Editing Role of Thioesterase II in the Biosynthesis of the Polyketide Pikromycin

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Polyketides are a large and structurally diverse class of natural products which possess a wide range of biological activities throughout medicinal and agricultural fields. Despite their structural diversity, polyketides are assembled by a common mechanism of Claisen condensations of simple malonate derivatives by polyketide synthases (PKSs) in a manner very similar to fatty acid biosynthesis. Type I PKSs are gigantic multifunctional modular proteins, which catalyze the biosynthesis of the polyketide moieties of various secondary metabolites in *Streptomyces*. Each module is responsible for one cycle of polyketide chain elongation and contains a set of discrete catalytic domains of ketosynthase (KS), acyltransferase (AT), and acyl carrier protein (ACP). Ketoreductase (KR), dehydratase (DH), and enoyl reductase (ER) domains may also be present, allowing structural variation in the level of processing of the β-ketoacyl chain. The fully extended polyketide chain bound to the PKS as an acyl ACP thioester is often released and cyclized by a thioesterase domain (TEI) covalently linked to the last extending module of the PKS.

In many cases, additional genes encoding a TE have been found within a polyketide biosynthetic gene cluster, for example, the tylosin PKS of *Streptomyces fradiae*, pikromycin PKS of *Streptomyces venezuelae*, rifamycin PKS of *Amycolatopsis mediterranei*, and the erythromycin PKS (DEBS) of *Saccharopolyspora erythraea*. These genes encoding a discrete protein were named as TEII to differentiate from the chain releasing TEI domains in modular polyketide synthases. Sequence analysis has revealed that these thioesterases are probably structurally and evolutionarily related. As TEI domains have been shown to be a necessary and sufficient factor for the release and cyclization of the polyketide chain in vivo and in vitro, the role of the TEII enzyme encoded within many PKS gene clusters has presented an intriguing question.

One proposal has been an editing role in which TEII removes aberrant groups attached by thioester linkages ACP domains within the PKS extension modules, which might otherwise block the normal chain elongation process. Recent studies on KS domains have shown they can catalyze a decarboxylation reaction of the ACP-bound dicarboxyl extender unit even when the KS active site cysteine is not primed with incoming acyl intermediates. Polyketide chains grow by decarboxylative condensations between a β-ketoacyl chain bound to the KS domain and a carboxylated extender unit (malonyl-, methylmalonyl-, ethylmalonyl-ACP, etc.). If the extender unit is decarboxylated to a non-activated acyl group (acetyl-, propionyl-, butyryl-ACP, etc.), the intermediate acyl chain cannot be processed to downstream domains through the normal condensation reaction. The

polyketide biosynthetic process would thus be derailed resulting in low yields of the fully extended polyketide product. TEII catalyzed cleavage of these ACP bound acyl residue would allow the activated dicarboxylic acid extender units to be loaded onto the PKS, restoring polyketide production.

In this study, we have generated recombinant TEII ($PikAV$) from $S.$ $venezuelae$ and performed the first catalytic study of such an enzyme with physiologically relevant substrates, various acyl- and carboxylated acyl-ACP thioesters. The pikromycin TEII exhibits a very high K_m for all ACP substrates and likely is predominantly dissociated from the PKS, minimizing its potential to block the normal biosynthetic process. TEII is active with a wide range of ACP bound thioesters consistent with an editing role for all of the modules within PKS. However, this lack of rigorous substrate specificity allows the TEII to remove acyl-ACP thioesters in the PKS loading module required to initiate the process, or the activated carboxylated acyl ACP thioesters in the extension modules. Thus either an excess or lack of TEII may lead to decreases in polyketide production. In $vivo$ experiments with overexpressed $pikAV$ in pikromycin producing cultures of $S.$ $venezuelae$ are shown to be consistent with this observation.

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