

Mechanistic Studies of Class II Lanthipeptide Synthetases

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Lanthipeptides are a class of peptidic natural products that contain intramolecular thioether linkages. In class II lanthipeptides, a single, bifunctional enzyme, LanM, dehydrates select serine and threonine residues, yielding dehydroalanine and dehydrobutyrine intermediates, respectively. The dehydroamino acids subsequently undergo a Michael-type addition via a cysteine thiol to form (methyl)lanthionine rings. Recent insights from the study of a human **lanthipeptide cyclase-like** enzyme, LanCL1, provided a deeper understanding of analogous processes in eukarya. The crystal structure of LanCL1 complexed to the product of a thia-Michael addition of glutathione to a dehydrobutyrine-containing peptide was solved. This revealed a histidine in the enzyme active site that may stabilize the enolate intermediate of the addition reaction. While this residue is not conserved among all cyclase homologs, all residues at that position can stabilize a negative charge. To investigate the residue further, we performed systematic mutation of that residue in a LanM enzyme and assessed the impact on cyclization.

