Crystal Structure of the Mycobacterium tuberculosis β-Ketoacyl-Acyl Carrier Protein Synthase III*

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Mycobacterial β-ketoacyl-ACP synthase III (KasB) is a multifunctional cysteine protease that catalyzes the elongation of fatty acyl-CoA substrates by β-branching and β-oxidation. Recently isolated kasB mutants of Mycobacterium tuberculosis exhibit enhanced drug resistance and are less susceptible to the drugs isoniazid and ethambutol. Understanding the structure of this enzyme thus has potential for the development of new therapeutic agents. The 2.1-Å crystal structure of kasB, from a kasB null mutant of M. tuberculosis, has been solved in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

An estimated annual incidence rate of 8 million people and an annual mortality rate of 3 million (1992) continue to make tuberculosis a serious worldwide health problem (1). The appearance of drug-resistant strains of M. tuberculosis and the human immunodeficiency virus pandemic have exacerbated this situation (2, 3). Effective treatment of tuberculosis infections requires the identification of both new drugs and drug targets. Fatty acid biosynthesis in pathogenic microorganisms is essential for cell viability and has recently attracted considerable interest as a target for development of new therapeutic agents (4–6). In these organisms, de novo fatty acid biosynthesis from an acetyl-CoA or related starter unit is typically catalyzed by a type II or disassociated fatty-acid synthase, composed of discrete enzymes (7). In contrast, de novo fatty acid biosynthesis in mammals and other higher organisms is catalyzed by a type I or associated fatty-acid synthase, composed of one or more multifunctional polypeptides (8).

Mycobacteria are unusual in that they possess both a type I and a type II fatty-acid synthase (Fig. 1) (9, 10). The type I fatty-acid synthase is responsible for formation of 16–24-carbon length fatty acids, which are then elongated to form long chain high molecular mass mycolates (11). These acids are high molecular mass α-alkyl-β-hydroxy fatty acids with the general structure R-CH(OH)-CH(R′)-COOH (where R is a meromycolate chain (50–56 carbons) and R′ is a significantly shorter chain (22–26 carbons)), which are key components of the mycobacterium cell wall. Triclosan and isoniazid are commonly used antibacterial agents that target mycolate biosynthesis (12). In the case of isoniazid, prevention of mycolate biosynthesis results from inhibition of the enoyl-acyl carrier protein (ACP)1 reductase (InhA) and possibly the ketoacyl-ACP synthase (KasA) (13–15). This latter enzyme is apparently responsible for catalyzing the decarboxylative condensation between an acyl-ACP and a malonyl-ACP in the carbon chain extension steps in mycolate biosynthesis and has also been shown to be inhibited by thiolactomycin (4, 15). A crystal structure has not been reported for KasA, but a hypothetical structure has been presented as an aid in drug design (4).

A ketoacyl-ACP synthase activity is also presumably needed to initiate the first decarboxylative condensation in mycolate biosynthesis. In other type II systems, this activity is provided by a β-ketoacyl synthase III (FabH, ketoacyl-ACP synthase III), which catalyzes a decarboxylative condensation between an acyl-CoA or similar substrate and malonyl-ACP (16, 17). By analogy, a ketoacyl-ACP synthase III activity that utilizes longer chain acyl-CoA substrates would be a link between the type I and type II fatty-acid synthases of M. tuberculosis.

In this study, we report that we have identified an M. tuberculosis FabH (mtFabH) that is able to preferentially use long chain acyl-CoA substrates such as myristoyl-CoA over acetyl-CoA.

At least two different mechanisms for generating the acyl-CoA substrates of KasA have been reported for KasA, but a hypothetical structure has been presented as an aid in drug design (4).

1 The abbreviations used are: ACP, acyl carrier protein; mtFabH, M. tuberculosis FabH; sgFabH, S. glaucescens FabH; ecFabH, E. coli FabH; SPA, scintillation proximity assay; PEG, polyethylene glycol.

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**EXPERIMENTAL PROCEDURES**

**Materials**—The following reagents were used: N-hydroxysuccinimido- diobiotin (Pierce); Escherichia coli acetyl carrier protein, imidazole, dithio- reitol, and malonyl-CoA (Sigma); [3H]acetyl-CoA (specific activity, of 60 Ci/mmol; Moravek Biochemicals, Inc.; [9,10-3H]myristoyl-CoA (specific activity, 60 Ci/mmol; American Radiochemical Chemicals); streptavidin-coated yttrium silicate scintillation proximity fluorospheres (SPA beads; Amersham Pharmacia Biotech); microbiological media (Difco); restriction enzymes and T4 DNA ligase (New England Biolabs Inc.); pET vector and expression strains (Novagen); Ni2+-agarose resin (QIA- GEN Inc.); and crystal screen kits 1 and 2 and polyethylene glycol (PEG) (4000; Hampton Research).

**Expression Plasmid of the M. tuberculosis fabH Gene in E. coli**—The putative fabH gene (Rv0533c) was amplified from M. tuberculosis (H37Rv) chromosomal DNA. The forward primer 5′-CAGATAGGACG-CATATGACGGAGATCG-3′ was designed to introduce an Ndel restriction site (underlined) at the start of the 5′-end of fabH. A BamHI site was created (underlined) downstream of the fabH stop codon in the reverse primer 5′-ATCCCTGCTGATGATGACGATCCC-3′. Polymerase chain reaction was performed using the GeneAmp® XL polymerase chain reaction kit (PerkinElmer Life Sciences). The resulting polymerase chain reaction product was eluted from agarose gel using Qiax (QIAGEN Inc.), digested with Ndel and BamHI, and ligated into Ndel/BamHI-digested pET15b to create pXH8. The insert coding sequence of FabH was verified by DNA sequence analysis.

**Purification of His-tagged mtFabH**—The pXH8 plasmid was used to transform E. coli BL21(DE3) pLysS cells (Novagen), and transformants were grown in LB medium to an absorbance at 600 nm of 0.35–0.4, with 0.5 mM isopropyl-β-D-thiogalactopyranoside, and incubated for an additional 3 h at 37 °C. Cells were harvested by centrifugation at 10,000 × g for 10 min at 4 °C and stored at −20 °C overnight. Lysis was performed with lysozyme according to the QIAexpressionist protocol, and lysate was then frozen at −70 °C until used. Thawed cells were sonicated at 12,000 × g for 30 min at 4 °C, and the supernatant was loaded onto a Ni2+-nitrilotriacetic acid-agarose column, which was then washed with 50 mM imidazole in 50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10% glycerol, and 3 mM β-mercapto- ethanol. mtFabH was eluted with 200 mM imidazole in the same buffer and dialyzed overnight against 50 mM Tris-HCl (pH 8.0), 10% glycerol, and 3 mM β-mercaptoethanol with either 300 or 50 mM NaCl at 4 °C. Purified protein was concentrated in a Centricon tube (Amicon, Inc.) with a molecular mass cutoff of 30,000 Da to 4–8 mg/ml as determined by the Bradford assay. Dithiothreitol was added to protein concentrates to 2 mM, and these concentrates were stored at 4 °C for 3–5 days. For longer storage of the enzyme, glycerol was added to 40–50%, and the aliquoted protein was stored at −20 °C.

**Radioactive FabH Assays**—Radioactive FabH assays using an SPA format and biotinylated malonyl-ACP were conducted essentially as described previously (18) using radioactive myristoyl-CoA. The standard reaction mixture contained the following components in a final volume of 20 μl: 0.15 μg of mtFabH or sgFabH (from Streptomyces glaucescens), 100 μM sodium phosphate buffer, 1% Triton X-100 (pH 7.0), 2.2 μM biotinylated malonyl-ACP, and 0.17 μM [9,10-3H]myristoyl-CoA (0.20 μCi; specific activity, 60 Ci/mmol). The reaction was initiated by the addition of [9,10-3H]myristoyl-CoA and incubated at 37 °C for various time periods. For each assay, 50 μl of the SPA bead solution (10 mg/ml) was added once the reaction was terminated.

**Enzyme Crystallization and Characterization**—Crystallization trials were performed by the vapor diffusion method, initially using Hampton crystal screen kits (20). Quasi-crystalline aggregates were obtained by monitoring a loss of malonyl-ACP. Malonyl-ACP (20 μM) was combined individually with different putative substrates (250 μM acetyl-CoA, propionyl-CoA, butyryl-CoA, octanoyl-CoA, lauroury-CoA, myristoyl-CoA, or palmitoyl-CoA) in 0.1× sodium phosphate buffer (pH 7.0) containing 1 mM dithiothreitol, 0.1% Triton X-100 in a final volume of 20 μl. The reaction was initiated by the addition of 0.8 μM of mtFabH, incubated at 37 °C for 60 min, and terminated on ice. The reaction mixture was then analyzed on a conformationally sensitive 13–15% polyacrylamide gel containing 2.5 μm urea, on which malonyl-ACP and the corresponding 3-ketoacyl-ACP products of a FabH-catalyzed reaction are readily resolved (19).

**X-ray Intensity Data Collection**—Form 1 crystals were flash-cooled in a cryoprotectant solution containing 80% reservoir solution and 20% PEG 400. Diffraction intensity data were collected at −170 °C on a
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Raxis II image plate detector with osmic confocal optics and a rotating anode source at 50 kV and 100 mA at a detector-to-crystal distance of 50 mm. Oscillation data frames were reduced, integrated, scaled, and merged with the HKL package (21). Merged intensity data were converted to structure factor amplitudes using the program Truncate (22).

Form 2 crystals were cryoprotected in 15% glycerol and 20% PEG 400 in crystallization reservoir solution and flash-frozen, and a half-sphere of crystals was reduced and described above. These data were extended to 2.1-Å resolution; showed no evidence of twinning; and were indexed in space group P2₁, with unit cell dimensions a = 64.1 Å, b = 54.8 Å, and c = 89.2 Å and β = 90.3°. The Matthews coefficient was consistent with a dimer of FabH in the asymmetric unit.

Structure Determination—A polyalanine chain based on residues 1–317 of a monomer of the refined structure of ecFabH (6, 23) (Protein Data Bank code 1EBL) was used as a search model for molecular replacement. In constructing the search model, the 4-residue insertion following residue 202 and the 1-residue insertion at position 263 of the ecFabH sequence were excluded. A cross-rotation search was carried out using data between 15 and 4 Å with the fast direct protocol (24) as implemented in CNS Version 1.0 (25). The solutions corresponding to the 15 highest peaks from the cross-rotation search were used as input in a translation search (26) as implemented in CNS Version 1.0. The presence of a dimer in the asymmetric unit was confirmed by cross-rotation and translation searches using a polyalanine chain based on the ecFabH dimer structure. In these searches, we noted that the orientations of the best monomer and dimer solutions were nearly identical and that the values of the monitor function in the translation search for the dimer were significantly higher for the best dimer solutions than for the best monomer solutions.

Model Building and Refinement—All model building was done using O Version 7.0 (27). An initial 2.8-Å SIGMAA weighted 2mFo – dFc (28) electron density map calculated using phases based on the best molecular replacement dimer solution was not readily interpretable. Two cycles of spot averaging of this map using a mask based on the ecFabH monomer with the Ave program (29) resulted in significant improvements in the quality of the map. The resulting map was readily interpretable and permitted placement of many side chains that were not present in the initial search model.

The model was iteratively refined via simulated annealing based on torsion-angle dynamics and a maximum likelihood target function (30) using CNS Version 1.0. Each refinement cycle was followed by manual rebuilding into mFo – dFc SIGMAA weighted cross-validated maps and mFo – dFc SIGMAA weighted cross-validated composite omit maps (31). Non-crystallographic symmetry was enforced via positional restraints between symmetry-related molecules. These restraints were initially assigned a weight of 300 kcal/mol Å, which was reduced to 37.5 kcal/mol Å in the final stages of the refinement. In the final stages of the refinement, non-crystallographic symmetry restraints on temperature factors were omitted from the restrained atomic B-factor refinement. Model phases were iteratively extended in steps to 2.1 Å over several cycles of refinement. During iterative rebuilding, residue geometries were monitored with the programs OOPs (32) and WHATCHECK (33).

In the final stages of the refinement, 278 solvent molecules, 7 glycerol molecules, and a ligand modeled as lauric acid (in monomer A) were added based on the presence of peaks with intensity >3σ in a SIGMAA weighted difference Fourier map. In the final stages of iterative rebuilding, SIGMAA weighted cross-validated difference Fourier maps calculated in the absence of solvent molecules contoured at 3 σ were used to assist in locating model errors. Coordinates in the refined structure were estimated using Crickshank's diffraction data precision indicator as implemented in the SPCHECK program (34). Refinement statistics are summarized in Table I.

RESULTS

Catalytic Activity of mtFabH with Long Chain Acyl-CoA Substrates—The translated open reading frame (Rv05366) of the

### Table I

| Data collection | Resolution (Å) | Rmerge | Refinement |
|----------------|---------------|--------|------------|
| Resolution (Å) | 2.1           | 0.12   |            |
| Rmerge | 0.262         | 0.281  | 0.319      |
| Rwork   | 0.222         | 0.281  | 0.319      |
| Rfree   | 0.974         | 0.978  | 0.978      |
| Complement, overall | 0.974 | 0.978  | 0.978      |
| Complement, highest resolution shell | 0.974 | 0.978  | 0.978      |
| Rotation angle (Å) | 31.7 | 32.089 | 32.089 |
| Bond length (Å) | 0.007 | 0.007  | 0.007      |
| Bond angles | 1.4° | 1.4°   | 1.4°       |
| Dihedral angles | 23.5° | 23.5°  | 23.5°      |
| Improper angles | 0.84° | 0.84°  | 0.84°      |
| Diffraction data precision indicator | 0.32 | 0.32   | 0.32       |

### Notes

| a | Rmerge = ΣΣ|Ih| – |Ih|/ΣΣ|Ih|, where Ih is the mean intensity of reflection h. All data with I > 3σ are included. |
| b | For reflections with Ih > 0, where Ih is the mean intensity of reflection h. |
| c | ω = (Ψ – Ψmin + σω)1/2, the expected minimum distance between two resolved atom peaks (34). |
| d | Calculated with reflections with F > 2σ. |
| e | Root mean square deviation. |

M. tuberculosis genome (35) was identified as having high sequence similarity to the identified FabH proteins of E. coli (ecFabH) and S. glaucescens FabH (sgFabH) (Fig. 2) (16, 17). The sequence of putative mtFabH contained all of the signature sequences for FabH enzymes, leading to the prediction that the protein would catalyze the condensation of an acyl-CoA substrate with a malonyl-ACP substrate. The phenylalanine residue (Phe37) proposed from the crystal structure of the E. coli ketoacyl-ACP synthase III to be important in restricting the acyl-CoA substrate specificity to carbon chain lengths of 2 or 3 (6) is a threonine in both mtFabH and sgFabH. sgFabH has been shown to accept a much greater range of acyl-CoA substrates than ecFabH (17), indicating that mtFabH might similarly be able to utilize longer acyl-CoA substrates.

To test this hypothesis, mtFabH was expressed, purified, and characterized as a recombinant protein with an N-terminal His tag. SDS-polyacrylamide gel electrophoresis analysis of the recombinant protein showed a molecular mass of 37,000 Da, and gel exclusion chromatography showed a native molecular mass of 76,600 ± 2500 Da, indicating that, like ecFabH and sgFabH, mtFabH is a homodimer. The recombinant protein was shown in an SPA to be active with the long chain saturated acyl-CoA.
the presence of short chain acyl-CoA substrates (C₂–C₄). In a
countrol experiment using sgFabH, a loss of malonyl-ACP
could be observed using these shorter chain acyl-CoA sub-
strates (data not shown). However, a loss of malonyl-ACP
substrate was observed when longer chain substrates (C₆–
C₁₆) were provided in the mtFabH enzyme assay. An inde -
pendent analysis recently carried out using a coupled assay
methodology has reported a similar substrate range for mt-
FabH, with the apparent preference for the substrate lauroyl-
CoA (36). These observations are consistent with the pro-
posed role of this enzyme in initiating mycolate fatty acid
biosynthesis and prompted attempts to crystallize and solve
the structure of this unique FabH.

Structure of mtFabH—The refined electron density map for
mtFabH was well resolved and continuous in all regions except
for fragmented density in molecule B from residues 24 to 50.
The amino-terminal extensions carrying the histidine tag, five
side chains in monomer A, and 32 side chains (14 between
residues 24–50) in monomer B have weak or missing electron
density.

As implied by the successful use of the ecFabH structure as
a search model in the molecular replacement solution of the
mtFabH structure, the backbone folds of these two molecules
are closely similar (Fig. 5). Excluding the two interior sequence
insertions at residues 202 and 263 and the amino- and carbox-
yl-terminal extensions of mtFabH (Fig. 2), the root mean
square deviation in main chain coordinates for the monomer
structures of ecFabH and mtFabH is 1.37 Å. The active-site
residues Cys112, His244, and Asn274 implicated in catalysis are
similarly disposed in mtFabH and ecFabH, as is the oxyanion
hole. The CoA/malonyl-ACP-binding channel and the interac-
tions that stabilize its structure are also almost identical in the
two enzymes. Differences occur at residues 144 and 210, which
are both prolines in mtFabH and arginine and asparagine,
respectively, in ecFabH. The side chain of Arg144 is spatially
replaced by the side chain of Lys141 in mtFabH, thereby main-
taining stabilization of the loops defining the channel.

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residues 24–50) in monomer B have weak or missing electron
density.
and their functional and biological significance, if any, is not obvious.

The 4-residue insertion at position 202 in mtFabH relative to ecFabH distorts the local conformation of this loop in a surprising way and creates a number of stabilizing intermonomer contacts. The alteration of this loop L9 (see nomenclature in Ref. 23), consisting of residues 191–204, by the 4-residue insertion at position 202 has the effect of inducing a new α-helix at positions 194–202 in mtFabH (Fig. 5). This new α-helix lies at the distal end of the putative acyl-binding channel inferred from the position of the acetyl group in the ecFabH crystal structure, and its possible functional significance is discussed below (Fig. 6). Differences in sequence between ecFabH and mtFabH upstream of the insertion site are consistent with the existence of an α-helix at this position in the latter, but not in the former. In ecFabH, Asn193, Asn198, and Pro199 would inhibit helix formation, whereas in mtFabH, these residues are Ile, Phe, and Ala, respectively.

The extended loops created as a result of the 4-residue sequence insertion converge at the non-crystallographic 2-fold symmetry axis relating the two monomers to make a number of stabilizing intermonomer contacts. The extended loops created as a result of the 4-residue sequence insertion converge at the non-crystallographic 2-fold symmetry axis relating the two monomers to make a number of stabilizing intermonomer contacts. The alteration of this loop L9 (see nomenclature in Ref. 23), consisting of residues 191–204, by the 4-residue insertion at position 202 has the effect of inducing a new α-helix at positions 194–202 in mtFabH (Fig. 5). This new α-helix lies at the distal end of the putative acyl-binding channel inferred from the position of the acetyl group in the ecFabH crystal structure, and its possible functional significance is discussed below (Fig. 6). Differences in sequence between ecFabH and mtFabH upstream of the insertion site are consistent with the existence of an α-helix at this position in the latter, but not in the former. In ecFabH, Asn193, Asn198, and Pro199 would inhibit helix formation, whereas in mtFabH, these residues are Ile, Phe, and Ala, respectively.

The extended loops created as a result of the 4-residue sequence insertion converge at the non-crystallographic 2-fold symmetry axis relating the two monomers to make a number of interactions that would stabilize the mtFabH dimer (Fig. 6). At their nexus, they create a small hydrophobic core about the 2-fold symmetry axis consisting of Phe198, Ile196, and Trp195 from each monomer, with the two Trp195 indole rings stacking on each other. The sequence differences relative to ecFabH at the positions creating this intermonomer hydrophobic locus in mtFabH are as follows: Asn198 → Phe, Arg199 → Ile, and Asp195 → Trp. These changes, plus the extra interactions at the amino termini of the mtFabH dimer, result in an additional 1384 Å² of contact area between the two monomers relative to ecFabH.

The single alanine insertion in mtFabH at residue 263 causes a local difference in conformation at a β-turn, which results in two less hydrogen bonds relative to ecFabH: Asn264(N) to the peptide oxygen of Asp239 and Asp239 to the peptide nitrogen of Leu288. These differences are compensated by formation of an ion pair between Arg261 and the carbonylate of Asp239. These changes are far from both the active site and the binding site of the enzyme and from the dimer interface, and their functional and biological significance, if any, is not obvious.

The electron density map of mtFabH shows significant continuous density in the site of monomer A corresponding to the location of the pantothentic acid moiety of CoA observed in the ecFabH structure (denoted binding channel 1) (Fig. 7). We have modeled this density as a lauric acid group extending from the active-site Cys112 to the open mouth of this binding channel. If this density represents an acyl group, it could possibly form a linkage with the active-site Cys112 sulfur, but both tenuous electron density and suboptimal stereochemistry argue against this. We believe that this group is a hydrophobic molecule taken up by mtFabH during purification, and its identity is currently under investigation.

Electron density in the other binding channel (binding channel 2) for long chain fatty acid, inferred by analogy to the Cys112-acetylated ecFabH complex, contains five discrete peaks assigned as solvent molecules. Two features of this site can explain the distinct substrate specificities of mtFabH and ecFabH. The presence of Phe87B (where B is monomer B) in this fatty acyl-binding site of ecFabH obstructs binding of straight fatty acid chains longer than ~4 carbons, thereby accounting for the selectivity of the E. coli enzyme for acetyl over longer chain substrates (6). In mtFabH, residue 87B is a threonine, whose smaller size permits binding of longer chain fatty acids (Fig. 8). The -O of Thr87B side chain is hydrogen-bonded to a bound solvent, thereby orienting the side chain methyl group toward the position of the acyl substrate and contributing to the hydrophobicity of its environment. This channel is also blocked in ecFabH by Arg199B and Leu191A (where A is
monomer A), which in mtFabH are isoleucine and glutamine, respectively, and by Ile^{203A} and Leu^{205A}, which are displaced in mtFabH by the changes around the insertion at position 202.

The end of the putative acyl-binding channel (channel 2) distal to the active-site Cys^{112} in mtFabH is capped by the α-helix at positions 194–202 induced just before the 4-residue insertion (Fig. 9). The Arg^{2024A} side chain, which is hydrogen-bonded to the peptide carbonyl oxygen of Pro^{144A}, blocks the end of this substrate channel, as do, to a lesser extent, the side chains of Gln^{191A}, Ile^{196B}, Phe^{198A}, Ala^{199B}, and Gln^{200B}. In the ecFabH structure, this area is open to solvent, but the inner part of the channel proximal to the active site is blocked by other residues as described above, preventing binding of longer chains.

**DISCUSSION**

Initiation of fatty acid biosynthesis in all type II systems studied to date requires the action of a specialized condensing enzyme, FabH (7, 17, 38). This enzyme catalyzes the condensation of an acyl-CoA substrate with malonyl-ACP to generate a 3-ketoacyl-ACP product. This product is reduced to an acyl-

The crystal structure of mtFabH reported here confirms its close similarity to the structure of ecFabH, which has recently been determined (6, 23). The active-site regions of both proteins are very similar, and both have a CoA/malonyl-ACP-binding site (binding channel 1). These observations are consistent with the fact that, in both enzymes, the latter half of the catalytic process involves release of CoA and a decarboxylative condensation between an acylated enzyme and malonyl-ACP. There are, however, several notable differences between the ecFabH and mtFabH dimer structures. The latter has a larger number of stabilizing intermonomer interactions than ecFabH as a result of sequence extensions at the amino terminus and of a 4-residue internal insertion. The amino terminus creates an arm that extends from each monomer and makes contacts with the opposite monomer of the dimer, whereas the internal insertion creates an added contact area in the dimer interface with stabilizing hydrogen bond and hydrophobic contributions. Although the significance of these observations is unclear, it is worth noting that a similar amino acid extension and internal insertion are observed in sgFabH (Fig. 2).

Finally, there are several unique features of mtFabH that have bearing on its distinct specificity and its potential role in regulating mycolate biosynthesis. In particular, sequence differences in the inferred binding channel 2 for long chain fatty acids between ecFabH and mtFabH explain the inability of ecFabH to utilize acyl-CoAs with chains longer than ~4 carbons in the acyl group. The mtFabH crystal structure now offers one plausible explanation why this enzyme can accept longer acyl-CoA substrates and also a structural basis for its apparent upper limit of 16 carbons in acyl-CoA chain length (36). As described above, the type I fatty-acid synthase in mycobacteria produces a bimodal (C_{14:0}–C_{16:0} and C_{24:0}–C_{26:0}) distribution of acyl-CoA fatty acids. It has been unclear whether one or both of these fatty acid products act as substrates for the synthesis of the long chain meromycolic acid (C_{50}–C_{56})-derived component of mycolates by the type II fatty-acid synthase (36). The specificity of mtFabH and the crystal structure now indicate that only the shorter acyl-CoA products (C_{14:0}–C_{16:0}) obtained from the type I fatty-acid synthase are elongated (Fig. 1). The longer chain acyl-CoA products (C_{24:0}–C_{26:0}) would thus be excluded from chain elongation and would remain available to be utilized, presumably in the coenzyme A form, as substrates for formation of the α-alkyl chain of the

**FIG. 8.** Modeled position of the myristoyl group in binding channel 2 showing the position of residue 87B, which is a threonine in mtFabH (blue) and a phenylalanine in ecFabH (green). Residue 87 is proposed to contribute to fatty acid chain length specificity for these two enzymes. T87B, Thr^{87B}; F87B, Phe^{87B}.

**FIG. 9.** Magnified view of the distal end of the myristoyl-binding site in mtFabH at the junction of the inserts of each monomer. The insert and preceding α-helix are shown in red, and the modeled myristoyl group (Ma and Mb in each subunit) is shown in lavender. Note the space in the FabH binding site for two more carbons on the end of myristoyl. R2024A, Arg^{2024A}; F198A, Phe^{198A}; A199B, Ala^{199B}; Q200B, Gln^{200B}; I196B, Ile^{196B}.

features that differentiate it from similar enzymes involved in de novo fatty acid biosynthesis.
mycolates (Fig. 1). The availability of these substrates might be markedly reduced if mtFabH used them to initiate meromycolic acid biosynthesis. Thus, we speculate that a combination of the bimodal distribution of fatty acids made by the type I fatty-acid synthase and the substrate specificity of mtFabH ensure the appropriate equimolar distribution of dramatically different chain length acyl thioester substrates required for mycolate biosynthesis.

It is widely accepted that mycolate biosynthesis is the main target of several front-line therapies for treating mycobacterial infections and that all of the enzymes in the unusual type II fatty-acid synthase involved in this process represent attractive targets for drug development (14). An important component of this system yet to be specifically targeted is mtFabH, which appears likely to play a key role in initiating and regulating mycolate biosynthesis. The availability of the structure of this enzyme and an SPA suitable for high throughput screening should facilitate the design, discovery, and development of much needed novel antimycobacterial agents.

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