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Crystallization and preliminary X-ray crystallographic analysis of UDP-*N*-acetylglucosamine acyltransferase from *Helicobacter pylori*

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Lipid A, a constituent of lipopolysaccharides, is essential for the growth and virulence of most Gram-negative bacteria. This makes its biosynthetic enzymes potential targets for development of new antibacterial agents. The first step of lipid A biosynthesis is catalyzed by the enzyme UDP-*N*-acetylglucosamine acyltransferase (LpxA). LpxA from the pathogenic bacterium *Helicobacter pylori* has been overexpressed in *Escherichia coli* and crystallized at 297 K using ammonium sulfate and sodium/potassium tartrate as precipitants in the presence of a detergent. Diffraction data to 2.1 Å resolution have been collected from a native crystal. The crystal belongs to space group $P6_322$, with unit-cell parameters $a = b = 90.69$, $c = 148.20$ Å. The asymmetric unit contains one subunit of LpxA, with a crystal volume per protein mass (V_M) of 2.87 Å^3 Da^{-1} and a solvent content of 57.1%.

1. Introduction

Lipid A serves as the hydrophobic anchor of lipopolysaccharides (LPSs), immunogenic glycolipids that make up the outer surface of the outer membranes of Gram-negative bacteria (Raetz, 1996). It is essential to the growth of most Gram-negative bacteria (Galloway & Raetz, 1990; Onishi *et al.*, 1996) and is also necessary for maintaining the integrity of the outer membranes as a barrier to toxic chemicals (Vaara, 1993; Nikaido, 1996). Therefore, inhibitors of lipid A biosynthesis would be potentially useful for the development of new antibacterial drugs against Gram-negative bacteria (Wyckoff, Raetz *et al.*, 1998).

The first step of lipid A biosynthesis is catalyzed by a cytoplasmic enzyme, UDP-N-acetylglucosamine (UDP-GlcNAc) acyltransferase (LpxA), which is encoded by the *lpxA* gene. It transfers an *R*-3-hydroxyacyl chain from *R*-3-hydroxyacyl carrier protein (ACP) to the glucosamine 3-OH of UDP-GlcNAc. There is some preference for the length and modification of the acyl chain among the different LpxAs. *E. coli* LpxA prefers *R*-3-hydroxymyristoyl-ACP to *R*-3-hydroxydecanoyl-ACP by a factor of ~1000, whereas *Pseudomonas aeruginosa* LpxA, which shows 54% sequence identity to *E. coli* LpxA, has an opposite preference (Dotson *et al.*, 1998; Wyckoff, Lin *et al.*, 1998). LpxA from *Chlamydia trachomatis* prefers non-hydroxylated myristoyl ACP (Sweet *et al.*, 2001). *E. coli* G173M mutant LpxA and the reciprocal *P. aeruginosa* M169G LpxA showed reversed substrate selectivities (Wyckoff, Lin *et al.*, 1998). Chemical modification and site-directed mutagenesis studies on the active site of *E. coli* LpxA indicate that His125 may be a catalytic residue, possibly acting as a general base (Wyckoff & Raetz, 1999).

The crystal structure of *E. coli* LpxA has been reported at 2.6 Å resolution (Raetz & Roderick, 1995). It is a homotrimer of 262-residue subunits, with each subunit composed of an N-terminal domain containing a left-handed parallel β-helix and a C-terminal domain containing four α-helices. However, this structure provided no clues to the mode of substrate binding at the active site or the catalytic mechanism, as it was determined in the absence of substrates or inhibitors.

H. pylori is a Gram-negative bacterium connected with the onset of gastritis, peptic ulcer and gastric cancer. Because of its importance as one of the major human pathogens, complete genome sequences of strains 26695 and J99 have been reported (Tomb *et al.*, 1997; Alm *et al.*, 1999). LpxA from *H. pylori* is a 270-residue protein which shows 38.4% sequence identity to *E. coli* LpxA. As the first step toward its structure elucidation, we report here its overexpression, crystallization and preliminary X-ray crystallographic data.

2. Experimental

2.1. Overexpression and purification

The *lpxA* gene encoding LpxA from *H. pylori* strain 26695 (HP1375) was amplified by the polymerase chain reaction (PCR) using the genomic DNA as template. The forward and reverse oligonucleotide primers were

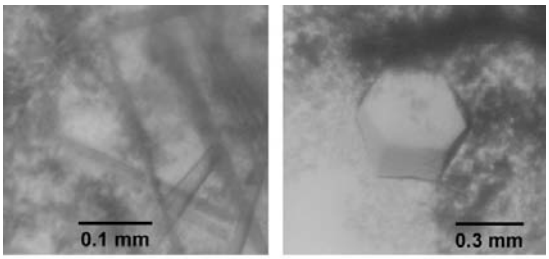

Figure 1
(*a*) Needle-shaped crystals of *H. pylori* LpxA grown in the absence of detergent. (*b*) A hexagonal plate crystal grown in the presence of the detergent 1-*s*-octyl-β-D-thioglucoside. Its approximate dimensions are 0.4 × 0.4 × 0.2 mm.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.21–2.10 Å).

X-ray wavelength (Å)	0.9794 (Photon Factory, BL-18B)
Temperature (K)	100
Resolution range (Å)	40.0–2.1
Space group	$P6_322$
Unit-cell parameters (Å)	$a = b = 90.69$, $c = 148.20$
Total/unique reflections	233961/21632
Completeness (%)	99.8 (99.8)
Mean $I/\sigma(I)$	6.9 (1.8)
$R_{\rm merge}$† (%)	7.9 (41.7)

† $R_{\rm merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h, i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h, i)$ for all i measurements.

designed using the published genome sequence (Tomb *et al.*, 1997). The PCR product was digested with *Nde*I and *Xho*I and was then inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a (Novagen). This construction adds a hexahistidine tag at the C-terminus of the recombinant LpxA. The protein was overexpressed in *E. coli* C41(DE3) cells (Miroux & Walker, 1996). The cells were grown in Luria–Bertani medium to an OD_{600} of 0.6 at 310 K and expression of the recombinant enzyme was induced with 1 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) at 310 K. Cell growth continued at 310 K for 6 h after IPTG induction and cells were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was suspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl and 50 m*M* imidazole) and was homogenized by sonication. The crude lysate was centrifuged at 36 000*g* (18 000 rev min^{-1}; Hanil Supra 21K rotor) for 1 h at 277 K and the cell debris was discarded. The first purification step utilized the C-terminal histidine tag by using an Ni^+-chelated Hi-trap chelating column (Amersham Biosciences). The next step was gel filtration on a Superdex-75 column with an elution buffer consisting of 50 m*M* Tris–HCl pH 8.0 and 150 m*M* NaCl. Further purification was achieved by ion-exchange chromatography on Source 15Q resin packed inside a HR10/10 column (Amersham Biosciences) previously equilibrated with 50 m*M* Tris–HCl pH 8.0. The protein was eluted with a linear gradient of 0–1.0 *M* NaCl in the same buffer. The final purification step was gel filtration on a HiLoad 16/60 Superdex-200 prep-grade column (Amersham Biosciences) previously equilibrated with buffer containing 20 m*M* potassium phosphate pH 7.0 and 200 m*M* NaCl. The purified protein solution was concentrated using a YM10 membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient of 11 880 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

2.2. Crystallization and X-ray data collection

A dynamic light-scattering experiment was performed on a DynaPro-801 instrument from Protein Solutions (Charlottesville, Virginia). The data were measured at room temperature with 1 mg ml^{-1} protein in 20 m*M* potassium phosphate pH 7.0 and 200 m*M* NaCl.

Crystallization was performed by the hanging-drop vapour-diffusion method using 24-well VDX tissue-culture plates (Hampton Research) at 297 K. A hanging drop was prepared by mixing 2 µl of the protein solution and 2 µl of reservoir solution. The protein concentration was 20 mg ml^{-1} before mixing with the reservoir solution. Each hanging drop was placed over 0.5 ml of the reservoir solution. Initial searches for the crystallization condition were performed using Crystal Screen I (Jancarik & Kim, 1991), Crystal Screen II and MembFac screening solutions (Hampton Research).

For X-ray data collection, a crystal was transferred to a solution consisting of 2.4 *M* ammonium sulfate, 0.2 *M* sodium/potassium tartrate and 30%(*v*/*v*) glycerol within a minute in four steps before being flash-frozen. X-ray diffraction data were collected at 100 K with an ADSC Quantum 4R CCD detector at the BL-18B experimental station of the Photon Factory, Tsukuba, Japan. The synchrotron X-ray wavelength was 0.9794 Å. The crystal was rotated through a total of 52°, with 1.0° oscillation per frame. The raw data were processed and scaled using the program *MOSFLM* (Leslie, 1997).

3. Results

The recombinant *H. pylori* LpxA with a C-terminal hexahistidine tag has been overexpressed in *E. coli* as a soluble fraction, with a yield of about 15 mg purified protein per litre of culture. The native molecular mass was estimated to be 85.6 kDa by dynamic light-scattering analysis, with a polydispersity of 5%. It indicates that the enzyme exists as a trimer (the calculated monomer mass including the His_6 tag is 30.6 kDa). Crystals were obtained using a reservoir solution consisting of 2.4 *M* ammonium sulfate and 0.2 *M* sodium/potassium tartrate. In the absence of a detergent, only thin needle-shaped crystals appeared (Fig. 1), which were too fragile to be characterized by X-ray diffraction. Addition of 1-*s*-octyl-β-D-thioglucoside to the hanging drop to a final concentration of 10%(*v*/*v*) yielded larger hexagonal-plate crystals (Fig. 1), which grew to approximate dimensions of 0.4 × 0.4 × 0.2 mm within 3 d.

X-ray diffraction data have been collected from a hexagonal crystal. A total of 233 961 measured reflections were merged into 21 632 unique reflections with an $R_{\rm merge}$ (on intensity) of 7.9%. The merged data set is 99.8% complete to 2.1 Å resolution. The systematic absences indicate that the crystal belongs to space group $P6_322$. The unit-cell parameters are $a = b = 90.69$ (2), $c = 148.20$ (2) Å, where the estimated standard deviations are given in parentheses. The presence of one subunit of LpxA in the asymmetric unit gives a crystal volume per protein mass ($V_{\rm M}$) of 2.87 Å^3 Da^{-1}, with a corresponding solvent content of 57.1% (Matthews, 1968). The statistics of data collection are summarized in Table 1. The structure has been solved by molecular replacement and reveals electron density corresponding to a detergent molecule bound at the active site. The structural details will be described in a separate paper.

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