Poster Presentation

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Characterization of transglutaminase homologues found in andrimid biosynthesis

J. Reimer[1], T. Schmeing[1]

[1]*McGill University, Department of Biochemistry, Montreal, Canada*

Nonribosomal peptide synthetases (NRPSs) are multimodular enzymes that synthesize products with diverse structures and activities ranging from antibiotics to industrial solvents. They are arranged into an assembly line of modules where each module is responsible for incorporating a specific monomer into the final nonribosomal peptide (NRP). Diversity in NRPs stems from the fact that NRPSs utilize not only the 20 proteinogenic amino acids, but also include nonproteinogenic amino acids, fatty acids, and alpha-hydroxy acids as building block substrates. Andrimid is a NRP antibiotic that inhibits membrane biosynthesis by blocking bacterial acetyl coenzyme A carboxylases. It is synthesized in a hybrid NRPS-polyketide synthase (NRPS-PKS) using a fatty acid, phenylalanine, valine, and glycine. A remarkable feature of this synthetic system is that instead of a normal condensation domain, it uses two atypical free-standing proteins with homology to transglutaminases to catalyze the formation of the first and second amide bonds. We are characterizing the action of transglutaminase homologues (TGH) in andrimid synthesis using biochemical assays and X-ray crystallography. Initial investigations of the andrimid biosynthetic cluster found in Panteao agglomerans focused on the TGH, AdmF, which catalyzes the formation of the first amide bond. Crystallization trials have been initiated on AdmF in its apo form and in complex with its interacting binding partner, the peptide carrier protein domain AdmI. To date, only a few andrimid producing bacteria have been discovered. Using genome mining, a biosynthetic cluster homologous to the andrimid biosynthetic cluster found in Panteao agglomerans was identified in Vibrio coralliilyticus. The two TGHs, CoraF and CoraS, were cloned, expressed and purified, and crystallization trials are underway. Our progress in biochemical and biophysical characterization of AdmF, CoraF, and CoraS will be presented.

Keywords: nonribosomal peptide synthetases, biomolecular engineering, natural products