.6 and 200 m*M* caesium sulfate at 293 K.

4. X-ray data collection

For diffraction measurements at cryogenic temperature, all crystals were picked up in nylon loops (Hampton Research) and rapidly cooled in liquid ethane without any cryoprotectants. The native data from the monoclinic crystal were collected by the rotation method with an oscillation angle of 2.0° at beamline BL38B1 at SPring-8 using an ADSC Quantum 4R detector at 100 K. Annealing of the frozen crystal improved the reflection quality (Yeh & Hol, 1998). The wavelength of the incident X-ray was 0.998 Å and the crystal-to-detector distance was 150 mm. The crystallographic data and processing statistics are shown in Table 1. Assuming two mLolB molecules in the asymmetric unit, the Matthews coefficient V_M was calculated to be 2.2 Å^3 Da^{-1} (the corresponding solvent content is 44%), which is consistent with those for protein crystals (Matthews, 1968).

Native data from the hexagonal crystal were collected using the rotation method with an oscillation angle of 2.0° at beamline BL44B2 at SPring-8 using a MAR CCD165 detector at 90 K. The wavelength of the incident X-rays was 1.1 Å. The crystal-to-detector distance was 140 mm. The crystallographic data and processing statistics are also given in Table 1. Assuming one mLolB molecule in the asymmetric unit, the Matthews coefficient V_M was calculated to be 2.3 Å^3 Da^{-1}, which corresponds to a solvent content of 47%.

Table 1
Crystallographic data statistics for mLolB.

Values in parentheses refer to the highest resolution shell.

	Monoclinic	Hexagonal
Space group	$P2_1$	$P6_322$
Unit-cell parameters		
a (Å)	37.2	71.4
b (Å)	112.4	71.4
c (Å)	47.8	133.9
β (°)	111.4	
Resolution range (Å)	30.0–1.90 (1.97–1.90)	30.0–2.20 (2.28–2.20)
Observed reflections	87187	211377
Unique reflections	28181	10647
Redundancy	3.1	19.9
Completeness (%)	97.9 (95.0)	98.2 (93.4)
R_{sym}† (%)	6.2 (27.9)	7.3 (25.3)

† $R_{sym} = \sum_{hkl}\sum_i |I_{hkl,i} - \langle I_{hkl}\rangle|/\sum_{hkl}\sum_i I_{hkl,i}$.

Table 2
Crystallographic data and statistics for selenomethionylated mLolB.

Values in parentheses refer to the highest resolution shell.

	Low remote	Edge	Peak	High remote
Wavelength (Å)	1.0500	0.9804	0.9798	0.9200
Space group	$P2_1$			
Unit-cell parameters				
a (Å)	37.3			
b (Å)	112.5			
c (Å)	47.5			
β (°)	110.6			
Resolution range (Å)	30.0–2.50 (2.59–2.50)			
Observed reflections	72837	75563	77198	74135
Unique reflections	12501	12560	12564	12460
Redundancy	5.8	6.0	6.1	5.9
Completeness (%)	97.2 (98.6)	97.1 (99.0)	97.2 (99.0)	97.1 (99.4)
R_{sym}† (%)	7.5 (22.7)	6.8 (20.6)	6.4 (18.2)	7.0 (19.1)

† $R_{sym} = \sum_{hkl}\sum_i |I_{hkl,i} - \langle I_{hkl}\rangle|/\sum_{hkl}\sum_i I_{hkl,i}$.

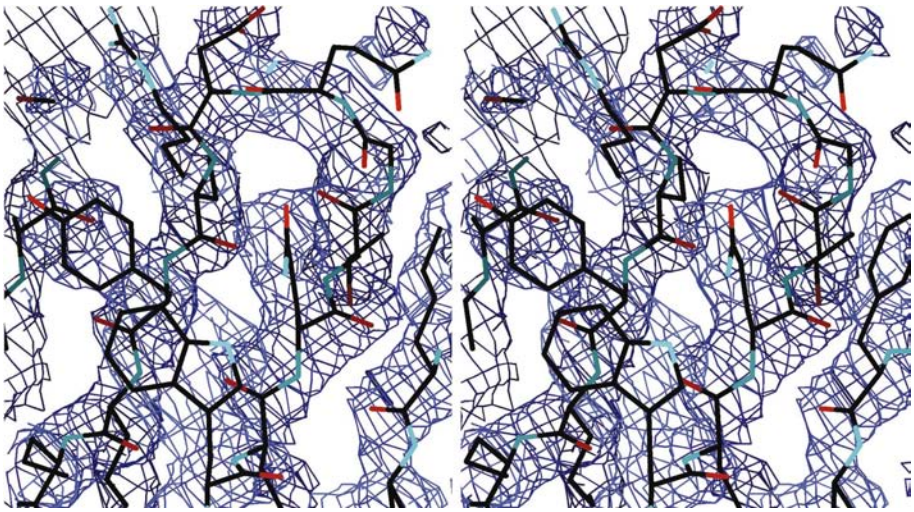

Figure 2
A stereoview of the electron-density map corresponding to a loop region of mLolB after density modification. The map was calculated at 2.5 Å resolution and contoured at the 1σ level. The current model is shown in stick representation. The figure was prepared using *XTALVIEW* (McRee, 1999).

Data from the selenomethionyl derivative for multiwavelength anomalous dispersion (MAD) phasing were measured at beamline BL44B2 at SPring-8 using a MAR CCD165 detector at 90 K. Four data sets were collected from one crystal. Peak (0.9798 Å) and edge (0.9804 Å) wavelengths were determined based on XAFS measurements. Low- and high-energy remote wavelengths were selected at 1.05 and 0.92 Å, respectively. The crystal-to-detector distance was 140 mm. The crystallographic data and processing statistics are shown in Table 2.

All data were processed and scaled using the *HKL*2000 program package (Otwinowski & Minor, 1997) and the *CCP*4 software suite (Collaborative Computational Project, Number 4, 1994).

5. MAD phasing

A Harker section ($v = 0.5$) of the anomalous difference Patterson map calculated by peak data showed no strong peaks (maximum level; +4σ). However, all of the ten Se atoms in the asymmetric unit (two mLolB molecules) were found using the program *SOLVE* (Terwilliger & Berendzen, 1999). The overall figure of merit at 2.5 Å resolution was 0.50 and the *Z* score was 41.98. The averaged occupancy of the ten Se atoms was 0.81, which was consistent with the value from the MALDI–MS experiments (0.85). Density modification was subsequently performed using the program *RESOLVE* (Terwilliger, 2000) with an overall figure of merit of 0.55. The quality of the electron-density map was sufficient for identification of each amino-acid residue. Loops as well as α-helices and β-strands showed clear electron density (Fig. 2).

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