Biochemical Characterization of Acyl Carrier Protein (AcpM) and Malonyl-CoA:AcpM Transacylase (mtFabD), Two Major Components of Mycobacterium tuberculosis Fatty Acid Synthase II*

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Malonyl coenzyme A (CoA)-acyl carrier protein (ACP) transacylase (MCAT) is an essential enzyme in the biosynthesis of fatty acids in all bacteria, including Mycobacterium tuberculosis. MCAT catalyzes the transacylation of malonate from malonyl-CoA to activated holo-ACP, to generate malonyl-ACP, which is an elongation substrate in fatty acid biosynthesis. To clarify the roles of the mycobacterial acyl carrier protein (AcpM) and MCAT in fatty acid and mycolic acid biosynthesis, we have cloned, expressed, and purified acpM and mtfabD (malonyl-CoA:AcpM transacylase) from M. tuberculosis. According to the culture conditions used, AcpM was produced in Escherichia coli in two or three different forms: apo-AcpM, holo-AcpM, and palmitoylated-AcpM, as revealed by electrospray mass spectrometry. The mtfabD gene encoding a putative MCAT was used to complement a thermosensitive E. coli fabD mutant. Expression and purification of mtFabD resulted in an active enzyme displaying strong MCAT activity in vitro. Enzymatic studies using different ACP substrates established that holo-AcpM constitutes the preferred substrate for mtFabD. In order to provide further insight into the structure-function relationship of mtFabD, different mutant proteins were generated. All mutations (Q9A, R116A, H194A, Q243A, S91T, and S91A) completely abrogated MCAT activity in vitro, thus underlining the importance of these residues in transacylation. The generation and characterization of the AcpM forms and mtFabD opens the way for further studies relating to fatty acid and mycolic acid biosynthesis to be explored in M. tuberculosis. Since a specific type of FabD is found in mycobacterial species, it represents an attractive new drug target waiting to be exploited.

Mycobacterium tuberculosis is able to survive and replicate within the hostile environment of host macrophages that produce microbicidal molecules, usually sufficient to kill other bacteria. The relative impermeability of the mycobacterial cell wall contributes largely to the intrinsic resistance of the bacterium to these microbicidal factors (1). The cell wall is based on a conventional peptidoglycan, covalently linked to an arabinogalactan esterified with mycolic acids. Mycobacterial mycolic acids are high molecular weight (C60-C90) α-alkyl, β-hydroxy fatty acids and represent 40–60% by weight of the mycobacterial cell envelope (2). Mycolic acids form the basis of a complex lipid bilayer outer membrane, which constitutes a permeability barrier of extremely low fluidity (3). The inner leaflet is made up of the highly structured covalently bound mycolic acids arranged perpendicular to the cell wall arabinogalactan, and the outer leaflet is made up of other complex free lipids (1–4). Due to the essential role of mycolic acids in intracellular survival of M. tuberculosis, the biosynthesis and assembly of these structures offer potential targets for chemotherapeutic intervention. Several components of the mycolic acid biosynthetic pathway, such as enoyl-ACP reductase, have already been described as targets for important antitubercular drugs (5–9).

Fatty acid biosynthesis in mycobacteria involves at least two different types of enzyme systems, fatty acid synthase (FAS)I and FAS-II (10). FAS-I is a single polypeptide with multiple catalytic activities generating short-chain acyl CoA esters (11), which then serve as precursors for elongation by other fatty acid and polyketide synthases (PKS). In contrast to FAS-I, FAS-II consists of dissociable enzyme components, which act upon a substrate bound to an acyl carrier protein (ACP), recently designated as AcpM in M. tuberculosis (12). FAS-II is incapable of de novo fatty acid synthesis, but elongates myristoyl-ACP and palmitoyl-ACP to long chain fatty acids ranging from 24 to 56 carbons in length (10). We have recently shown that mtFabH, a β-ketoacyl-ACP synthase, forms a pivotal link between the type I and type II FAS elongation systems in M. tuberculosis. Indeed, mtFabH uses lauroyl-CoA (C12) and myristoyl-CoA (C14) to generate myristoyl-ACP (C14) and palmitoyl-ACP (C16), respectively, the preferred substrates of the FAS-II system (13). We have also demonstrated that the β-ketoacyl-ACP synthase A (KasA) belongs to the FAS-II sys-

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1 The abbreviations used are: FAS, fatty acid synthase; ACP, acyl carrier protein; ES-MS, electrospray mass spectrometry; Kas, β-ketoacyl-ACP synthase; MCAT, malonyl-CoA:ACP transacylase; ml, M. leprae; mt, M. tuberculosis; PKS, polyketide synthase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis.
and catalyzes the first elongation step by condensing a two-carbon unit from malonate (malonyl-AcpM) to a pre-existing carbon chain esterified to the phosphopantetheine moiety of AcpM. The reaction requires activated malonate in the form of malonyl-AcpM as a substrate. In bacteria, transacylation of holo-ACP with malonate involves malonyl-CoA and the enzyme malonyl-CoA:ACP transacylase (MCAT) encoded by fabD (14). Analysis of the M. tuberculosis genome (15) revealed that acpM (Rv2244) is genetically linked to Rv2243 whose product is homologous to FabD proteins from various microorganisms (16–18). Both acpM and mtfabD belong to the same transcriptional unit that also includes kasA and kasB.

In this study, we overexpressed, purified, and characterized M. tuberculosis AcpM and mtFabD. Overexpression of AcpM in Escherichia coli produced holo-AcpM, apo-AcpM, and palmitoylated-AcpM. The relative abundance of each form could be modulated by the growth conditions, a useful feature in generating valuable substrates (holo-AcpM and palmitoylated-AcpM) for future studies in fatty acid and mycolic acid biosynthesis in M. tuberculosis. Furthermore, we provide evidence that the product encoded by mtfabD catalyzes MCAT activity both in vivo and in vitro using E. coli holo-ACP and M. tuberculosis holo-AcpM, respectively, as substrates for transacylation.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—All cloning steps were performed in E. coli Top10 (Invitrogen). Liquid cultures of recombinant E. coli were grown in LB broth at 37 °C with either 150 μg/ml ampicillin or 25 μg/ml kanamycin, according to the selection marker present on the plasmid. Strain LA2-89, a thermosensitive E. coli fabD mutant (fabD89), was a generous gift from A. R. Stuitje (16). E. coli C41(DE3), which expresses the T7 RNA polymerase, was used as a host for the overproduction of M. tuberculosis AcpM and mtFabD and was kindly provided by B. Miroux and J. E. Walker (19).

Plasmids and DNA Manipulation—Restriction enzymes and T4 DNA ligase were purchased from Roche, and Vent DNA polymerase was purchased from New England Biolabs. All DNA manipulations were performed using standard protocols, as described by Sambrook et al. (20).

Cloning of M. tuberculosis AcpM in E. coli—The acpM genes (Rv2244) was amplified by PCR from M. tuberculosis H37Rv genomic DNA using the following primers: acpM-sense, 5'-cgt cag cag aga aat cat-3' (containing a NdeI restriction site, underlined); and acpM-antisense, 5'-cga att atc act tgg act ccg cca a-3' (containing an EcoRI restriction site, underlined). The resulting McAT activity both in vivo and in vitro using E. coli holo-ACP and M. tuberculosis holo-AcpM, respectively, as substrates for transacylation.

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Expression and Purification of the M. tuberculosis AcpM—An overnight culture of E. coli C41(DE3) carrying pET28a::mtfabD was used to inoculate a large volume of LB broth supplemented with 25 μg/ml ampicillin and 150 μg/ml kanamycin and incubated at 37 °C under shaking until the optical density reached 0.75. The culture was then induced with 1 mM isopropyl-β-D-thiogalactoside and grown on LB medium containing 2 g/liter NaCl and supplemented with 150 μg/ml ampicillin. Plates were incubated at either 37 °C (permissive temperature) or 40 °C (non-permissive temperature).

mtFabD Mutagenesis—Different mtFabD mutant proteins were constructed as templates for the QuikChange site-directed mutagenesis kit (Stratagene) with the following primers: Q9A, 5'-ttg ctc gca ccc gga gag tgg cag g-3' and 5'-cgt cag cag aga aat cat-3' (containing a NdeI restriction site, underlined); and acpM-antisense, 5'-cga att atc act tgg act ccg cca a-3' (containing an EcoRI restriction site, underlined). The 365-base pair PCR product was purified, digested with NdeI and EcoRI, and ligated into pET28a (Novagen) that had been digested with the same enzymes. The DNA insert was sequenced to verify the absence of PCR artifacts. The resulting plasmid designated pET28a::acpm was used to transform E. coli C41 (DE3) for overproduction of the recombinant protein.

MCAT Assay—mtFabD was assayed by its ability to catalyze the transfer of the malonyl group from [2-14C]malonyl-CoA to holo-AcpM. Briefly, a reaction mixture containing 10 μM malonyl-CoA (9.2 μM unlabeled malonyl-CoA and 0.8 μM [2-14C]malonyl-CoA [36,000 cpm]), 5 mM diethiothreitol, 50 mM potassium phosphate buffer (pH 6.8), and 10 mM mtFabD in a final volume of 100 μl was incubated for 5 min at room temperature during which the malonyl-CoA substrate did not become rate-limiting. The reaction was subsequently quenched by the addition of 500-5000 μM trichloroacetic acid. Precipitation was completed by incubation on ice for 10 min. Centrifugation at 18,000 × g for 10 min yielded a pellet that was washed with 10% ice-cold trichloroacetic acid and resuspended in 20 μl of 2% SDS containing 20 μl aqueous NaOH. The total cpm of radiolabeled material in the suspension was measured by scintillation counting using 5 ml of EconoStain.

The apparent Km values of mtFabD for M. tuberculosis AcpM were

2 L. Kremer and G. S. Besra, unpublished results.
mately growing cells were induced with 1 mM isopropyl-
β-D-thiogalactopyranoside and incubated either for an additional 3 h at 37 °C (sample A) or overnight at 16 °C in fresh Terrific Broth medium (sample B). After lysis of the cells and centrifugation, the different clarified soluble lysates were loaded onto Ni²⁺-chelating chromatography columns. M indicates the molecular mass marker; lane 1, purified AcpM from sample A; lane 2, purified AcpM from sample B; lane 3, purified holo-AcpM obtained by loading fractions from the Ni²⁺-affinity column onto a thiopropyl-Sepharose 6B column. Proteins were separated on a 15% SDS-PAGE and visualized by staining with Coomassie Blue.

determined by initial velocity measurements under standard conditions using variable concentrations of holo-AcpM. The apparent Kₘ value of mtFabD for malonyl-CoA was determined using 20 μM holo-AcpM and variable concentrations of malonyl-CoA with a fixed activity of [2-14C]malonyl-CoA as described in the standard assay.

**Protein Analysis**—Proteins were separated by SDS-PAGE on a Mini-Pretean II system (Bio-Rad, Hertfordshire, United Kingdom) and stained with Coomassie Blue R350 (Amersham Pharmacia Biotech, Uppsala, Sweden). Protein concentrations were determined using the BCA protein assay reagent kit (Pierce Europe, Oud-Beijerland, Netherlands).

Electrospray Mass Spectrometry (ES-MS) and N-terminal Sequencing—Mass spectrometry was carried out on a Micromass Platform II single quadrupole mass spectrometer. The sample was delivered into the mass spectrometer by an Agilent 1050 liquid chromatograph after in-line desalting. Edman degradation was carried out on an Agilent Edman sequencer.

**RESULTS**

Expression and Purification of AcpM—In this study, we have cloned the *M. tuberculosis* acpM into an *E. coli* expression vector, allowing AcpM to be produced as a His-tagged protein. Different culture conditions were used, as described under “Experimental Procedures,” to generate sample A and sample B. Recombinant proteins were overexpressed and purified by affinity chromatography using a Ni²⁺-affinity column. Analysis by SDS-PAGE of the eluted fractions from sample A showed the presence of three distinct protein bands (Fig. 1, lane 1), which were subjected to automated N-terminal sequencing. The sequence obtained, GGSHHHHHHHGLVPRGSFM, was consistent with the expected N terminus of AcpM (minus the N-terminal Met residue). This was confirmed by ES-MS of the intact protein and was also a characteristic feature of the proteins analyzed from sample B by ES-MS. The major mass spectral peaks from sample A are shown in Fig. 2. The calculated mass of the apo-AcpM (based on the amino acid sequence and taking into account the N-terminal data) is 14,556 Da. The holo-AcpM form of the protein (after addition of the 4'-phosphopantetheine group, 339 Da) has a molecular mass of 14,895 Da. The analysis by ES-MS demonstrates that the major product of sample A is apo-AcpM with significant amounts of holo-AcpM and palmitoylated-AcpM assuming an error of ±2 Da (Fig. 2). In contrast, sample B contains predominantly holo-AcpM and palmitoylated-AcpM with a minor peak observed at 14,554 Da for apo-AcpM, by ES-MS analyses (data not shown). Analysis by PAGE (Fig. 1, lane 2), supported by the above ES-MS analysis, confirms the identity of the middle band as apo-AcpM. Subsequent purification of sample B using thiopropyl-Sepharose 6B chromatography yielded the top band (Fig. 1, lane 3), which by ES-MS analysis (data not shown) was confirmed as holo-AcpM and a substrate for mtFabD (see below). Therefore, the identity of the lower band was deduced as palmitoylated-AcpM. Interestingly, treatment of either sample A or B with dithiothreitol cleaved the acyl group from palmitoylated-AcpM, yielding higher concentrations of holo-AcpM (data not shown), also confirming the identity of the lower band by PAGE analysis as an acyl-AcpM product. Altogether, the N-terminal sequencing and ES-MS analysis demonstrate that both samples contain AcpM, and appear to contain apo-, holo-, or palmitoylated forms, although present in different relative proportions. Sample A consists of apo-AcpM > palmitoylated AcpM > holo-AcpM, whereas sample B consists of holo-AcpM > palmitoylated AcpM >> apo-AcpM. In addition, these differences in intensities do appear to correspond to those seen on PAGE analysis (Fig. 1).

Amino Acid Sequence Alignment of Rv2243 with Various FabD Proteins—The predicted product of the first open reading frame (Rv2243) of the *M. tuberculosis* kasAB operon is a 30.7-kDa protein that bears strong similarity with MCAT (FabD) from various microorganisms as shown in Fig. 3. Rv2243 is 29% and 51% identical to its *E. coli* and *Streptomyces coelicolor* A3 FabD, respectively. A high resolution crystal structure of *E. coli* FabD has been reported (22) and has revealed that Ser-92 and His-201 are involved in catalysis. In addition, the main chain carboxyl of Gln-250 serves as a hydrogen bond acceptor in an interaction with His-201. Two other residues, Arg-117 and Gln-11, have also been shown to be located in the active site (22). The five catalytic residues belonging to the *E. coli* FabD (Gln-11, Ser-92, Arg-117, His-201, Gln-250) are fully conserved among the different organisms and are referred to as Gln-9, Ser-91, Arg-116, His-194, and Gln-243 in the mycobacterial protein (Fig. 3). Interestingly, the catalytic Ser-91 is located at the center of a GHSVG pentapeptide, which corresponds to the GXGXG signature motif of serine-dependent acylhydrolases (23).

Complementation of an *E. coli* fabD Mutant—To provide evidence of the function of *M. tuberculosis* Rv2243, complementation experiments were undertaken. The gene was amplified by PCR and cloned into pUC18. The construct, named pUC18::mtFabD, was used to transform *E. coli* LA2-89 temperature-sensitive mutant deficient in MCAT activity. This strain carries an amber mutation in the *fabD* gene together with a supE tRNA suppressor (24). The complementation of strain LA2-89 with pUC18::mtFabD at 37 °C (permissive temperature) and 40 °C (non-permissive temperature) are shown in Fig. 4. The complemented strain was able to grow at both 37 °C and 40 °C, whereas the control strain (LA2-89 transformed with pUC18) failed to grow at the non-permissive temperature of 40 °C, directly demonstrating that Rv2243 possesses MCAT activity in *E. coli*.

Enzymatic Activity of Purified *M. tuberculosis* mtFabD—A hexa-histidine (His₆)-tagged mtFabD protein was overexpressed in *E. coli* and purified by affinity chromatography as shown in Fig. 5. Its observed molecular mass of 33 kDa is consistent with the calculated molecular mass of mtFabD (30.7 kDa) plus the 6-His containing N-terminal extension. The purified protein was used to determine its enzymatic characteristics. Initial velocities were first measured as a function of malonyl-CoA concentration (Fig. 6A). The double-reciprocal plot analysis indicated that mtFabD has an apparent Kₘ for malonyl-CoA of 12.6 μM (Fig. 6A). In a second series of experiments, the concentration of malonyl-CoA was kept constant, whereas increasing concentrations of holo-AcpM were added to the reaction mixture. Under these conditions, an apparent Kₘ
for holo-AcpM of 14.1 μM was obtained (Fig. 6B). Holo-AcpM constituted a better substrate than E. coli holo-ACP, since it was more rapidly malonylated by M. tuberculosis MCAT (Fig. 6C). Nevertheless, the mycobacterial mtFabD was able to convert the E. coli holo-ACP into malonyl-ACP, confirming the previous data obtained in vivo in the complemented fabD mutant LA2-89 strain.

Construction and Activity of Various mtFabD Mutant Proteins—Extensive analysis of acyltransferases from various fatty acid or polyketide synthases has demonstrated that a serine, such as Ser-92 in the E. coli FabD protein (16, 22, 25), is the active nucleophilic residue of these enzymes. During its transfer from malonyl-CoA to ACP, the malonyl moiety is transiently attached to this serine to form a stable malonyl-serine enzyme intermediate (26). Nucleophilic attack of this ester by the sulfhydryl of ACP yields malonyl-ACP. An alignment of different FabD enzymes from various organisms suggested the assignment of Ser-91 as the active residue in mtFabD (Fig. 3). Therefore, we constructed and analyzed a number of mtFabD mutant proteins (Fig. 7). In two of these mutant proteins, designated S91A and S91T, in which either Ala or Thr substituted the active Ser, no MCAT activity could be detected, indicating that Ser-91 plays a key role in catalysis (Fig. 7B). Based on the three-dimensional structure of the E. coli protein, four other residues were also shown to play a critical role in catalysis. Alignments presented in Fig. 3 revealed that these residues are fully conserved among various microorganisms. We replaced Gln-9, Arg-116, His-194, and Gln-243 individually by alanine, and the purified proteins were analyzed for MCAT activity (Fig. 7, A and B). MCAT activity of all mutants was abolished, confirming the importance of these amino acids in transacylation (Fig. 7B). Incubation of the wild-type mtFabD and mutant mtFabD proteins with 14C-labeled malonyl-CoA and analysis by SDS-PAGE autoradiography showed that the malonyl group could be covalently attached to some of the proteins, i.e. Q9A and R116A, in addition to wild-type mtFabD (Fig. 7C).

DISCUSSION

It has been previously shown that FabD is a critical enzyme involved in fatty acid biosynthesis in all bacteria, by catalyzing the transacylation reaction of malonate from malonyl-CoA to holo-ACP; malonyl-ACP is a key substrate for elongation of fatty acids. In M. tuberculosis, mycolic acids are known to be essential for viability and pathogenicity (10), but details of the mycolic acid biosynthetic pathway are not well understood. In this study, we have cloned, purified, and characterized both mtFabD and AcpM from M. tuberculosis in order to investigate their role in mycolic acid biosynthesis.

When ACP encoding genes from various organisms are ex-
pressed in *E. coli*, the apo-protein without an attached 4′-phosphopantetheine prosthetic group dominates. For instance, the ACPs from *Sinorhizobium meliloti* and *Streptococcus pneumoniae* have been overproduced and isolated from *E. coli* as apo-versions of ACP (27, 28). On the other hand, overexpression in *E. coli* of the ACPs from *Bacillus subtilis* (17) and *Pseudomonas aeruginosa* (29) generated the recombinant ACP containing the 4′-phosphopantetheine group. We show here that, depending on the culture conditions used, two or three different AcpM forms can be isolated in various amounts from recombinant *E. coli*, corresponding to apo-, holo-, and palmitoylated AcpM. Also, N-terminal amino acid sequence analysis of purified AcpM revealed that the N-terminal methionine was probably removed by an aminopeptidase (30), as has been observed with other ACPs (17, 29, 31, 32). When cells were incubated overnight at 16 °C in fresh Terrific Broth medium, the apo-AcpM was almost completely converted to holo-AcpM by ligation of 4′-phosphopantetheine. The amount of palmitoylated-AcpM, regardless of the induction conditions used, is unusual and may reflect the high affinity of holo-AcpM to this fatty acid. This is an important feature of the heterologous *E. coli*.

![Protein sequence comparisons of MCAT from different microorganisms](image1)

**FIG. 3.** Protein sequence comparisons of MCAT from different microorganisms. *Bs*, *B. subtilis*; *Ec*, *E. coli*; *Mt*, *M. tuberculosis*; *Sc*, *S. coelicolor* A3. Dots represent conserved residues involved in catalysis.

![Complementation of E. coli fabD89 temperature-sensitive MCAT mutant](image2)

**FIG. 4.** Complementation of *E. coli* fabD89 temperature-sensitive MCAT mutant. LA2-89 strain was transformed with either pUC18 or pUC18::mtfabD, and plates incubated overnight at either 37 °C (permissive temperature) or at 40 °C (non-permissive temperature).

![Purification of mtFabD](image3)

**FIG. 5.** Purification of mtFabD. mtFabD was expressed as a His-tagged protein and purified by Ni²⁺-chelate chromatography as described under “Experimental Procedures.” Purity of the recombinant protein was analyzed by 10% SDS-PAGE and visualized by Coomassie Blue staining. *M*, molecular mass marker; lane 1, purified mtFabD.
coli system, in that it obviates the need for the mycobacterial ACP synthase protein to convert inactive apo-AcpM into its activated holo-product for use in subsequent acylation reactions. In addition, the generation of palmitoylated-AcpM and the ease of separation of holo-AcpM from palmitoylated-AcpM provide valuable substrates for studies relating both to mtFabD and to KasA. In this regard, we have recently shown that palmitoylated-AcpM is the preferred substrate of KasA, which is involved in fatty acid and meromycolic acid elongation.2

Until recently, only ACPs expressed constitutively and involved in the biosynthesis of essential fatty acids were known. The discovery of specialized ACPs for the biosynthesis of polyunsaturated fatty acids in Rhizobium meliloti (33) and in Rhizobium leguminosarum (34) were thought to represent unusual cases involved in complex secondary metabolism. Recently, a second ACP was isolated from R. leguminosarum and was shown to be involved in the transfer of 27-hydroxoyctacosanoic acids during lipid A biosynthesis (35). AcpM from M. tuberculosis, which presents the highest sequence identity to FabC from Streptomyces glaucescens (36), is an unusual ACP in re-
loration to its size compared with the E. coli ACP. AcpM consists of a longer polypeptide that may be important for its specializes role in transferring very long chain meromycolic acids (up to 56 carbons) to the different lipogenic centers of the FAS-II enzyme system. Yuan et al. (37) have recently demonstrated that modification of the meromycolic acid chain, through the addition of methyl groups, occurs in parallel with the synthesis of the AcpM-bound meromycolate chain. Treatment of M. tuberculosis by isoniazid was accompanied by a marked up-regulation of both KasA and AcpM (9, 12) and studies based on microarray hybridization have also shown that mtFabD, acpM, kasA, kasB, and accD6 are induced by treatment with either isoniazid or ethionamide (38). In addition, acpM is located in the same transcriptional unit, between mtFabD and kasA. Altogether, this suggests that the expression of acpM may also be regulated. Interestingly, analysis of the M. tuberculosis genome reveals the presence of at least two other putative ACPs, Rv0033 and Rv1344. Preliminary studies with Rv0033 (mtAcp1) have shown that it is produced as the apo-form of the protein in E. coli (data not shown). Further characterization of these putative ACPs and their participation in general fatty acid biosynthesis is currently under investigation.

The open reading frame Rv2243 encodes MCAT and complements the temperature-sensitive fabD89 mutation in E. coli LA2-89, indicating that mtFabD is able to interact with the components of the E. coli FAS-II system to reconstitute fatty acid biosynthesis. In vitro, mtFabD catalyzed transacylation of M. tuberculosis holo-AcpM more efficiently than that of the E. coli holo-ACP. In E. coli, the active site of FabD is located in a cleft between two subdomains (22). The nucleophile Ser-92 is located in a sharp turn between a β-strand and an α-helix within the major subdomain. Mutants of the mtFabD protein in which either Thr or Ala substituted the Ser-92 completely highlighting their importance in structure-function relationship. However, two of the mutants (Q9A and R116A) were still able to bind to malonyl-CoA, although to a lesser extent than wild-type mtFabD.

The M. tuberculosis genome is unusual in that it encodes for at least 18 different PKSs that may be involved in the formation of complex lipids. Recently, it has been shown that cell wall-associated polyketides play an important role in mycobacterial virulence (39, 40). However, the genetics and biochemical steps involved in these elaborate pathways remain largely unknown. The existence of a functional connection between fatty acid metabolism and the polyketide antibiotic tetracenomycin C has recently been demonstrated in S. glaucescens. It has been shown that FabD constitutes a link between both pathways (36, 41). The same conclusion was also reached about the possible role in actinorhodin synthesis of MCAT isolated from S. coelicolor (18, 42). Therefore, it would be interesting to investigate whether mtFabD may act on both the FAS-II system involved in mycolic acid biosynthesis and in certain PKS systems.

Interestingly, another gene encoding a putative MCAT is present in the M. tuberculosis genome. This gene (Rv0649, fabD2) encodes a protein of 224 amino acids with a molecular mass of 23.6 kDa and displays 28% identity with Rv2243. Surprisingly, this enzyme does not contain the characteristic GXXGX motif of mtFabD, suggesting that other residues replacing the active serine in this motif may be involved in catalysis. This hypothesis is strengthened by recent studies on the MCAT from S. coelicolor (42), where it was observed that a S97A mutant was still covalently labeled by malonyl-CoA, indicating that the serine nucleophile is dispensable for MCAT activity. This suggests that the MCAT from S. coelicolor possesses an alternative nucleophile group that is capable of substituting for the active site serine in the S97A mutant. Thus, further studies are required to determine whether FabD2 also possess MCAT activity. However, analysis of the Mycobacterium leprae genome (43), which is now regarded as a “minimal genome” has revealed that, although mtFabD was present and highly homologous to its M. tuberculosis counterpart, there was no gene homologous to fabD2 in M. leprae. This suggests that mtFabD represents the essential enzyme possessing MCAT activity involved in fatty acid and mycolic acid biosynthesis in M. tuberculosis. Thus, the discovery of molecules that specifically inhibit mtFabD and mtFabD may lead to the development of new therapeudic anti-leprosy and anti-tubercular agents.

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Biochemical Characterization of Acyl Carrier Protein (AcpM) and Malonyl-CoA:AcpM Transacylase (mtFabD), Two Major Components of *Mycobacterium tuberculosis* Fatty Acid Synthase II

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