Crystallographic Studies of β-Ketoacyl-Acyl Carrier Protein Synthase III (FabH)

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Introduction

β-Ketoacyl-acyl carrier protein (ACP) synthase III (KAS III or FabH) plays an essential and regulatory role in bacterial fatty acid biosynthesis. It initiates fatty acid elongation cycles and is involved in the feedback regulation of the biosynthetic pathway via product inhibition. FabH catalyzes the carbon-carbon condensation reaction between a CoA-attached acetyl group and an ACP-attached malonyl group, yielding the final product acetoacetyl-ACP. In bacteria, fatty acid biosynthesis enzymes (FAS) are discrete proteins. In human, they are encoded in a single polypeptide and have little sequence homology to their bacterial counterparts. Because they are essential for bacteria, bacterial FAS provide excellent molecular targets for developing selective and broad-spectrum antibacterial compounds. It is known that the broad-spectrum antibiotic triclosan inhibits the enoyl-ACP reductase (FabI) of FAS; the antituberculosis drug isoniazid may target both FabI and a β-ketoacyl-ACP synthase; the antibiotic agent cerulenin binds both KAS I (FabB) and KAS II (FabF); and the antibiotic compound thiolactomycin inhibits all three FAS condensing enzymes (KAS I-III).

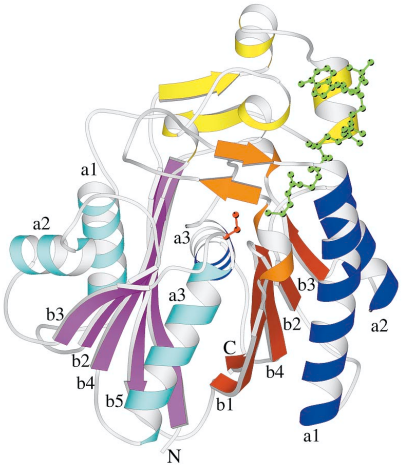

FIG. 1. Ribbon diagram of a FabH monomer. The N- and C-terminal halves have a similar fold. The core secondary structural elements are labeled as b1-b5 (N-magenta, C-red) and a1-a3 (N-cyan, C-blue). Those of insertion regions are drawn in yellow (N) and orange (C). Cys112 is shown in a red ball-and-stick drawing, and CoA is shown in green.

Results and Discussion

Escherichia coli FabH is a 35 kDa protein and acts most likely as a 70 kDa homodimer in solution. While FabB and FabF are 38% identical in amino acid sequences, FabH shows no overall sequence homology to either FabB or FabF. We solved the crystal structure of *E. coli* FabH and FabH-ligand complexes,[1-3] with the best quality structure determined at 1.1 Å resolution. These structures show that FabH has the same five-layered αβαβα core fold (Fig. 1) seen in the FabB and FabF, suggesting that the three condensing enzymes are evolutionarily related. Despite this overall similarity, the catalytic triad of FabH, Cys112, His244 and Asn274, are different from the Cys-His-His triads in FabB and FabF. The acetylated-FabH structure established the catalytic role of Cys112. The acetyl oxygen atom in FabH is hydrogen bonded to the backbone nitrogen atoms of Cys112 and Gly306, giving the exact position of the oxyanion hole. The acetyl methyl group projects into a hydrophobic pocket formed by the side chains of Leu142, Phe157, Leu189, Leu205 and Phe87' of the other monomer. The small size of this pocket explains why FabH cannot activate large acyl chains. Our structures also revealed the CoA binding mode in the substrate binding tunnel, with the CoA adenosine ring sandwiched between the side chains of Trp32 and Arg151, and hydrogen bonded to the carbonyl oxygen of Arg151 and Thr28 Oγ1 (Fig. 2). The observed FabH dimer in these structures buries about 3000 Å^2 of accessible surface area. A structure of apo FabH revealed significant conformational changes, exemplified by the disordering of four essential loops and over 50% of the dimer interface. Clearly, the structures have advanced our understanding of FabH catalytic mechanism and may provide important insights into inhibitor design.

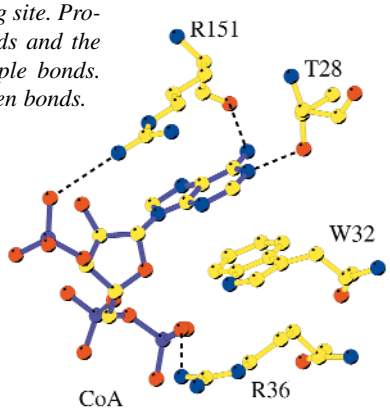

FIG. 2. The CoA adenine binding site. Protein residues are in yellow bonds and the CoA molecule is drawn in purple bonds. The dashed lines denote hydrogen bonds.

Acknowledgment

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References

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