IDENTIFICATION OF IMPORTANT RESIDUES FOR THE INTERACTION BETWEEN STEAROYL-ACYL CARRIER PROTEIN DESATURASE AND FERREDOXIN

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Stearoyl-acyl carrier protein desaturase (Δ9D) catalyzes the O_2 and NADPH-dependent desaturation of stearoyl-acyl carrier protein (18:0-ACP) to yield oleoyl-ACP (18:1-ACP). Δ9D is a homodimeric enzyme that contains a catalytically essential diiron cofactor in each subunit. The catalytic pathway of this enzyme consists of acyl-ACP binding, electron transfer from ferredoxin to form a diferrous center, and O_2 activation as essential prerequisite to product formation. In this multiprotein pathway, the interaction between the different components represents a potential point for the regulation of enzyme catalysis. It has been shown that binding of acyl-ACP induces changes in the diiron site of the chemical reduced Δ9D, and it has been postulated that these rearrangements enhanced the reactivity with O_2. The addition of 18:0-ACP to chemically reduced Δ9D in the presence of oxygen results in stoichiometric formation of peroxo-cycled Δ9D without the formation of 18:1-ACP and without release of H_2O_2. The formation of 18:1-ACP is observed only when 18:0-ACP and Fd are present. This observation indicates that in addition to electron transfer; the binding of ferredoxin plays a significant role in catalytic desaturation.

The requirement of specific protein-protein interaction between Fd and Δ9D is unique among the members of the diiron enzyme super family. In this work we investigate the structural components involved in the interaction between these two proteins. A combination of cross-linking, mass spectroscopy, site-directed mutagenesis, and steady state assays have been employed to identify the amino acids involved in this interaction and to determine their contribution to catalysis. The reaction of activated carboxylic groups of Fd with primary amines of Δ9D was observed with oxidized and reduced Fd. Analysis of the structure of Δ9D identified two possible pathways for effective electron transfer; in these proposed regions the residues K60, K56 and K40 are conserved and in closed proximity to interact with the carboxylic groups of Fd. The Δ9D mutants K60A, K56A, and K56A/K60A show a decrease in the Km for Fd of 6, 25 and 250-fold, respectively. Despite the observed effect on the Km for Fd, the mutant enzymes cross-liked to Fd with similar efficiency as wt Δ9D. Interestingly, cross-linking was abolished only in the case of pre-incubation of Δ9D K56A/K60A with 18:0-ACP. This indicates that 18:0-ACP modulates the interaction between Δ9D and Fd. HPLC analysis of tryptic peptides and mass spectroscopy is currently used to identified all the residues involved in the interaction between these proteins. Mapping the interaction points will provide insight into the role of Fd binding in Δ9D activity. Funded by NIH GM 50853 to BGF.