Cofactors in Fatty Acid Biosynthesis—Active Site Organizers and Drug Targets

The structure of *E. coli* β-ketoacyl-acyl carrier protein reductase (FabG) in its binary complex with NADPH reveals how cofactor-induced conformational changes configure the active site for catalysis. It provides another target for drug design in the fatty acid biosynthesis pathway.

It has been over 40 years since the components of the fatty acid biosynthesis pathway were identified and 20 years since the detailed mechanisms of the steps of this essential pathway in bacteria and eukarya were worked out. The multiple enzyme activities that catalyze the steps of fatty acid synthesis, collectively called fatty acid synthase (FAS), are closely related and intersect with the fatty acid biosynthetic pathways (Campbell and Cronan, 2001).

The most recently determined structures of enzymes in the FAS system have been the β-ketoacyl-acyl carrier protein reductase (FabG), which reduces the ACP-linked β-ketoacyl group to a β-hydroxyacyl group. Structures of this enzyme have been determined for the *Brassica Napus* and *E. coli* enzymes and of the apoenzymes of *M. tuberculosis* and *E. coli* have been determined. In this issue of *Structure*, the structure of the binary cofactor complex of *E. coli* FabG with NADP is described. Through comparison of this complex with the apoenzyme and with a point mutant structure, a complex set of conformational changes induced by cofactor binding is revealed. These changes resemble the Ser-Tyr-Lys catalytic triad and also a water chain that is added to play an essential role as a proton wire in restoring the catalytic conformation at the end of the reaction. These conformational changes also suggest a basis for the coordinated positive (acyl-ACP) and negative (NADPH) cooperativity of the tetrameric FabG coupled to NADPH binding.

It is noteworthy in this FabG binary complex structure that binding of the nucleotide and nicotinamide moieties of NADPH are inferred to occur sequentially and that the participation of the NADP cofactor in catalysis is not limited to chemistry in the nicotinamide ring. The structure of the Tyr151Phe mutant in complex with NADP shows that the nucleotide of NADP binds in the same way as in the wild-type, but the nicotinamide and ribose groups are disordered. The implication is that the nucleotide binds first and subsequent conformational changes create the nicotinamide binding site and the catalytic configuration at the active site. Comparison of the Tyr151Lys mutant binary complex structure with that of the wild-type provides multiple critical roles for the ribose hydroxyl groups of NADP in organizing the active site residues and in catalysis. These hydroxyl groups form hydrogen bonds to the Tyr151 and Lys156 residues of the catalytic triad and also are part of the proton wire extending from the ordered water chain that is essential for catalytic turnover.

Consequent of the fact that the polyketide biosynthetic pathways in organisms like *streptomyces* are closely related and intersect with the fatty acid biosynthetic pathways (Campbell and Cronan, 2001).
This intimate involvement in catalysis of parts of the NADP cofactor structure beside the nicotinamide ring invites comparison with the two best characterized and widely used drugs that disrupt microbial fatty acid biosynthesis, isoniazid and triclosan. Both of these compounds interact with the NAD binding site of the other reductase in the fatty acid biosynthesis pathway, the enoyl-acyl carrier protein reductase, FabI. Isoniazid is a pro-drug that must undergo activation to a free radical prior to its acylation of the NAD$^+$ radical generated during catalysis. It binds in a complementary site adjacent to the NAD, forming hydrogen bonds to the nicotinamide and its ribose and to the protein, and a stacking interaction with a phenylalanine sidechain (Rozwarski et al., 1998). Triclosan also interacts with the NAD of FabI through stacking interaction on the pyridine ring and hydrogen bonds to the ribose and catalytic residues in the active site (Levy et al., 1999). Furthermore, a conformational change that closes the active site and creates new binding interactions of the enzyme with triclosan appears important for the activity of this inhibitor (Qiu et al., 1999).

This limited number of cases may be highlighting the vulnerability of enzymes to inhibitors targeted at cofactor binding sites. In contrast to such drugs as methotrexate, a folate analog inhibitor of dihydrofolate reductase, isoniazid, and triclosan interact with, but don’t replace, the enzyme bound cofactor. The binding sites and chemistry of these two drug inhibitors with FabI are so complex that current rational drug design methods are unlikely to have predicted them, especially in the absence of knowledge about the protein conformational changes accompanying cofactor binding. Perhaps drug designers should direct more effort toward targeting enzyme-cofactor complexes like that of FabG with NADP, factoring in the conformational changes of the enzyme and the multiple unique roles of the cofactor itself.

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Selected Reading