Abstract

Fatty acid synthesis type-II pathway is ubiquitous among bacteria and plants. The pathway consists of several enzymes for fatty acid elongation. In most bacteria, FAS-II pathway can only elongate fatty acyl chain up to a length of 18 carbon units. But in some bacteria, such as *Mycobacterium tuberculosis*, FAS-II pathway could lead to the synthesis of fatty acid chains up to a length of C_{80}. Fatty acid synthesis in *M. tuberculosis* is crucial because the pathway supplies mycolic acid which is a major component of its cell envelope. The enzyme β-ketoacyl-ACP or FabG is involved in the second step of fatty acid elongation. There are two conserved FabG genes among Mycobacterial species, FabG1 and FabG4. FabG1 is involved in FAS-II pathway but the function of FabG4 in fatty acid metabolism is still unknown. Unlike *M. tuberculosis*, *Staphylococcus aureus* uses only the FAS-II pathway for the synthesis of fatty acids as extending to 18 carbon units. Staphylococcal FabG1 is involved in FAS-II pathway. In the current work, attempts were made to unravel structural and functional aspects of Mycobacterial FabG4 (*Mt*FabG4) and Staphylococcal FabG1 (*Sa*FabG1). *Mt*FabG4 polypeptide is unusually long compared to other characterized FabGs. Such large FabG proteins are mostly found in bacteria and are designated as high molecular weight FabGs (HMwFabGs). Kinetic analysis shows that *Mt*FabG4 is an NADH utilizing β-ketoacyl reductase whereas *Sa*FabG1 is a typical NADPH utilizing β-ketoacyl reductase. Both are functional as dimers. Crystal structure of *Mt*FabG4 NADH complex shows that the *Mt*FabG4 protomer consists of two distinct domains: N-terminal flavodoxin domain (dimerization domain) and C-terminal β-α-β dinucleotide binding domain (catalytic domain). The C-terminal residues of the two catalytic domains of FabG4 contribute to the dimeric interface, where to conserved Arginine residues (Arg146 and Arg445) are involved in a complex hydrogen bonding network. Mutational analysis shows that the conserved C-terminus is important for orientating active site Tyr360 and Lys364 such that they are optimal for catalysis. The influence of C-terminal segments is mediated via two catalytic loops (loop-I and loop-II). *Mt*FabG4:NAD^+:Hexanoyl-CoA complex indicates that both the catalytic loops are important for substrate binding. Hexanoyl-CoA is a substrate mimetic and hence the structure of the complex reveals the mode of substrate binding to FabGs. *Sa*FabG1 is a single domain protein and forms dimer in solution but crystallizes as a tetramer. Fluorescence resonance energy transfer (FRET) between the single Trp104 of the protomer and NADPH suggests that *Sa*FabG1 functional dimer is made of protomers related by the two fold designated as the Q-axis in the crystal structure and the cooperativity of NADPH binding is also due to the interactions at this interface.

Keywords: High Molecular weight FabG, Fluorescence Resonance Energy Transfer, *M. tuberculosis*, *S. aureus*, Hexanoyl-CoA, Cooperativity.