Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic analysis of β-hydroxyacyl ACP dehydratase (FabZ) from *Plasmodium falciparum*

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Received 11 July 2003
Accepted 7 October 2003

The malarial parasite *Plasmodium falciparum* synthesizes fatty acids by the type II mechanism. In this cycle, the dehydration of the β-hydroxyacyl acyl carrier protein is catalyzed by FabZ. Purified FabZ has been crystallized using the hanging-drop vapour-diffusion and microbatch techniques. The crystals are orthorhombic, with space group $I222$ or $I2_12_12_1$ and unit-cell parameters a = 71.78, b = 81.99, c = 97.49 Å. A complete data set to a resolution of 2.5 Å has been collected under cryoconditions (100 K) using a MAR imaging-plate detector system mounted on a rotating-anode X-ray generator.

1. Introduction

Fatty acids are essential components of phospholipids and sphingolipids, which make up the cellular and organellar membrane. In living systems, fatty-acid biosynthesis occurs by two distinct pathways. In fungi, mammals and some mycobacteria, fatty-acid biosynthesis is accomplished by multifunctional proteins; each reaction is catalyzed by a distinct domain of these large proteins. This is known as type I or associative fatty-acid biosynthesis (type I FAS). In contrast, plants and most bacteria synthesize fatty acids by a different mechanism known as the type II or dissociative fatty-acid synthesis system (type II FAS; Raetz, 1990), in which each reaction is catalysed by a distinct enzyme.

In type II FAS, FabZ is the primary dehydratase involved in fatty-acid elongation. However, in anaerobic bacterial systems, the dehydration of β-hydroxyacyl acyl carrier protein (ACP) to *trans* 2-enoyl ACP is the key step in driving the synthesis of both saturated and unsaturated fatty acids. This step is catalyzed by two dehydratases, namely β-hydroxyacyl ACP dehydratase (FabZ) and β-hydroxydecanoyl ACP dehydratase (FabA) (Heath & Rock, 1995; Kass *et al.*, 1967; Kass & Bloch, 1967; Brock *et al.*, 1967). Both the enzymes effectively catalyze the dehydration reaction, with subtle differences in substrate specificities. FabA is most effective on acyl ACPs of 9–11 C atoms in length (Helmkamp *et al.*, 1968; Endo *et al.*, 1970; Morisaki & Bloch, 1971). Compared with FabA, FabZ is less specific and is more effective on short-chain and long-chain saturated and unsaturated acyl ACPs. In *Escherichia coli*, it was reported that FabZ has a specific activity that is 4.4-fold higher than that of FabA towards β-hydroxybutyryl ACP. Although both FabA and FabZ participate in the initial steps of the fatty-acid elongation cycle, FabZ is most efficient towards short-chain acyl ACPs and the overall activity decreases as the chain length increases (Heath & Rock, 1996).

The crystal structure of FabA from *E. coli* revealed that the bent chain of *cis*-unsaturated fatty acids does not fit into the 6 Å diameter wormhole that gives access to the active-site histidine residue (Leesong *et al.*, 1996). Thus, FabZ is the primary dehydratase involved in the elongation of the unsaturated branch of the pathway. Reduced FabZ activity selectively increases the level of intermediates in the saturated branch of the pathway, showing its specificity for long-chain fatty acids (Heath & Rock, 1996).

In contrast to humans, the malarial parasite *Plasmodium falciparum* synthesizes fatty acids by the dissociative mechanism, like bacteria and plants, making the pathway a potential target for the development of novel antimalarials (Waller *et al.*, 1998, 2000; Roos *et al.*, 1999). The parasite appears to lack the *fabA* gene. Thus, FabZ is the only dehydratase involved in the fatty-acid elongation cycle in *P. falciparum*.

In this paper, we report the purification and preliminary crystallographic studies of FabZ from *P. falciparum*. FabZ has a subunit molecular weight of 16 kDa (144 amino-acid residues) and exists as a dimer as determined by gel filtration. Comparison with *E. coli* FabA (subunit MW 18.8 kDa), which also is a functional dimer, shows that the sequence homology of these two proteins is confined to a 25-residue stretch close to the active histidine, 11 out of these 25 residues being identical. The active histidine residue of FabA is conserved in the FabZ sequence. Nevertheless, the size and the nature of the substrate-binding pocket appears to play a crucial role in determining

Figure 1
A crystal of FabZ from *P. falciparum*. The dimensions of the crystal are 0.45 × 0.15 × 0.05 mm.

the substrate chain length. Structure determination of FabZ will provide insights into its exquisite specificity and exact mechanism of action.

2. Materials and methods

2.1. Expression and purification

FabZ from *P. falciparum* was expressed in *E. coli* BL21 (DE3) cells as a fusion protein with an N-terminal His tag using the pET-28a(+) (Novagen) vector. The cultures were grown at 310 K in Luria broth (Hi-media, Delhi, India) until A_{600} reached 0.6 and then induced using 1 m*M* isopropyl β-D-thiogalactopyranoside and incubated for a further 12 h at 285 K. Cells were harvested, resuspended in lysis buffer containing 20 m*M* Tris pH 7.5, 0.5 *M* NaCl and 5 m*M* imidazole and lysed by sonication. The supernatant obtained was applied to a Ni–NTA metal affinity column (His-bind resin, Novagen) and protein was eluted using a step gradient of 0.3–0.5 *M* imidazole. The fractions were tested for purity by SDS–PAGE. The typical yield of the protein was 15 mg per litre of *E. coli* culture.

2.2. Crystallization

Crystallization was carried out at 293 K using the hanging-drop vapour-diffusion method by mixing 2 µl of approximately 10 mg ml^{-1} protein with 2 µl of well solution. Initial screening was performed using Hampton Research Crystal Screens I and II (Jancarik & Kim, 1991). Small crystals were seen in three different conditions. Based on these conditions, the pH range 4–9 was screened as the initial crystals appeared at pH 4.6 and 8.0. Slightly larger crystals were obtained in the pH 4–5 range with 20–30% PEG 4000 in 0.1 *M* acetate buffer and 0.2 *M* sodium acetate, ammonium sulfate or magnesium chloride as precipitants. Further improvement in the size and quality of the crystals was accomplished by refining these conditions and employing the microbatch method (diffusion through silicone oil) for crystallization. Crystals appeared in 2–3 d and reached maximum dimensions within a week. While the needle-shaped crystals obtained in the magnesium chloride condition did not diffract, those from the sodium acetate condition showed weak diffraction to 3.0 Å. The best crystals (Fig. 1) were obtained using ammonium acetate as the precipitant and diffracted to 2.5 Å resolution. A complete data set was collected using a single crystal obtained using the following conditions: 3 µl 12.5 mg ml^{-1} protein solution mixed with 3 µl 30% PEG 4000 and 0.2 *M* ammonium acetate in 0.2 *M* acetate buffer pH 4.5.

Table 1
Data-collection statistics.

Values in parentheses correspond to the last resolution shell (2.59–2.50 Å).

Space group	$I222/I2_12_12_1$
Unit-cell parameters	
a (Å)	71.78
b (Å)	81.99
c (Å)	97.49
Resolution (Å)	20–2.5
No. of reflections	58691
No. of unique reflections	10203
Completeness (%)	99.5 (99.9)
Average $I/\sigma(I)$	25.0 (5.1)
$R_{\rm merge}$ (%)	6.1 (38.0)

3. Data collection and preliminary X-ray analysis

X-ray diffraction data were collected using a MAR 345 imaging plate. The X-ray beam (Cu $K\alpha$ radiation) from a Rigaku Ultrax-18 rotating-anode X-ray generator operating at 50 kV and 80 mA was focused with an Osmic mirror system. A complete data set was collected to 2.5 Å resolution under cryo-conditions (100 K). The crystal was soaked in mother liquor containing 20% glycerol for 5 min and immediately flash-cooled using a nitrogen-gas stream. A total of 166 frames were collected with 1° oscillation and 1° frame width with an exposure time of 10 min per frame. The crystal-to-detector distance was 120 mm. The data were processed with *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystals belonged to space group $I222$ (or $I2_12_12_1$), with unit-cell parameters a = 71.78, b = 81.99, c = 97.49 Å. Details of data collection and processing are given in Table 1. The value of the Matthews coefficient (Matthews, 1968) is 2.2 Å^3 Da^{-1} and the solvent content is 45% assuming a dimer in the asymmetric unit.

Although the functional similarity between FabZ and FabA suggests similar structures for the two proteins, the absence of overall sequence homology between *P. falciparum* FabZ and *E. coli* FabA indicates that significant differences might exist between the two structures. Initial attempts to determine the structure of FabZ by the molecular-replacement method using the available coordinates of FabA (PDB code 1mka) and the program *AMoRe* (Navaza, 1994) did not yield a clear solution. The structure determination is also being attempted by the multiple isomorphous replacement method.

The intensity data were collected at the X-ray facility for structural biology at the Indian Institute of Science, supported by the Department of Science and Technology (DST) and the Department of Biotechnology (DBT), India. This work is supported by grants from the DBT to NS.

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