Engineering Modified Lacticin 481 Using the Lantibiotic Synthetase LctM

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The lantibiotics are a class of ribosomally synthesized and post–translationally modified peptide antibiotics, which are characterized by their high content of amino acid residues lanthionine (Lan) and 3-methyllanthionine (MeLan), and typically (but not always) unsaturated amino acids 2,3-dehydroalanine (Dha) and (*Z*)-2,3-dehydrobutyrine (Dhb). The bifunctional enzyme LctM forms the modifications present in the lantibiotic lacticin 481 which is produced by Gram positive *Lactococcus lactis* bacteria. In this study, we explore the substrate specificity of the lantibiotic synthetase LctM in dehydrating non-proteinogenic serine analogs in semisynthetic substrates. Thus, a series of non-proteinogenic threonine analogs were designed and stereoselectively synthesized. They were further incorporated into the peptide substrates for lacticin 481 synthase using solid phase peptide synthesis (SPPS) and expressed protein ligation (EPL). The utility of LctM toward the *in vitro* synthesis of novel constrained peptides containing non-proteinogenic threonine analogs displays a high degree of substrate promiscuity. In these peptides the threonine analogs in which the γ-methyl group was replaced by ethyl, vinyl, ethynyl, allyl, and propynyl were indeed dehydrated by the lantibiotic synthetase LctM, and provide a blueprint for the design of peptide therapeutics.