Characterization of Azoline Biogenesis in Ribosomal Peptide Natural Products: Substrate Handling and Mechanism

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The thiazole/oxazole-modified microcins (TOMMs) are a recently grouped class of ribosomally synthesized posttranslationally modified peptide natural products. These peptidic natural products occupy a large chemical and functional space but are linked through the biosynthesis of the eponymous azole moieties. Previous work has demonstrated that an evolutionarily conserved, heterotrimeric enzyme complex (TOMM synthetase) is responsible for azole biogenesis. Although multiple groups have investigated these biosynthetic enzymes over the last 15 years, the exact roles of each member of the TOMM synthetase and the mechanism of azoline formation remained enigmatic. Using the biosynthetic machinery from a novel TOMM cluster from *Bacillus* sp. Al Hakam, it was discovered that cyclization is catalyzed via the ATP-dependent phosphorylation of the carbonyl oxygen. Moreover, a detailed dissection of the synthetase complex demonstrated that this modification is performed by the YcaO homolog (D protein) that exists in all characterized TOMM clusters. Finally, through a combination of saturation mutagenesis of the modification enzymes and the use of substrate analogs, the role of the E1-ubiquitin ligase homolog (C protein) in the cluster has begun to be addressed.