Identification, Substrate Specificity, and Inhibition of the Streptococcus pneumoniae β-Ketoacyl-Aeryl Carrier Protein Synthase III (FabH)*

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In the bacterial type II fatty acid synthase system, β-ketoacyl-acyl carrier protein (ACP) synthase III (FabH) catalyzes the condensation of acetyl-CoA with malonyl-ACP. We have identified, expressed, and characterized the Streptococcus pneumoniae homologue of Escherichia coli FabH. S. pneumoniae FabH is ~41, 39, and 38% identical in amino acid sequence to Bacillus subtilis, E. coli, and Hemophilus influenzae FabH, respectively. The His-Asn-Cys catalytic triad present in other FabH molecules is conserved in S. pneumoniae FabH. The apparent $K_{\text{m}}$ values for acetyl-CoA and malonyl-ACP were determined to be 40.3 and 18.6 μM, respectively. Purified S. pneumoniae FabH preferentially utilized straight short-chain CoA primers. Similar to E. coli FabH, S. pneumoniae FabH was weakly inhibited by thiolactomycin. In contrast, inhibition of S. pneumoniae FabH by the newly developed compound SB418011 was very potent, with an $IC_{50}$ value of 0.016 μM. SB418011 also inhibited E. coli and H. influenzae FabH with $IC_{50}$ values of 1.2 and 0.59 μM, respectively. The availability of purified and characterized S. pneumoniae FabH will greatly aid in structural studies of this class of essential bacterial enzymes and facilitate the identification of small molecule inhibitors of type II fatty acid synthase with the potential to be novel and potent antibacterial agents active against pathogenic bacteria.

Fatty acid biosynthesis in bacteria, plants and animals is carried out by the ubiquitous fatty acid synthase (FAS)1 system. In the type I system of animals, including humans, FAS is a homodimer of two large polypeptides, each comprised of several distinct enzyme domains and an integral acyl carrier protein (ACP) (1, 2). In the type II systems of bacteria (3), plants (4), and protozoa (5), the FAS components, including the ACP, exist as discrete proteins. The corresponding enzymes of the type I and II FASs are related in structure and function but generally lack overall sequence homology. The essentiality of type II FAS for bacterial viability together with major differences between it and type I FAS suggest that broad-spectrum anti-bacterial drugs may be obtained by screening for inhibitors of the bacterial components (6–8).

In the type II FAS system, β-ketoacyl-ACP synthase (KAS) enzymes are central to the initiation and elongation steps and play a pivotal role in the regulation of the entire pathway (9). KAS I (FabB), II (FabF), and III (FabH) catalyze the condensation of malonyl-ACP with either acetyl-CoA (in the case of FabH) or the growing ACP-linked acyl chain to form the corresponding β-ketoacyl-ACP substrate for the subsequent reduction step in the elongation cycle catalyzed by FabG. FabH acts via a ping-pong mechanism (10, 11) and is unique among KAS in that it utilizes acetyl-CoA as an acyl group donor whereas FabB (12) and FabF (13) both utilize acyl-ACPs as primers. Also, unlike FabB and FabF, which are sensitive to both cerulenin and thiolactomycin (TLM), FabH is insensitive to cerulenin and much less sensitive to TLM (3, 12, 14, 15). FabH also possesses acetyl-CoA:ACP transacylase activity in vitro, albeit at a rate ~200-fold lower than that of the condensing activity. The physiological role of the transacylase reaction is unknown.

The crystal structure of Escherichia coli FabH was recently solved (16–18) and shown to display a five-layered core structure that, despite the lack of overall sequence similarity, is similar to that of FabF (19) and other condensing enzymes of known structure (20, 21). Consistent with our biochemical data (22), both in the presence and absence of acetyl-CoA, the structure has a quasi-2-fold symmetry (16, 17). In addition, co-crystallization of FabH with acetyl-CoA provided direct evidence that Cys-112 is the catalytic nucleophile (16, 17).

Interestingly, despite the similar folds, the substrate specificities for FabH from various bacterial species appear to be quite different. E. coli FabH was most selective for acetyl-CoA and inactive with longer chain acyl-CoAs (23). In contrast, Bacillus subtilis FabH displayed low activity with acetyl-CoA but was active with 4- to 8-carbon straight-chain acyl-CoA and all branched-chain acyl-CoA molecules (24), whereas the order of reactivity of Streptomyces glaucescens FabH was butyryl-CoA→acetetyl-CoA→isobutyryl-CoA (25). Recently, Choi et al. (26) demonstrated that the FabH homolog of Mycobacterium tuberculosis prefers long-chain (C10-C16) acyl-CoA substrates over either short- or branched-chain primers, thus distinguish-
Characterization of *S. pneumoniae* FabH

**TABLE I**

| Primers used in the present study | Sequence |
|---|---|
| EKGS | AGAGGGTTTACACCAATCT |
| EKGI0 | GACTGGCTCCATCATTCTC |
| FabH3 | CGGAGTACCAATTGTTCACTACCAGTATTAC |
| FabH5 | TGGGAATTCTTAGGGTTTCACAAAATGTCAGGT |
| SPYOH1 | GACGGCTCCAAGTAAATTC |
| SPYOH2 | AAGACTGGAGGATGACCTA |

Purification of *S. pneumoniae* FabH—All lysis and purification steps were carried out at 4 °C. 10 g of *E. coli* cells over-expressing tagged *S. pneumoniae* FabH were resuspended in 300 ml of lysis buffer (buffer A) containing 20 mM Tris-HCl (pH 8.0), 300 mM sodium chloride, 0.2 mM phenylmethylsulfonyl fluoride, 40 mM imidazole, and 5 mM 2-mercaptoethanol. Membranes were lysed by 10,000 p.s.i. using a microfluidizer (Microfluidics Corporation). Cell debris were removed by centrifugation (Sorvall RC-5B) at 35,000 × g for 30 min. The supernatant was applied to a 15-ml Ni-NTA metal affinity column (Qiagen) at 2 ml/min equilibrated in buffer A. The column was washed with 10-column-volumes of buffer A and eluted with 20 mM Tris-HCl (pH 8.0), 300 mM sodium chloride, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM 2-mercaptoethanol, and 300 mM imidazole. Fractions containing FabH were pooled, dialyzed, and filtered through 4 liters of buffer B containing 20 mM Tris-HCl (pH 8.0) and 5 mM 2-mercaptoethanol, and applied to a 15-ml Source Q column (Amersham Pharmacia Biotech) equilibrated in buffer B. The column was washed with 10-column-volumes of buffer B and then eluted with a 20-column-volume linear gradient of 0–1.0 M sodium chloride in buffer B. FabH, which eluted at 300–400 mM NaCl, was next applied to a Superdex 200 size exclusion column (2.6 × 60 cm, Amersham Pharmacia Biotech) equilibrated in 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, and 5 mM DTT. Yield of purified protein was ~10 mg/g wet weight of *E. coli*. SDS-PAGE, native isoelectric focusing (IEF), N-terminal sequence, and amino acid composition analyses were performed as described (22).

**Size Exclusion Chromatography**—The apparent molecular weight of *S. pneumoniae* FabH was determined by size exclusion chromatography on analytical Superdex 200 or Superdex 75 (1.6 × 30 cm, Amersham Pharmacia Biotech) column equilibrated in 20 mM HEPES (pH 7.0), 300 mM NaCl, 10 mM 2-mercaptoethanol, and 1 mM EDTA. The column was calibrated with the following molecular mass standards (Bio-Rad): thyroglobulin (668 kDa), y-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), and vitamin B12 (1 kDa). A 200-μg (3 mg/ml) sample of purified *S. pneumoniae* FabH was injected at a flow rate of 0.5 ml/min.

**Enzyme Assays**—*S. pneumoniae* FabH-directed acetoacetyl-ACP formation was specifically measured using [3H]acetyl-CoA and malonyl-ACP. Malonyl-ACP was generated de novo by malonyl-CoA acyl carrier protein transacylase (FabH) (12). The substrate [3H]acetyl-CoA is soluble in 10% trichloroacetic acid whereas the resulting [3H]acetoacetyl-ACP is not. Final reaction conditions in 25 μl were typically as follows: 100 mM sodium phosphate (pH 7.0), 1 mM DTT, 175 μM ACP, 200 μM malonyl-CoA, 15 ng of purified *S. aureus* FabD, 40 μM acetyl-CoA, 0.5 μM [3H]acetoacetyl-ACP (specific activity 7 Ci/mmol, Moravek Biochemicals), and 4 μM *S. pneumoniae* FabH. ACP, malonyl-CoA, and FabH were preincubated with buffer alone for 10 min at 37 °C prior to addition of acetyl-CoA and FabH. FabH was added last to start the reaction, and reactions were then incubated at 33 °C for 10–15 min. 10% trichloroacetic acid was added to stop the reaction, and stopped reactions were incubated on ice for 20 min prior to filtration. Reaction mixtures were filtered and washed twice with 10% trichloroacetic acid on Packard GF/C filter plates using a Packard Filtermate harvester. The filter plates were then dried completely at 60 °C, and the radioactivity was quantified using Wallac Supermix scintillation mixture and a Wallac Microbeta 1450 liquid scintillation counter.

Malonyl-ACP formation was determined in the filtration assay format as described above for FabH. Final reaction conditions typically were 100 mM sodium phosphate (pH 7.0), 1 mM DTT, 50 μM malonyl-CoA, 0.5 μM [3H]malonyl-ACP (specific activity 56 mCi/mmol, Moravek Biochemicals), 15 ng of FabD, and 25 μM ACP. Reactions were stopped, filtered, and quantified as for the FabH filtration assay.

To assay different non-radiolabeled acetyl-CoAs as primers for FabH, a spectrophotometric assay was employed. *α*-Ketoglutarate dehydrogenase has been used previously to specifically quantify free CoA (27) and was used as coupling enzyme to follow FabH activity. Reaction conditions in 50 μl of final volume were as follows: 100 mM sodium phosphate (pH 7.0), 1 mM DTT, 500 μM NAD, 1 mM *α*-ketoglutaric acid, 0.025 units of *α*-ketoglutarate dehydrogenase, 75 μM various acyl-CoAs, 20 μM malonyl-ACP, and 4 μM *S. pneumoniae* FabH. Assays were started by the addition of FabH and followed spectrophotometrically at 340 nm.

For inhibition studies, cerulenin (0.011–668 μM), TLM (0.0534–200 μM), SB418011 (0.0001–1 mM), and SB418011 (0.0001–1 mM) were dissolved in dimethyl sulfoxide (Me2SO) and tested for their ability to inhibit *S. pneumoniae*, *E. coli*, *H. influenzae* FabH, and human FAS proteins using the filtration assay as described above. I(C50) values were determined by GraFit version 4.09 fitting the data to the equation: √ν = Vc/(1 + IC(C50)).

*G. Van Aller, S. Khandekar, and J. Lonsdale, unpublished data.*

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*D. Gentry, M. Grooms, S. Khandekar, and J. Lonsdale, unpublished results.*
The concentration of FabH in the assay reactions was: 4 nM S. pneumoniae FabH, 0.5 nM E. coli FabH, and 1 nM H. influenzae FabH, respectively. The concentrations of ACP (75 μM for S. pneumoniae, 20 μM for E. coli, and 62.5 μM for H. influenzae FabH, respectively), malonyl-CoA (100 μM for S. pneumoniae, 40 μM for E. coli, and 90 μM for H. influenzae FabH, respectively), and acetyl-CoA (40 μM for S. pneumoniae, 10 μM for E. coli, and 18 μM for H. influenzae FabH, respectively) were based on the turnover ratio of FabD and FabH, respectively. IC50 values against human FAS were determined spectrophotometrically by monitoring the oxidation of NADPH to NADP at 340 nm. The reaction mixture contained 50 mM potassium phosphate (pH 6.5), 4 mM DTT, 11 μM acetyl-CoA, 190 μM NADPH, and 10 mM human FAS. The reaction was started by the addition of 75 μM malonyl-CoA and incubated at 20 min at 25 °C.

Circular Dichroism (CD) Structure and Stability Analysis—Far-UV CD spectra were recorded on a Jasco J-710 CD instrument using a 0.1-cm path length cuvette cell. Data (seven accumulations) were collected using a time constant of 4 s, at 50 nm/min, and with a 1-nm spectral bandwidth. A buffer-only spectrum was subtracted from the FabH spectrum, and the resulting ellipticity data, θ (in degrees), were converted to difference molar extinction coefficient data as described (28). Concentration of residues was determined by absorbance at 210 nm—taking the measurements. Dynamic light scattering analysis was performed on purified protein (1–6 mg/ml) using a DynaPro-MSTC instrument (Protein Solutions, Inc.) as described previously (22).

RESULTS AND DISCUSSION

Sequence Analysis of the fabH Gene and Its Product—The S. pneumoniae fabH gene was cloned via PCR taking advantage of conserved genomic structure between S. pneumoniae and S. pyogenes as described under “Materials and Methods.” PCR of S. pneumoniae genomic DNA using all four possible primer combinations yielded products around the expected size of 1.0–1.2 kilobase pairs. Sequencing of the PCR products confirmed the presence of the N terminus of fabH. In this way two S. pneumoniae contigs were joined and the full-length sequence of fabH was obtained. The open reading frame encodes a protein of 324 amino acids (Fig. 1). The calculated molecular mass of the encoded protein is 34,901 Da, and the isoelectric point is 4.9.

The alignment in Fig. 1 was manually constructed with the aid of the sequence homology search algorithm PSI-BLAST. A FabH sequence profile was generated via five iterations of PSI-BLAST using the S. pneumoniae amino acid sequence as a query against an all-bacteria protein database. The profile was then used to search a Homo sapiens-specific protein database. The alignment was optimized by manual inspection using the x-ray crystal structure of E. coli FabH (16) and the predicted secondary structure of the eukaryotic FAS-β-ketoacyl synthase domain. The species shown are a subset of the alignment containing 43 members of the FabH family representing 38 different species including green plants and Plasmodium falciparum.

S. pneumoniae FabH is ~41, 39, and 38% identical to E. coli, H. influenzae FabH, respectively and only similar to the ω-ketoacyl synthase domain of human and chicken FAS molecules. The active site of E. coli FabH comprises Cys-112, His-244, and Asp-274 (16, 17). These residues are conserved in S. pneumoniae FabH (Cys-112, His-249, and Asn-274) (Fig. 1, highlighted in green) that protrude into the active site of the opposite dimer-pair, are not rigorously conserved in E. coli FabH, and with the corresponding eukaryotic FAS domains from H. sapiens and G. gallus. The conserved cysteine (Cys-112), histidine (His-244), and asparagine (Asn-274) active site residues are in bold face. Secondary structure labeling is based on the E. coli crystal structure (16), similar, h, homologous.
(Leu-142, Phe-157, Leu-189, Leu-205, and Phe-87). The E. coli FabH residues, Trp-32 and Arg-151, are invariant within the FabH family, presumably preserving their side-chain interactions with the adenine ring of CoA as suggested in E. coli but different in human and chicken FAS. In addition, the residues that form hydrogen bonds to pantotheinate, Gly-209 and Asn-247, are invariant in the FabH family but again are different in eukaryotic FAS. Although the high degree of sequence conservation within the bacterial FabH protein family easily distinguishes this member of the β-ketoacyl synthase superfamily from FabF and the eukaryotic fatty acid synthases, distant similarities can be found using sensitive search algorithms such as PSI-BLAST. Structural similarities between FabF and FabH are easily recognizable and, from this analysis, one would predict a similar structure for the corresponding domain in eukaryotic FAS.

Purification and Characterization of FabH S. pneumoniae—
FabH was expressed in E. coli as an N-terminal decahistidine-tagged fusion protein. FabH expressed well and was purified from E. coli cell lysate using a combination of Ni-NTA, anion exchange, and preparative Superdex 200 size exclusion chromatography as described under “Materials and Methods.” The overall yield of the purified FabH was ~10 mg/gm (wet weight) of E. coli cells. Purified FabH was nearly homogeneous as judged by SDS-PAGE (Fig. 2A). N-terminal sequence analysis confirmed the 34-kDa band as N-terminal decahistidine-tagged S. pneumoniae FabH. The amino acid composition was in complete agreement with the expected sequence.

### Table II

| Substrate        | Enzyme activity (nmoles/min/μg) |
|------------------|-------------------------------|
| Acetyl-CoA       | 5.04 ± 0.50                   |
| Butyryl-CoA      | 3.66 ± 0.32                   |
| Isobutyryl-CoA   | 1.38 ± 0.19                   |
| Isovaleryl-CoA   | 0.71 ± 0.12                   |
| Lauroyl-CoA      | ND                            |
| Palmitoyl-CoA    | ND                            |
agreement with the predicted composition based upon the primary amino acid sequence deduced from the nucleotide sequence of the expressed protein (not shown). Purified FabH migrated predominantly with a pI of ~6.1 under native conditions (Fig. 2B), which is in close agreement to its theoretical pI of 6.02 calculated from the amino acid sequence of decahistidine-tagged S. pneumoniae FabH.

Our previous studies showed that the molecular mass of E. coli FabH by size exclusion chromatography and dynamic light scattering performed under non-denaturing conditions was ~65 kDa, implying the non-covalent dimeric nature of the protein (22). In agreement with the solution studies, the crystal structure of the E. coli FabH in the presence and absence of acetyl-CoA revealed a non-covalent homodimer (16, 17). The homodimeric nature of the E. coli FabH was the same as that reported for the E. coli FabB (29) and FabF (19, 29) proteins and for the FabH of S. glaucescens (25).

To determine whether S. pneumoniae FabH has similar properties, purified protein was subjected to size exclusion chromatography and dynamic light scattering performed under nondenaturing conditions. On an analytical Superdex S200 column S. pneumoniae FabH eluted as a single species with a molecular mass of ~60 kDa (Fig. 2C); similar results were obtained using a Superdex S75 column (not shown). The size exclusion chromatography profile and SDS-PAGE analysis indicate a non-covalent homodimeric structure for S. pneumoniae FabH. S. pneumoniae FabH was monodisperse at lower concentration as judged by dynamic light scattering (not shown). However, unlike the E. coli FabH, at higher concentrations (>3 mg/ml), the S. pneumoniae FabH appeared to form soluble aggregates as evident from an increased polydispersity index (not shown). Similar results were obtained with purified untagged S. pneumoniae FabH indicating that the decahistidine tag was not the cause of the aggregation. It may be that the hydrophobic residues of S. pneumoniae FabH that are exposed to solvent are responsible for its aggregation. Further work, including modeling and site-directed mutagenesis, may help to determine whether the aggregation is an intrinsic property of S. pneumoniae FabH.

**CD Spectroscopy**—Our previous circular dichroism spectroscopic studies showed the presence of both α-helix and β-sheet elements in purified E. coli FabH (22). Consistent with these results, the crystal structure of E. coli FabH displayed a five-layered core structure, α-β-α-β-α, where each α comprises two α-helices and each β consists of a five-stranded, mixed β-sheet (16, 17). To determine its secondary structure and stability, S. pneumoniae FabH was subjected to CD spectroscopy (Fig. 3). The far-UV circular dichroism spectrum of S. pneumoniae FabH is shown as difference molar extinction coefficient/mole of peptide bond versus wavelength (Fig. 3A). Softsec computer software analysis predicted a α-helix content of 26% and a β-sheet content of 31%. These values are comparable with those obtained for E. coli FabH based on its crystal structure (39% α-helix, 28% β-sheet; Ref. 16) and indicate the presence of a similar core structure despite the overall low homology between these two enzymes. To determine its stability, GdnHCl denaturation was carried out monitoring the secondary structure of S. pneumoniae FabH using the CD signal at 220 nm as indicator. Purified FabH was diluted into denaturant to a 0.2 mg/ml final concentration at 20 °C and incubated for 10 min before taking measurements. The denaturation midpoint (1.3 M GdnHCl), m value (1.14 kcal/mol) and ΔG of unfolding (1.53 kcal/mol) revealed that purified S. pneumoniae FabH is moderately stable (Fig. 3B) and unfolds in a manner consistent with a single cooperative unfolding transition. In contrast, the

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**TABLE III**

Specific activity for S. pneumoniae FabH was determined using the spectrophotometric assay as described under “Materials and Methods.” Values represent the mean ± standard deviation, n = 3. ND, not determined.

| Acyl CoA substrate | E. coli FabH (Ref. 24) | B. subtilis FabH (Ref. 24) | S. glaucescens FabH (Ref. 25) | M. tuberculosis FabH (Ref. 26) | S. pneumoniae FabH (present study) |
|--------------------|------------------------|--------------------------|-------------------------------|-----------------------------|----------------------------------|
| Acetyl             | 465 ± 62               | 24 ± 0.9                 | 11.5 ± 0.2                    | <0.001                      | 5.0 ± 0.5                        |
| Butyryl            | 1 ± 0.1                | 8 ± 0.8                  | 20.5 ± 1.0                    | <0.001                      | 3.7 ± 0.3                        |
| Lauroyl            | ND                     | ND                       | 130.0 ± 2.6                   | <0.1                        | <0.1                             |
| Palmitoyl          | ND                     | ND                       | 12.5 ± 0.3                    | ND                          | ND                               |
| Isobutyryl         | <1                     | 58 ± 2                   | 3.4 ± 0.1                     | ND                          | 1.4 ± 0.2                        |
| Isovaleryl         | <1                     | 16 ± 0.8                 | ND                            | <0.001                      | 0.7 ± 0.1                        |

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**A. Cerulenin**

![Cerulenin](https://example.com/cerulenin.png)

**B. Thiolactomycin**

![Thiolactomycin](https://example.com/thiolactomycin.png)

**C. SB418011**

![SB418011](https://example.com/sb418011.png)

**Fig. 4. Structures of inhibitors used.** A, cerulenin; B, thiolactomycin (TLM); C, SB418011.

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*4 C. Silverman, S. Khandekar, unpublished data.*
denaturation midpoint (2.1 ± 0.1 M GdnHCl), m value (0.55 ± 0.21 kcal/mol) and ΔG of unfolding (1.2 ± 0.6 kcal/mol) revealed that purified E. coli FabH unfolds in a substantially less cooperative manner (not shown). These results imply that despite their overall similar core structures, E. coli and S. pneumoniae FabH have different structural folds. These differences may account for the aggregation of S. pneumoniae FabH observed at high concentrations. The crystal structure of S. pneumoniae FabH will greatly aid in dissecting the differences.

Enzyme Kinetics—The kinetic constants for acetoacetyl-ACP synthase activity were determined using the filtration assays described under “Materials and Methods.” The malonyl-ACP concentration in the FabH filtration assay was estimated to be 25% of the total ACP added based on the percent conversion calculated from the FabD assay alone. Using this method the apparent Km values for acetyl-CoA and malonyl-ACP were determined to be 40.3 ± 2.3 μM and 18.6 ± 1.5 μM, respectively. The maximum velocity of the reaction was 7.1 nmoles/min.

Substrate Specificity—FabH species purified from Gram-negative and Gram-positive bacteria, despite their overall similar catalytic mechanism, have displayed significantly different substrate specificities. To determine substrate specificity of Gram-positive S. pneumoniae FabH, purified protein was assayed spectrophotometrically as described under “Materials and Methods,” and the results are summarized in Table II. Purified S. pneumoniae FabH was able to utilize short- and branched-chain acyl-CoAs. Of the CoA substrates examined, the relative order of activity was determined to be acetyl— butyryl—isobutyryl—isovaleryl. Longer-chain substrate such as, lauroyl-CoA and palmitoyl-CoA, showed no detectable activity in this assay.

The substrate specificity of various FabH molecules are summarized in Table III. E. coli FabH preferred short-chain acyl-CoA primers, among which acetyl-CoA was the most preferred substrate (23, 24). The enzyme was inactive with longer-chain (longer than C4) primers as well as all branched-chain CoA primers. The smaller binding pocket observed in the crystal structures of E. coli FabH (16, 17) and FabH-CoA complex (16) support this finding. Additionally, observing CoA in the crystal indicated that the reaction product has a notable affinity for FabH (16). The B. subtilis FabH homologs, in contrast, were active with 4- to 8-carbon straight-chain acyl-CoAs and all branched-chain acyl-CoAs (24). Similarly, the S. glaucescens FabH efficiently utilized both butyryl-CoA and isobutyryl-CoA (25). Interestingly, FabH of M. tuberculosis (26) preferred only long-chain (CS-C20) acyl-CoA substrates indicating that its substrate-binding pocket is significantly different from those of other FabH molecules. Based on our results and those summarized in Table III, the binding site pocket of S. pneumoniae FabH appears to be more similar to those of S. glaucescens and B. subtilis FabH than those of E. coli and M. tuberculosis FabH molecules. Availability of a FabH crystal structure from a Gram-positive organism will be helpful in comparing the substrate binding pockets to account for the differences in the substrate specificity of various primers.

The fatty acid composition of bacteria appear to vary from species to species. For example, Gram-negative E. coli, in which FabH cannot use branched-chain acyl-CoA primers, does not produce branched-chain fatty acids (30). In contrast, Gram-positive bacilli and streptomyces, whose FabH components are capable of efficiently utilizing branched-chain acyl-CoAs as substrates, produce significant amounts of odd and even carbon-number branched-chain fatty acids (24, 31). Interestingly, S. pneumoniae, also a Gram-positive organism, does not produce significant amounts of branched-chain fatty acid structures (30, 31). In agreement with the fatty acid composition of S. pneumoniae, the activity of S. pneumoniae FabH with the branched-chain CoAs was significantly weaker than that with the straight-chain CoAs (Table II). Thus, as surmised by Choi et al. (24), substrate specificity of the FabH enzymes appears to be the determining factor in the biosynthesis of branched- or straight-chain fatty acids by type II fatty acid synthase.

Enzyme Inhibition—There is considerable potential for selective inhibition of the bacterial FAS by broad-spectrum bactericidal agents. The broad-spectrum antibiotic triclosan (6, 32) inhibits the FabI (enoyl-ACP reductase) component of bacterial FAS; the anti-tuberculosis drug isoniazid may inhibit both InhA and a β-ketoacyl-ACP synthase (7, 8, 33). E. coli FabB and F are sensitive to both cerulenin (29, 34) and TLM (14, 34), whereas the human FAS is sensitive to cerulenin but not TLM (9). E. coli FabH, unlike the other E. coli KAS enzymes, is insensitive to cerulenin and sensitive, albeit weakly, to TLM (12, 35). Likewise, M. tuberculosis FabH is inhibited by TLM but not by cerulenin (26). Earlier, TLM was shown to inhibit mycolic acid synthesis in mycobacteria (36) and the FabH of S. glaucescens (25).

To extend these studies to purified S. pneumoniae FabH and its E. coli and H. influenzae FabH homologues, conditions were set at Km concentrations for the substrates as described under “Materials and Methods.” The structures of the compounds tested are shown in Fig. 4 and the IC50 values are summarized in Table IV. In agreement with the earlier reports (14, 34), E. coli FabH in our studies was sensitive, albeit weakly, to TLM with an IC50 of 32.0 ± 12.0 μM, and likewise to those studies, cerulenin did not inhibit E. coli FabH in our hands (Table IV). In addition, similar to E. coli FabH, H. influenzae and S.

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**Table IV**

| Source            | SB418011 | TLM   | Cerulin |
|-------------------|----------|-------|---------|
| S. pneumoniae FabH| 0.016 ± 0.003 | 7.9 ± 1.1 | >200    |
| E. coli FabH      | 1.20 ± 0.40  | 32.0 ± 12.0 | >200    |
| H. influenzae FabH| 0.59 ± 0.05  | 5.8 ± 1.6  | >200    |
| Human FAS         | >200      | >200   | 45 ± 8  |

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pneumoniae FabH were not inhibited by cerulenin (Table IV). However, S. pneumoniae and H. influenzae FabH were comparatively more sensitive to TLM with IC50 values of 7.9 ± 1.1 μM and 5.8 ± 1.6 μM, respectively.

Recently, crystal structures of E. coli FabB-TLM and FabB-cerulenin binary complexes were reported (34). Unlike FabH, which contains a His-Asn-Cys catalytic triad, FabB contains a His-His-Cys triad in its active site. Using site-directed mutagenesis studies, Price et al. (34) showed that the two-histidine active site architecture of FabB is critical to cerulenin and TLM binding and inhibition. In our studies, we found that despite the conservation of His-Asn-Cys triad H. influenzae and S. pneumoniae FabH were 4- to 5-fold more sensitive to TLM than to E. coli FabH. It appears that in addition to the catalytic triad, other yet unidentified factors may be responsible for the inhibition of S. pneumoniae and H. influenzae FabH by TLM.

Given the resurgence of antibiotic resistance in pathogenic bacteria, there is an urgent need for novel broad-spectrum antibacterial agents. In bacteria, FabH appears to play a major role in the regulation of the overall rate of fatty acid biosynthesis (9, 17). Because none of the available small molecule inhibitors of bacterial FAS appear to be potent against FabH, we have initiated the development of high throughput screening assays to identify potent, small molecule broad-spectrum inhibitors of bacterial FAS. The availability of purified FabH with IC50 of >200 μM (Table IV). Together, these results strongly suggest that it is indeed feasible to obtain highly potent and broad-spectrum inhibitor(s) of this physiologically important enzyme target (17, 23).

In conclusion, we have cloned and characterized the S. pneumoniae homologue of E. coli FabH. The availability of purified S. pneumoniae FabH will be of great utility in kinetic and structural studies as well as in developing high throughput screening assays to identify and develop small molecule inhibitors of pathogenic bacteria.

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REFERENCES
1. Jayakumar, A., Tai, M. H., Huang, W. Y., Al Feel, W., Hsu, M., Abu-Elheiga, L., Chirala, S. S., and Wakil, S. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8685–8689
2. Chirala, S. S., Huang, W. Y., Jayakumar, A., Sakai, K., and Wakil, S. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5588–5593
3. Cronan, J. E., Jr., and Rock, C. O. (1996) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R. J. L., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds.), pp. 612–636, American Society for Microbiology, Washington, D. C.
4. Clough, R. C., Matthys, A. L., Barnum, S. R., and Jaworski, J. O. (1992) J. Biol. Chem. 267, 20992–20998
5. Waller, R. P., Keeling, P. J., Donald, R. G., Striepen, B., Handman, E., Lang, Unracs, K. N., Cowman, A. F., Beers, G. S., Roos, D. S., and McFadden, G. I. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12352–12357
6. Qu, X., Janson, C. A., Court, R. I., Smyth, M. G., Payne, D. J., and Abdel-Meguid, S. S. (1999) Protein Sci. 8, 2529–2532
7. Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Um, K. S., Wilson, T., Collins, D., de Liogier, G., and Jacobs, W. R., Jr. (1994) Science 263, 227–230
8. Mizowaki, K., Slayden, R. A., Zha, Y., Ramaswamy, S., Pan, X., Mead, D., Crane, D. D., Musser, J. M., and Barry, C. E., III (1998) Science 280, 1607–1610
9. Magnuson, K., Jackowski, S., Rock, C. O., and Cronan, J. E., Jr. (1993) J. Bacteriol. 175, 612–619
10. Jackowski, S., and Rock, C. O. (1987) J. Biol. Chem. 262, 7927–7931
11. Tsay, J. T., Oh, W., Larson, T. J., Jackowski, S., and Rock, C. O. (1992) J. Biol. Chem. 267, 6687–6694
12. Jackowski, S., and Rock, C. O. (1992) J. Bacteriol. 174, 508–513
13. Garwin, J. L., Klages, A. L., and Cronan, J. E., Jr. (1980) J. Bacteriol. 140, 1183–1186
14. Jackowski, S., Murphy, C. M., Cronan, J. E., Jr., and Rock, C. O. (1989) J. Biol. Chem. 264, 7624–7629
15. Vance, D., Goldberg, I., Mitsuhashi, O., and Bloch, K. (1972) Biochem. Biophys. Res. Commun. 44, 449–451
16. Qu, X., Janson, C. A., Konstantinidis, A. K., Nwagwu, S., Silverman, C., Smith, W. W., Khanderka, S., Lonsdale, J., and Abdel-Meguid, S. S. (1999) J. Biol. Chem. 274, 36465–36471
17. Davies, C., Heath, R. J., White, S. W., and Rock, C. O. (2000) Structure 8, 185–195
18. Qu, X., Janson, C. A., Smith, W. W., Head, M., Lonsdale, J., and Konstantinidis, A. K. (2001) J. Mol. Biol. 307, 182, 10996–11000
19. Huang, W., Jia, J., Edwards, P., Dehesh, K., Schneider, G., and Lindquist, Y. (1998) EMBO J. 17, 1183–1191
20. Moehle, M., Schneider, G., Edwards, P., Dehesh, K., and Lindquist, Y. (1999) J. Biol. Chem. 274, 6031–6034
21. Mathieu, M., Zeelen, J. P., Paupert, R. A., Erdmann, R., Kunau, W. H., and Wierenga, R. K. (1994) Structure 2, 797–808
22. Khanderka, S. S., Konstantinidis, A. K., Silverman, C., Janson, C. A., McNulty, D. E., Nwagwu, S., Van Aller, G. S., Doyle, M. L., Kane, J. F., Qiu, X., and Lonsdale, J. (2000) Biochem. Biophys. Res. Commun. 270, 100–107
23. Huntington, R. J., and Rock, C. O. (1996) J. Biol. Chem. 271, 10986–11000
24. Choi, K. H., Heath, R. J., and Rock, C. O. (2000) J. Bacteriol. 182, 365–370
25. Han, L., Lobo, S., and Reynolds, K. A. (1998) J. Bacteriol. 180, 4431–4446
26. Choi, K. H., Kramer, L., Besra, G. S., and Rock, C. O. (2000) J. Biol. Chem. 275, 28201–28207
27. Garland, P. B. (1964) Biochem. J. 92, 10e–12e
28. Woody, R. W. (1995) Methods Enzymol. 246, 34–71
29. Edwards, P., Nielsen, J. S., Metz, J. G., and Dehesh, K. (1997) FEBS Lett. 402, 62–66
30. Brez, J. L., Tornabene, T. G., and LaForce, F. M. (1979) J. Infect. Dis. 140, 443–452
31. Kaneda, T. (1991) Microbiol. Rev. 55, 288–302
32. McMurry, L. M., Oethinger, M., and Levy, S. B. (1998) FEMS Microbiol. Lett. 166, 305–309
33. Rozwarski, D. A., Grant, G. A., Barton, D. H., Jacobs, W. R., Jr., and Sacchetti, J. C. (1988) Science 230, 98–102
34. Price, A. C., Choi, K. H., Heath, R. J., Li, Z., White, S. W., and Rock, C. O. (2003) J. Biol. Chem. 278, 6551–6559
35. Hayashi, T., Yamamoto, O., Sasaki, H., Kawaguchi, A., and Okazaki, H. (1983) Biochem. Biophys. Res. Commun. 115, 1108–1113
36. Besra, G. S. (1998) Methods Mol. Biol. 101, 91–107
Identification, Substrate Specificity, and Inhibition of the *Streptococcus pneumoniae* β-Ketoacyl-Acyl Carrier Protein Synthase III (FabH)

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