Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray crystallographic studies of enoyl-acyl carrier protein reductase (FabI) from *Psuedomonas aeruginosa*

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Received 8 October 2010
Accepted 22 November 2010

During fatty-acid biosynthesis, enoyl-acyl carrier protein (enoyl-ACP) reductase catalyzes the reduction of *trans*-2-enoyl-ACP to fully saturated acyl-ACP *via* the ubiquitous fatty-acid synthase system. NADH-dependent enoyl-ACP reductase (FabI) from *Pseudomonas aeruginosa* has been purified and crystallized as an apoenzyme and in a complex form with NADH and triclosan. Triclosan is an inhibitor of FabI and forms a stable ternary complex in the presence of NADH. The crystals of native and complexed FabI diffracted to resolutions of 2.6 and 1.8 Å, respectively. The crystals both belonged to space group $P2_1$, with unit-cell parameters a = 117.32, b = 155.844, c = 129.448 Å, β = 111.061° for the native enzyme and a = 64.784, b = 107.573, c = 73.517 Å, β = 116.162° for the complex. Preliminary molecular replacement further confirmed the presence of four tetramers of native FabI and one tetramer of the complex in the asymmetric unit, corresponding to Matthews coefficients (V_M) of 2.46 and 2.05 Å^3 Da^{-1} and solvent contents of 50.1 and 40.1%, respectively.

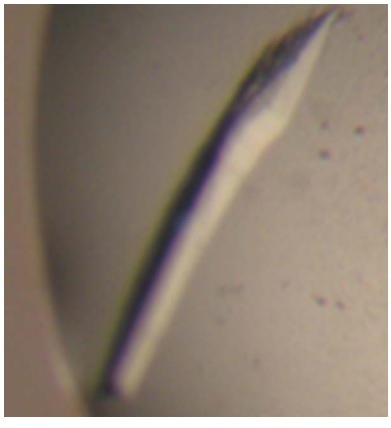
1. Introduction

Fatty-acid biosynthesis is a significant process in bacteria, plants and mammals. In particular, fatty acids are an essential component of cell envelopes, lipoproteins and phospholipids in bacteria. Most bacteria and plants synthesize fatty acids using a discrete and highly conserved group of proteins that each carry out a single reaction, which is called type II fatty-acid synthesis (FAS II; Menendez *et al.*, 2009; Priyadarshi *et al.*, 2009). In contrast, yeast and mammals employ type I fatty-acid synthesis (FAS I), which is catalyzed by the individual domains of a large polypeptide that serve as attachment points for pantetheine, an acyl carrier protein (ACP) prosthetic group (Heath *et al.*, 2001; Massengo-Tiasse & Cronan, 2009; Rafferty *et al.*, 1995). Even though FAS I and FAS II are similar in their catalytic mechanism, there are some differences that can be taken advantage of for the synthesis of antibacterial inhibitors (Zhang *et al.*, 2003; Davies *et al.*, 2000). One example is enoyl-ACP reductase (ENR), which plays an important role in FAS II and is an attractive target. ENRs, which include enoyl-ACP reductase (FabI), FabK, FabL and FabV, catalyze the conversion of *trans*-2-enoyl-ACPs to acyl-ACPs during the final steps of the elongation cycle (Heath & Rock, 1995; Hoang & Schweizer, 1999; Zhu *et al.*, 2010).

FabI is a target for triclosan, which is a chlorophenol with broad-spectrum antimicrobial activity. Triclosan is commonly used in antibiotic products as well as in surgical scrubs, cosmetics, dental care products and toys (Heath *et al.*, 2000). Triclosan tightly binds to FabI as an inhibitor of bacterial fatty-acid biosynthesis and prevents the elongation of the nascent fatty-acid chain (Webber *et al.*, 2008; Zhang *et al.*, 2006). The FabI–triclosan complex is stabilized in the presence of NADH (Heath *et al.*, 2002).

Pseudomonas aeruginosa, a Gram-negative aerobic rod bacterium, is an opportunistic pathogen that causes disease (Stover *et al.*, 2000). *P. aeruginosa* is a common cause of morbidity in hospitalized patients and the increased resistance among recovered strains of *P. aeruginosa* has become problematic (Carmeli *et al.*, 1999; McGowan, 2006).

Several FabI crystal structures have been reported, but there are still only a few reports of the structure of the FabI–NADH–triclosan



complex for pathogenic Gram-negative rod bacteria. Therefore, more detailed investigations are required for comparison with existing FabI structures and to improve the understanding of antibiotics for use against resistant *P. aeruginosa*. As the first step toward elucidating its structure, we report the crystallization and preliminary X-ray crystallographic analysis of FabI from *P. aeruginosa* in the native form and in a complexed form.

2. Materials and methods

2.1. Cloning and expression

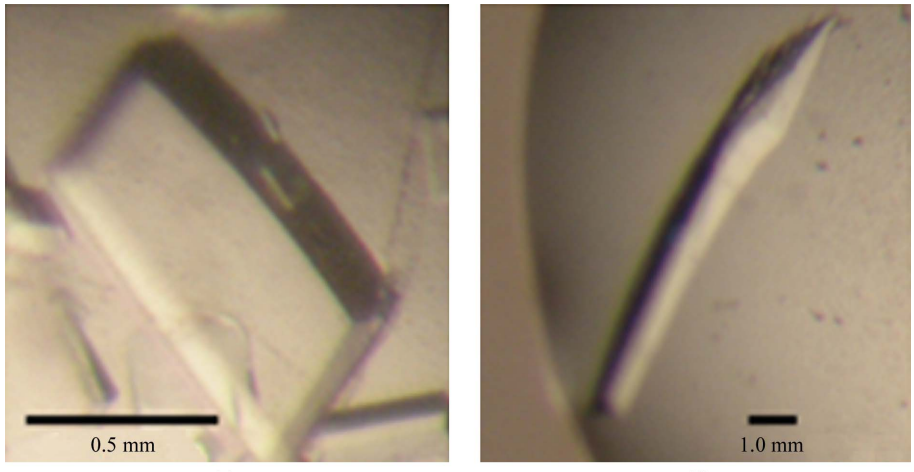
The *fab*I gene was amplified by polymerase chain reaction (PCR) from *P. aeruginosa* genomic DNA using the forward primer 5′-**GGA ATT CCA TAT** GGG ATT TCT CAC AGG AAA A-3′ and the reverse primer 3′-**CCG CTC GAG** GTC GTC GTC CAG C-5′ (*Nde*I and *Xho*I recognition sites are shown in bold). The PCR-amplified DNA fragment was purified, digested with *Nde*I and *Xho*I and ligated into a pET-22b vector (Novagen, USA), which has six consecutive histidines at its C-terminus. The recombinant plasmid was transformed into the chemically competent *Escherichia coli* BL21 (DE3) strain (Novagen) using a heat-shock technique and then cultured in Luria–Bertani medium containing 50 µg ml^{-1} ampicillin at 310 K to an optical density of 0.6 at 600 nm. Protein expression was induced by the addition of 0.2 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were allowed to grow for an additional 6 h at 30 K, which was followed by centrifugation at 5000*g* for 30 min at 277 K.

2.2. Purification

The harvested cell pallets were suspended in buffer *A* (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 5 m*M* imidazole) and lysed by sonication on ice, after which the lysate was centrifuged at 20 000*g* for 40 min. The supernatant was loaded onto an Ni^{2+}-chelated HiTrap chelating HP column (GE Healthcare, USA) equilibrated in buffer *A*. The bound protein was eluted with a linear gradient to buffer *B* (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 1 *M* imidazole). Fractions containing FabI were identified by SDS–PAGE and purified by gel-filtration chromatography on a HiLoad 16/60 Superdex 200 column (GE Healthcare, USA) which had been equilibrated with gel buffer (20 m*M* Tris–HCl pH 7.9, 200 m*M* NaCl, 2 m*M* DTT). The soluble fractions containing protein were pooled together and concentrated to 12 mg ml^{-1} using an Amicon Ultra-15 centrifugal filter device (Millipore, USA). This procedure yielded approximately 1 g FabI protein from a 1 l culture. The protein concentration was estimated using the Bradford assay and the purity was confirmed by 15% SDS–PAGE to be >95%.

2.3. Crystallization and preliminary X-ray analysis

Preliminary crystallization screens for the FabI enzyme were performed by the sitting-drop vapour-diffusion method (0.2 µl protein solution and 0.2 µl reservoir solution equilibrated against 100 µl reservoir solution) using several commercial screens: Wizard I, Wizard II (Emerald BioSystems), Crystal Screen, Crystal Screen 2 and PEG/Ion (Hampton Research). After screening, the crystals were optimized and grown using the hanging-drop vapour-diffusion method in 24-well VDX plates from Hampton Research under the following conditions: 6% Tacsimate, 8% polyethylene glycol 3350 for the native crystal and 0.1 *M* sodium malonate pH 5.0, 10%(*w*/*v*) polyethylene glycol 3350 for the FabI–NADH–triclosan complex crystal. Each hanging drop was made up of 1 µl protein solution and 1 µl reservoir solution and was equilibrated over 500 µl reservoir solution. For cocrystallization, NADH (0.05 m*M*) and triclosan (1 m*M*) were added to the protein solution using the hanging-drop vapour-diffusion method in 0.1 *M* sodium malonate, 10% polyethylene glycol 3350. The crystal dimensions of the native and FabI–NADH–triclosan complex crystals were 0.5 × 0.1 × 1.0 and 0.1 × 0.05 × 0.9 mm, respectively. The crystals were transferred to a cryoprotection solution consisting of 25% ethylene glycol in mother liquor. Data sets were collected from the native and FabI–NADH–triclosan complex crystals on beamlines 4A and 6C, respectively, at Pohang Light Source (PLS; Pohang, Republic of Korea) using an ADSC Quantum 210 CCD detector. A total range of 200° was covered with 1.0° oscillations and 30 s exposure per frame for the native crystal and a total range of 360° was covered with 1.0° oscillations and 5 s exposure per frame for the ternary-complex crystal. The wavelength of the synchrotron X-rays was 1.0000 Å on beamline 4A and 1.12713 Å on beamline 6C. The distance from the native crystal to the detector was 200 mm, whereas that from the ternary-complex crystal to the detector was 150 mm. The X-ray diffraction data showed that the native crystal diffracted to 2.6 Å resolution, while the FabI–NADH–triclosan complex crystals diffracted to 1.8 Å



(*a*) (*b*)

Figure 1
Crystals of FabI protein from *P. aeruginosa*. (*a*) Native FabI crystal; (*b*) FabI–NADH–triclosan complex crystal. The crystal dimensions of the native and ternary-complex FabI crystals were 0.5 × 0.1 × 1.0 and 0.1 × 0.05 × 0.9 mm, respectively.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

	Native	NADH–triclosan complex
X-ray source	PLS beamline 4A	PLS beamline 6C
Wavelength (Å)	1.0000	1.12713
Space group	$P2_1$	$P2_1$
Unit-cell parameters (Å, °)	a = 117.32, b = 155.844, c = 129.448, β = 111.061	a = 64.784, b = 107.573, c = 73.517, β = 116.162
Resolution range (Å)	50.0–2.6	50.0–1.8
Multiplicity	2.5 (1.6)	5.5 (2.6)
Total/unique reflections	280743/112311	412722/74526
Completeness (%)	84.9 (67.7)	89.1 (46.8)
Mean $I/\sigma(I)$	8.66 (1.8)	31.8 (4.0)
$R_{\rm merge}$† (%)	10.9 (32.5)	4.9 (18.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl)\rangle$ represent the diffraction-intensity values of the individual measurements and the corresponding mean values, respectively.

resolution. All data sets were indexed, integrated and scaled using the *HKL*-2000 software package (Otwinowski & Minor, 1997).

3. Results and discussion

The gene encoding FabI from *P. aeruginosa* was successfully cloned, expressed in *E. coli* and purified with a C-terminal His_6 tag by two steps of column chromatography: Ni column chromatography and gel filtration. The molecular weight of FabI was predicted to be 28 kDa from the sequence and this was confirmed by SDS–PAGE. Crystals that were suitable for X-ray analysis were obtained by the hanging-drop vapour-diffusion method under the following conditions: 6% Tacsimate, 8% polyethylene glycol 3350 for the native crystal (Fig. 1*a*) and 0.1 *M* sodium malonate pH 5.0, 10%(*w*/*v*) polyethylene glycol 3350 for the FabI–NADH–triclosan complex (Fig. 1*b*).

The native crystal of FabI diffracted to a resolution of 2.6 Å and belonged to the monoclinic space group $P2_1$, with unit-cell parameters a = 117.32, b = 155.84, c = 129.44 Å, β = 111.06°. Assuming the presence of four tetramers per asymmetric unit, the $V_{\rm M}$ value was calculated to be 2.46 Å^3 Da^{-1} (Matthews, 1968), with an estimated solvent content of 50.1%. The crystal of the FabI–NADH–triclosan complex diffracted to a resolution of 1.8 Å and belonged to the monoclinic space group $P2_1$, with unit-cell parameters a = 64.78, b = 107.57, c = 73.51 Å, β = 116.16°. The asymmetric unit contained one tetramer, corresponding to a $V_{\rm M}$ value of 2.05 Å^3 Da^{-1}, with an estimated solvent content of 40.1%.

The data-collection statistics are summarized in Table 1. Structure determination was performed by the molecular-replacement method using *MOLREP* from *CCP*4 (Collaborative Computational Project, Number 4, 1994) with the crystal structure of the *E. coli* enoyl reductase–NAD^+–triclosan complex (PDB code 1c14; 69% identity; Qiu *et al.*, 1999) as a search model. Further refinement of the model structures using experimental phases is currently in progress and the structural details will be reported separately.

We thank the staff at beamlines 4A and 6C of Pohang Light Source, Republic of Korea for assistance with data collection. This study was supported by a research fund (2010) from the Catholic University of Pusan.

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