Poster Presentation

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Mechanistic insights into substrate-specificity of mycobacterial type-III PKS

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Superfamily of type-III polyketide synthases (PKSs) catalyze iterative claisen-like decarboxylative condensation of coenzyme-A (CoA)-linked thioesters in plants, microbes and fungi to biosynthesize structurally diverse polyketide scaffolds. PKSs in conjunction with fatty acid synthases (FASs) generate long-chain lipids essential for the survival of mycobacteria. PKS18 shows broad specificity for acyl-CoA substrates (C6-C20) to produce tri- and tetra-ketide alpha-pyrones[1]. Earlier, our crystal structure of PKS18 revealed a 20Å long substrate-binding tunnel formed due to a conformational switch by subtle changes in the dihedral (psi-torsion) angles. Further, we showed a tunnel-blocking mutant, C205F, which resulted in altered substrate specificity[2,3]. Unlike plant type-III PKSs, active-site architecture in Mtb-PKS18 is strikingly different. With the advent of genome sequencing, type-III PKSs are identified in several organisms, e.g., Streptomyces griseus for melanin production, Azotobacter vinelandii for cyst formation, Neurospora crassa for resorcinolic lipid biosynthesis, and Dictyostelium discoideum for DIF-1 precursor synthesis, etc. In an attempt to elucidate the enzymatic mechanism, we present here the first snapshot of Mtb type-III PKS in complex with CoA, where C175A active-site mutant of PKS18 is crystallized in the presence of palmitoyl-CoA. We solved the structure by molecular-replacement method and refined to a resolution of 2.05Å (Rwork= 19%, Rfree= 25%). Analysis shows a 16Å long CoA-binding pocket oriented away from the dimeric interface. The bent conformation of CoA at the pyrophosphate shields the phosphopantotheine from the surface water and the phosphates point towards the solvent. The phosphopantotheine arm is held in place through Van der Waals and hydrogen bonding interactions with the adenine ring. At the entrance of the tunnel, basic residues R68 (replaced by lysine in other type-III PKS) and R71 participate in electrostatic interactions with the phosphates, stabilizing the bent conformation of CoA molecule. Such interactions are conserved among plant type-III chalcone synthases. K318 of Mtb PKS18 is not conserved among type-III PKS enzymes, which interacts with the phosphate of CoA that also connects the ribose and the pantetheine arm, a unique interaction observed only in Mtb type-III PKSs. The plasticity of such long binding pockets for catalysis in these condensing enzymes is being investigated to understand metabolite diversity.

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