Investigation of Substrate Specificity of the Bifunctional Lantibiotic Enzyme LctM

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Lantibiotics are a unique class of ribosomally synthesized peptide antibiotics produced by Gram-positive bacteria. These compounds are termed lantibiotics because they contain the thioether amino acids lanthionine (Lan) and/or methyllanthionine (MeLan), in addition to 2,3-didehydroalanine (Dha) and (*Z*)-2,3-didehydrobutyrine (Dhb), which arise from enzyme catalyzed post-translational modifications. These non-proteinogenic amino acids are introduced by the dehydration of serine and threonine residues followed by stereoselective intramolecular Michael addition of cysteines onto the unsaturated amino acids. Lacticin 481 synthetase (LctM) catalyzes the post-translational modifications required for the generation of the lantibiotic lacticin 481. The precursor peptide for lacticin 481, LctA, consists of a leader sequence and a structural region. Only the latter undergoes dehydration and cyclization by LctM resulting in generation of an intricate polycyclic peptide. The leader sequence is proteolytically removed with concomitant export of the mature lantibiotic outside the cell by the dedicated cysteine protease/ABC transporter protein LctT.

A detailed understanding of the mechanisms governing dehydration and cyclization will allow the utilization of LctM for the engineering of novel conformationally constrained therapeutic peptides. We have employed site-directed mutagenesis and expressed protein ligation (EPL) to create analogues of LctA to examine the substrate tolerance of LctM. These studies have revealed relaxed substrate specificity and uncovered guidelines for the positional and sequence specificity of dehydration. This work demonstrates that LctM offers versatile control over the introduction of unsaturated amino acids and thioether rings into peptides.