Engineering the Substrate Specificity and Catalysis from Crystal Structures of the Beta-subunit of Acyl-CoA Carboxylase

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The carboxylation of acyl-Coenzyme A is one of the key regulation checkpoints for the biosynthesis of fatty acids and polyketides. Acetyl-Co A carboxylases (ACC) and propionyl-CoA carboxylase (PCC) catalyze the carboxylation of acetyl- and propionyl-CoAs to generate malonyl- and methylmalonyl-CoA, respectively. Inhibitors of the ACCases have been identified as potential therapeutics for cancer and obesity, as well as herbicides and antibiotics. The crystal structures of the carboxyltransferase domain, AccB and PccB in *S. coelicolor*, are hexamers [1] that assemble into a ring shaped complex. The biotin-binding pocket has been identified where biotin and propionyl-CoA bind perpendicular to each other and are highly hydrophobic. Mutagenesis and kinetics studies of PccB and AccB allowed interconversion of their corresponding substrate specificity for acetyl-CoA, propionyl-CoA and butyl-CoA. The mutants structures show that dimer interaction is essential for enzyme catalysis, stability, and substrate specificity, which is highly conserved among biotin-dependent carboxyltransferases. ACCase mutants with relaxed substrate specificity can provide novel extender units, which can be fed into the polyketide biosynthesis pathway to generate "unnatural" natural products.

[1] Diacovich L., Mitchell D.L., Pham H., Gago G., Melgar M.M., Khosla C., Gramajo H., Tsai S.C., *Biochemistry*, 2004, **43(44)**, 14027-36.

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