Biosynthetic Engineering of Unnatural Natural Products

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Plantazolicin (PZN) is a ribosomally synthesized and posttranslationally modified peptide (RiPP) natural product which exhibits extraordinarily narrow-spectrum antibacterial activity against the causative agent of anthrax, *Bacillus anthracis*. During PZN biosynthesis, a cyclodehydratase catalyzes cyclization of cysteine, serine, and threonine residues in the precursor peptide to azoline heterocycles, and a dehydrogenase then catalyzes the oxidation of many of these azolines to thiazoles and (methyl)oxazoles. The final biosynthetic steps consist of leader peptide cleavage and enzymatic dimethylation of the nascent N-terminus. Using heterologously expressed and purified enzymes, the precursor peptide was fully cyclized and oxidized *in vitro*, concordant with the cyclization pattern found in the natural product. Using a suite of variant precursor peptides, the substrate tolerance of the synthetase complex was elucidated *in vitro*. Despite increased promiscuity *in vitro* compared to what has been previously observed *in vivo*, the PZN biosynthetic enzymes retained exquisite selectivity in catalyzing cyclization of mutant peptides only at positions which correspond to those cyclized in the natural product. A cleavage site was subsequently engineered to remove the leader peptide, yielding fully mature PZN variants after enzymatic dimethylation. Production of these novel variants through *in vitro* biosynthesis facilitates the determination of their antibacterial potency, thus expanding the growing picture of the PZN structure-activity relationship.