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Crystallization and preliminary X-ray crystallographic analysis of β-ketoacyl-ACP synthase I (XoFabB) from *Xanthomonas oryzae* pv. *oryzae*

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The proteins in the fatty-acid synthesis pathway in bacteria have significant potential as targets for the development of antibacterial agents. An essential elongation step in fatty-acid synthesis is performed by β-ketoacyl-acyl carrier protein synthase I (FabB). The organism *Xanthomonas oryzae* pv. *oryzae* (Xoo) causes a destructive bacterial blight disease of rice. The XoFabB protein from Xoo was expressed, purified and crystallized for the three-dimensional structure determination that is essential for the development of specific inhibitors of the enzyme. An XoFabB crystal diffracted to 3.0 Å resolution and belonged to the tetragonal space group $P4_1$, with unit-cell parameters $a = b = 82.2$, $c = 233.2$ Å. Assuming that the crystallographic structure contains four molecules in the asymmetric unit, the corresponding V_M would be 2.18 Å^3 Da^{-1} and the solvent content would be 43.5%. The initial structure was determined by the *MOLREP* program with an *R* factor of 44.0% and does contain four monomers in the asymmetric unit.

1. Introduction

Bacterial blight, which is caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a destructive disease of rice which has caused huge damage in most rice-growing countries (Ezuka & Kaku, 2000). However, no effective antibacterial agents against Xoo are available to date. In 2005 the whole genome sequence of Xoo was determined (Lee *et al.*, 2005) and approximately 100 different essential genes of Xoo were selected as targets for the development of antibacterial agents against Xoo (Payne *et al.*, 2004, 2007). The gene coding for β-ketoacyl-acyl carrier protein synthase I was one such target.

Fatty-acid synthesis (FAS) in bacteria has been clarified as type II, in which each catalytic step is performed by an individual enzyme (Rock & Jackowski, 2002). In contrast, the type I FAS found in eukaryotes (in particular in mammals) consists of a large single polypeptide chain for all catalytic steps (Smith *et al.*, 2003). In FAS type II pathways, the condensation of acyl groups onto the elongating fatty-acid chain is catalyzed by β-ketoacyl-acyl carrier protein synthases (KASs). Three enzymes related to KAS activity have been identified and called KAS I, KAS II and KAS III; these enzymes are also known as FabB, FabF and FabH, respectively (Heath & Rock, 2002; Garwin *et al.*, 1980). FabB can catalyze the condensation reaction of both saturated and unsaturated acyl-acyl carrier proteins (ACPs), whereas FabF can only carry out the condensation reaction of saturated acyl ACPs (Cronan & Rock, 1987). Since FabB plays a vital role in bacterial survival and has no counterpart in eukaryotes, it has been considered as a potential target for drug development. The crystal structure of FabB from *Escherichia coli* was solved a decade ago (Olsen *et al.*, 1999) and Price and coworkers reported that two natural compounds, cerulenin and thiolactomycin, could inhibit *E. coli* FabB (Price *et al.*, 2001). Although they inhibit the growth of several bacteria successfully, the limited selectivity of cerulenin and problems with the synthesis and stability of thiolactomycin have decreased

their usage as antibacterial drugs (Heath *et al.*, 2001; Price *et al.*, 2001). Recently, using a structure-based screening method, it has been suggested that aminothiazole is a promising compound that inhibits *E. coli* FabB (Pappenberger *et al.*, 2007).

In this study, we cloned the *XoFabB* gene from Xoo and the gene product (402 residues, 44 kDa) was expressed, purified and crystallized. We believe that the three-dimensional crystal structure of XoFabB will provide valuable information in the development of antibacterial agents against Xoo.

2. Materials and methods

2.1. Cloning

The *XofabB* gene coding for β-ketoacyl-acyl carrier protein synthase I from Xoo (ATCC10331) was amplified by PCR using the genomic DNA of Xoo as a template. The oligonucleotides used were 5′-GGG **CAT ATG** CGT CGC GTC GTC ATC ACC GGA AT-3′ for the forward primer and 5′-GG **GGA TCC** TCA GAC CCG GCC GAA CAC CAG G-3′ for the reverse primer (*Nde*I and *Bam*HI sites are shown in bold). The PCR fragments were digested with the restriction enzymes and then inserted into a pET11a vector (Novagen, Republic of Korea). The plasmid pET11a was modified to include the sequence MGHHHHHHSSENLYFQGH containing six histidine residues at the N-terminus of the cloned target protein to facilitate protein purification. The *XofabB* gene in the recombinant vector pET-XofabB was verified to be free of mutations by DNA sequencing (Macrogen, Republic of Korea).

2.2. Overexpression and purification

The recombinant vector pET-XofabB was transformed into *E. coli* BL21 (DE3) cells grown in Luria–Bertani medium supplemented with ampicillin (50 µg ml^{-1}). The transformant was cultured in a shaking incubator at 310 K. When the OD_{600} of the culture reached 0.6, 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce expression of the XoFabB protein. The culture was further incubated for 6 h at 303 K and the cells were harvested by centrifugation at 277 K for 30 min at 800*g*. The cell pellet was resuspended in ice-cold lysis buffer (25 m*M* Tris–HCl pH 7.5, 300 m*M* NaCl, 15 m*M* imidazole, 3 m*M* β-mercaptoethanol) and lysed by sonication on ice (Sonomasher, S&T Science, Republic of Korea). The lysate was centrifuged for 30 min at 25 000*g* (Vision VS24-SMTi V508A rotor) at 277 K to pellet the cellular debris. The supernatant containing soluble XoFabB was loaded onto a column containing Ni–NTA resin (Novagen) according to the the manufacturer's protocol. The unbound material which flowed through was discarded and non-specifically bound proteins were eluted with lysis buffer containing 30 m*M* imidazole. XoFabB was eluted using lysis buffer containing 250 m*M* imidazole. The fractions containing the target protein were collected and dialyzed for 12 h at 277 K in buffer *A* (25 m*M* Tris–HCl pH 7.5, 3 m*M* β-mercaptoethanol). The protein was further purified by anion-exchange chromatography using a Uno-Q column (Amersham, Republic of Korea) with buffer *B* (25 m*M* Tris–HCl pH 7.5, 1 *M* NaCl, 3 m*M* β-mercaptoethanol) as the high-salt buffer in a step gradient. XoFabB protein was eluted at an NaCl concentration of between 380 and 420 m*M*. The homogeneity of the purified protein was confirmed *via* SDS–PAGE: only one band of XoFabB was visible on 10% SDS–PAGE, at a position corresponding to 44 kDa (Fig. 1). The purified XoFabB was concentrated to 7 mg ml^{-1} in buffer *A* for crystallization purposes using a spin concentrator with a molecular-weight cutoff of 30 000 Da.

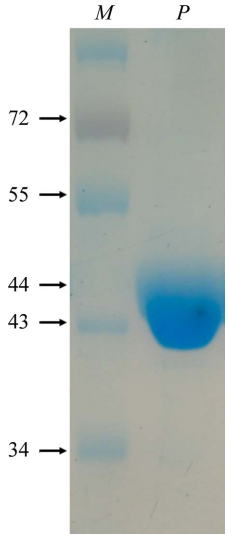

Figure 1
Purified XoFabB shown on 10% SDS–PAGE. Lane *M*, PageRuler Prestained Protein Ladder (Fermentas, Republic of Korea; labelled in kDa), lane *P*, protein.

2.3. Crystallization and X-ray data collection

Crystallization conditions were initially screened using the sitting-drop vapour-diffusion method with a Hydra II eDrop automated pipetting system (Matrix) at 287 K. Drops consisted of 0.5 µl XoFabB protein and 0.5 µl reservoir solution and were equilibrated against 70 µl reservoir solution at 287 K. The initial crystallization conditions tested were from Index and Grid Screen Ammonium Sulfate kits (Hampton Research, USA) and the Wizard kit (Emerald BioSystems, USA). After 2 d, needle-like crystals were observed in one condition from Grid Screen Ammonium Sulfate (condition No. 14; 0.1 *M* citric acid pH 5.0, 2.4 *M* ammonium sulfate; Fig. 2). Since the sitting drops did not produce good crystals, we tried using hanging drops and also tried the seeding method with various concentrations of the protein. The final well diffracting crystals were obtained from hanging drops consisting of 2 µl 5.5 mg ml^{-1} XoFabB and 2 µl reservoir solution (0.1 *M* citric acid pH 5.5, 2.2 *M* ammonium sulfate). An optimized crystal (0.3 × 0.05 × 0.03 mm; Fig. 3) was mounted in a loop and transferred to a cryoprotectant solution prior to cooling in liquid nitrogen. Several cryoprotectant solutions were created by mixing the reservoir solution with various concentrations of glycerol and ethylene glycol; reservoir solution with an additional 20%(*v*/*v*) ethylene

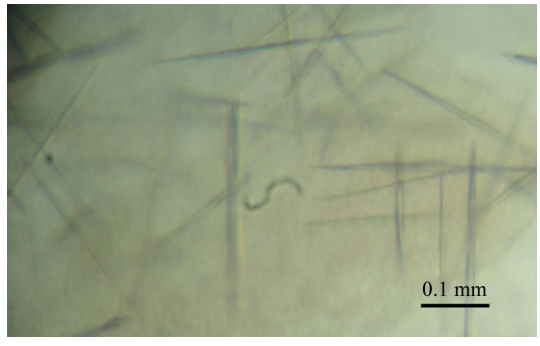

Figure 2
Initial crystals of XoFabB protein obtained by the sitting-drop vapour-diffusion method using condition No. 14 of the Grid Screen Ammonium Sulfate kit (0.1 *M* citric acid pH 5.0, 2.4 *M* ammonium sulfate) at 287 K.

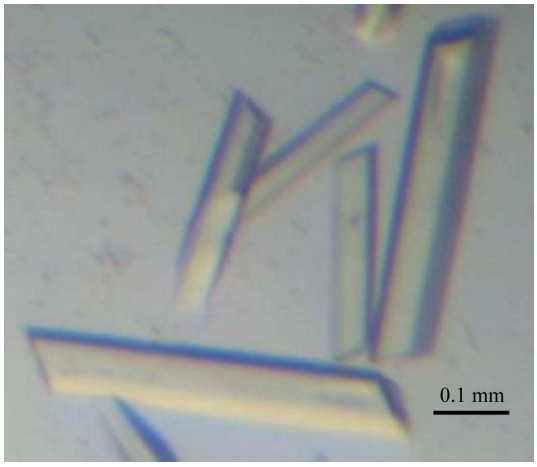

Figure 3
Optimized XoFabB crystals (0.3 × 0.05 × 0.03 mm) were obtained using a reservoir solution consisting of 0.1 *M* citric acid pH 5.5, 2.2 *M* ammonium sulfate at 287 K.

glycol was finally selected as the most suitable cryosolution. The crystal was analyzed on beamline BL26B1 of SPring-8 in Japan using a Jupiter210 (Rigaku/MSC) CCD detector. Diffraction data were collected to 3.0 Å resolution and were integrated and scaled using *HKL*-2000 (Otwinowski & Minor, 1997). The crystal belonged to a tetragonal primitive space group ($P4_1$, $P4_2$ or $P4_3$), with unit-cell parameters $a = b = 82.2$, $c = 233.2$ Å. Matthews coefficient analysis (Matthews, 1968) indicated that there might be four molecules present in the asymmetric unit ($V_M = 2.18$ Å^3 Da^{-1}), giving a solvent content of 43.5%. The final statistics of data collection and processing details are summarized in Table 1. Monomer *A* of the FabB crystal structure from *E. coli* (Olsen *et al.*, 1999) was used as a search model for molecular replacement (MR) using the *MOLREP* (Vagin & Teplyakov, 2010) and *Phaser* (McCoy *et al.*, 2007) programs. The resulting model was given by *MOLREP*, with an initial *R* factor of 44.0%.

3. Results and discussion

We expressed the β-ketoacyl-acyl carrier protein synthase I from Xoo (XoFabB) in *E. coli* with a 6×His-TEV tag and purified and crystallized it. Initial needle-shaped crystals were observed using condition No. 14 from Grid Screen Ammonium Sulfate in sitting drops at 287 K. After optimization of this crystallization condition, large single crystals were obtained and a complete diffraction data set was collected to 3.0 Å resolution. The initial space group derived from the auto-indexing program was *P*4 ($P4_1$, $P4_2$ or $P4_3$) or *P*422. Analysis of self-rotation functions calculated by *MOLREP* confirmed that the XoFabB crystal possessed one fourfold and two twofold crystallographic axes and an additional twofold noncrystallographic axis (Fig. S1[1]). The *MOLREP* and *Phaser* programs were used for MR with the *E. coli* β-ketoacyl-ACP synthase I structure (PDB entry 1dd8; 62% sequence identity; Olsen *et al.*, 1999) as a template. Although all possible space groups were used in MR, *MOLREP* only provided a solution in space group $P4_1$. The resulting model has four molecules in the asymmetric unit, with an initial *R* factor of 44.0%. The resulting electron-density maps are of high quality and no clashes were found between symmetry-related molecules (Fig. S3[1]). Currently, structure refinement is being carried out. We believe that the XoFabB structure will provide insights into its catalytic mechanism and will provide valuable information for the development of antibacterial agents against Xoo.

[1] Supplementary material has been deposited in the IUCr electronic archive (Reference: NJ5100).

Table 1
Data-collection statistics.

Values in parentheses are for the last shell.

Beamline	Spring-8 BL26B1
Wavelength (Å)	1.0
Resolution range (Å)	50.0–3.0 (3.05–3.00)
Space group	$P4_1$
Unit-cell parameters (Å)	$a = b = 82.2$, $c = 233.2$
Total No. of reflections	1169323
No. of unique reflections	30477
Completeness (%)	98.2 (95.9)
Molecules per asymmetric unit	4
Solvent content (%)	43.5
Average $I/\sigma(I)$	10.6 (2.4)
$R_{\rm merge}$† (%)	11.5 (38.2)
$R_{\rm p.i.m.}$‡ (%)	9.5 (35.5)
Multiplicity	4.2 (3.1)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$. ‡ $R_{\rm p.i.m.} = \sum_{hkl}[1/(N-1)]^{1/2} \times \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection *hkl*, $\sum_{hkl}$ is the sum over all reflections, $\sum_i$ is the sum over *i* measurements of reflection *hkl* and $\langle I(hkl)\rangle$ is the weighted average intensity of all observations *i* of reflection *hkl*.

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References

Cronan, J. E. & Rock, C. O. (1987). *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology*, edited by F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter & H. E. Umbarger, Vol. 1, pp. 474–477. Washington DC: American Society for Microbiology.
Ezuka, A. & Kaku, H. (2000). *Bull. Natl Inst. Agrobiol. Resour. (Jpn)*, **15**, 53–54.
Garwin, J. L., Klages, A. L. & Cronan, J. E. Jr (1980). *J. Biol. Chem.* **255**, 3263–3265.
Heath, R. J. & Rock, C. O. (2002). *Nat. Prod. Rep.* **19**, 581–596.
Heath, R. J., White, S. W. & Rock, C. O. (2001). *Prog. Lipid Res.* **40**, 467–497.
Lee, B. M. *et al.* (2005). *Nucleic Acids Res.* **33**, 577–586.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Olsen, J. G., Kadziola, A., von Wettstein-Knowles, P., Siggaard-Andersen, M., Lindquist, Y. & Larsen, S. (1999). *FEBS Lett.* **460**, 46–52.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pappenberger, G., Schulz-Gasch, T., Kusznir, E., Müller, F. & Hennig, M. (2007). *Acta Cryst.* D**63**, 1208–1216.
Payne, D. J., Gwynn, M. N., Holmes, D. J. & Pompliano, D. L. (2007). *Nature Rev. Drug Discov.* **6**, 29–40.
Payne, D. J., Gwynn, M. N., Holmes, D. J. & Rosenberg, M. (2004). *Methods Mol. Biol.* **266**, 231–259.
Price, A. C., Choi, K.-H., Heath, R. J., Li, Z., White, S. W. & Rock, C. O. (2001). *J. Biol. Chem.* **276**, 6551–6559.
Rock, C. O. & Jackowski, S. (2002). *Biochem. Biophys. Res. Commun.* **292**, 1155–1166.
Smith, S., Witkowski, A. & Joshi, A. K. (2003). *Prog. Lipid Res.* **42**, 289–317.
Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* D**66**, 22–25.