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Crystallization of *Escherichia coli* β-ketoacyl-ACP synthase III and the use of a dry flash-cooling technique for data collection

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β-Ketoacyl-acyl carrier protein (ACP) synthase III (FabH) is a condensing enzyme active in the fatty-acid biosynthesis pathway of bacteria. The enzymes of this pathway provide a set of targets for the discovery of previously unknown antibiotics. FabH from *Escherichia coli* has been crystallized in two crystal forms using the sitting-drop vapor-diffusion technique. The first form crystallized in the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 63.1, b = 65.1, c = 166.5 Å; the second form crystallized in the tetragonal space group $P4_12_12$, with unit-cell parameters $a = b$ = 72.7, c = 99.8 Å. A flash-cooling technique using no cryoprotectant was utilized in obtaining data from the second type of crystals.

1. Introduction

Fatty-acid biosynthesis in *E. coli* is an important step in membrane synthesis. The enzymes involved are individual polypeptides in this organism, whereas in higher organisms these enzymes are domains in multienzyme complexes. Fatty-acid biosynthesis is a cyclic procedure where two-carbon units are added to a growing chain. FabH is the initiating enzyme and catalyzes the condensation of acetyl-CoA with malonyl-ACP (acyl carrier protein) to yield acetoacetyl-ACP, CO_2 and CoASH. This condensation is followed by reduction, dehydration and further reduction; continuing cycles begin with condensation of the growing chain (acyl-ACP) with malonyl-ACP, a step also carried out by a β-ketoacyl-ACP synthase (Cronan & Rock, 1996). In addition to initiating the cycle, FabH is a target for feedback regulation of the biosynthetic pathway *via* product inhibition by acyl-ACPs of various lengths. Acyl-ACP inhibition increases with increasing chain length from 12 to 20 C atoms (Heath & Rock, 1996).

In recent years, the incidence of serious health issues caused by increasing resistance to available antibiotics has increased (Bax *et al.*, 1998; Travis, 1994). Thiolactomycin is a known antibacterial agent which inhibits bacterial fatty-acid synthases including FabH (Magnuson *et al.*, 1993). Therefore, FabH should be a reasonable target for the design of inhibitors with the potential to become new antibacterial agents.

2. Methods and results

2.1. Protein expression and purification

Previously, *E. coli* FabH expressed with a His-tag was purified (Heath & Rock, 1996). Here, a non-His-tagged *E. coli* FabH was used which had been overexpressed in *E. coli* DH10B cells using the pET29 (Novagen) vector and purified using Q-Sepharose, MonoQ and hydroxyapatite chromatographic steps at 277 K (Khandekar *et al.*, 2000).

2.2. Crystallization

The sitting-drop vapor-diffusion method was used for all crystallizations.

Initial crystallization conditions were found using the sparse-matrix method of Jancarik & Kim (1991) and then optimized. Protein concentration was 11 mg ml^{-1} in 20 m*M* Tris–HCl at pH 8.0, 50 m*M* NaCl and 1 m*M* dithiothreitol. Droplets were formed using 2 µl protein and 2 µl reservoir solution and were equilibrated against 0.3 ml reservoir solution. Form I crystals grew from a reservoir of 20% PEG 8000 (Fluka) and 0.1 *M* HEPES at pH 7.5 as small plates which required separation for data collection.

A second crystal form (form II, Fig. 1) was grown with protein at 10 mg ml^{-1} in 20 m*M* Tris pH 7.4, 50 m*M* NaCl and 2 m*M* dithiothreitol and a reservoir solution containing 0.1 *M* magnesium chloride, 0.05 *M* Tris–HCl pH 8.5 and 15% PEG 4000 (Fluka). These crystals grew as tetragonal bipyramids, 0.2 mm on a side. Both crystal forms were obtained at room temperature (293–294 K) and grew in about a week.

2.3. Data collection

X-ray diffraction experiments were carried out at beamline 17-ID (IMCA) at the Advanced Photon Source (APS). Crystals had to be flash-cooled at cryogenic temperatures for data collection at this powerful synchrotron beamline. The form I crystals were flash-cooled

in a cryoprotectant solution containing 80% crystallization mother liquor and 20% glycerol. They diffracted to better than 2.0 Å resolution and a data set 97.1% complete to 1.95 Å resolution has been collected. There are a total of 292 572 integrated measurements, giving 50 198 unique reflections (sixfold redundancy). The mosaicity of the crystal is 0.7° and the $R_{\rm merge}$ is 0.077. The crystal belonged to the orthorhombic $P2_12_12_1$ space group, with unit-cell parameters $a = 63.1$, $b = 65.1$, $c = 166.5$ Å and one FabH dimer per asymmetric unit. The crystal has a V_M value of 2.45 Å^3 Da^{-1}, suggesting a 50% solvent content.

The form II crystals were much more difficult to flash-cool; the crystallization mother liquor (15% PEG 4000) formed ice upon flash-cooling. Additions of glycerol or ethylene glycol, oil, higher concentrations of PEG 4000 *etc*. caused the crystals to dissolve or the mosaicity to increase to unacceptable levels. To flash-cool these crystals, a standard nylon loop from Hampton Research was used, with the loop size carefully chosen to be smaller than the diameter of the crystal (<0.1 mm). The loop encircled one tip of the diamond-shaped crystal and the crystal was lifted out of the mother liquor so that the crystal sat above the loop such that no mother liquor was visible inside the loop. The crystal was then flash-frozen in a nitrogen stream at 100 K. In half of the trials, ice rings were seen in the diffraction images; this did not prohibit data acquisition. In the other half of the trials, the images were clear and showed no ice diffraction.

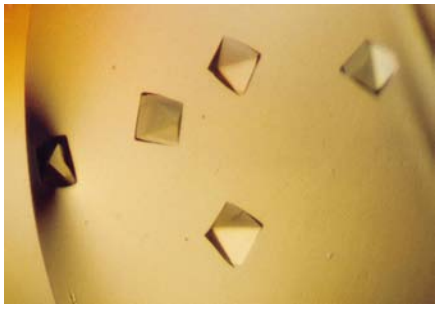

Figure 1
Crystals of *E. coli* FabH, form II. The size of the largest crystal is 0.2 mm on an edge.

Similar to the oil method described by Hope (1990), this technique works by excluding or minimizing the mother liquor from the crystal surface. There is no oil used in this method; the use of oil in this case destroyed the crystal. This method has also been used for crystals grown under high-salt conditions, *e.g.* the crystals of varicella-zoster virus protease grown from 2.5 *M* NaCl, a structure which was solved at 3.0 Å at room temperature (Qiu *et al.*, 1997). These crystals grew as hexagonal rods and diffracted to 1.9 Å with synchrotron radiation using this technique for flash-cooling with a loop size matching the diameter of the crystal (unpublished data). Since no changes were introduced into the crystal solution, this technique is ideal for crystals that are sensitive to environmental changes, such as FabH.

A data set for the form II crystal has been collected to 1.9 Å resolution and 99.9% compleness. There are a total of 303 763 measurements, resulting in 21 724 unique reflections (14-fold redundancy). The mosaicity of the crystal is 0.9° and the $R_{\rm merge}$ is 0.060. The crystal belongs to the tetragonal space group $P4_12_12$, with unit-cell parameters $a = b = 72.7$, $c = 99.8$ Å. There is one FabH monomer per asymmetric unit, with a V_M of 1.9 Å^3 Da^{-1} and about 35% solvent content. Structures for both crystal forms have been determined (Qiu *et al.*, 1999); these will provide a structural basis for inhibitor design.

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