

Structural and Biochemical Investigation of Terminal Alkene Formation in Allylmalonyl-CoA Biosynthesis

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Polyketide natural products are replete with diverse and complex chemical structures. This vast chemical diversity is generated through the condensation and subsequent reduction of small malonyl-CoA-derived extender units in an assembly line-like fashion. Of these extender units, allylmalonyl-CoA is of particular interest for incorporation into bioproducts because of the reactivity of the alkene handle it contains. This alkene handle is formed by TcsD, an acyl-CoA dehydrogenase (ACAD). ACADs are a large family of flavoenzymes that catalyze the oxidation of fully saturated acyl-CoA thioesters to their α,β -unsaturated counterparts. However, TcsD catalyzes the formation of a γ,δ -olefin on an α,β -unsaturated substrate, 2-pentenoyl-CoA. Herein, we interrogate the unusual regiochemistry of this transformation. We present biochemical data from experiments using natural substrates and substrate analogs to probe the mechanism of TcsD. In addition, we report high resolution (1.5 Angstroms) structural data from the first solved crystal structure of this enzyme. We use this combination of biochemical and structural data to propose a logical mechanism by which TcsD controls this novel ACAD activity. By understanding the activity of TcsD, we can better engineer strains to heterologously produce allylmalonyl-CoA for introduction into novel bioproducts such as polymer precursors.

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