Nine Posttranslational Modifications During the Biosynthesis of Cinnamycin and the Tailoring Enzymes

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Lantibiotics are ribosomally-synthesized and post-translationally modified antimicrobial peptides that are characterized by the thioether crosslinks lanthionine (Lan) and methyllanthionine (MeLan). Cinnamycin is a nineteen amino acid lantibiotic that contains one Lan and two MeLan crosslinks. Cinnamycin also contains an unusual lysinoalanine (Lal) bridge formed from the ε-amino group of lysine 19 and a serine residue at position 6, and an *erythro*-3-hydroxy-L-aspartic acid resulting from the hydroxylation of L-aspartate at position 15. These modifications are critical in mediating the interactions of cinnamycin with its target, phosphatidyl ethanolamine. Herein, we investigated the biosynthetic machinery using both *in vitro* studies and heterologous expression in *Escherichia coli*. CinX is an α-ketoglutarate/iron(II)-dependent hydroxylase that carries out the hydroxylation of aspartate 15 of the precursor peptide CinA. In addition, CinM catalyzes dehydration of four Ser and Thr residues and subsequent cyclization of Cys residues to form the three (Me)Lan crosslinks. The order of the post-translational modifications catalyzed by CinM and CinX is interchangeable *in vitro*. CinX did not require the leader sequence at the N-terminus of CinA for activity, but the leader peptide was necessary for CinM function. Although CinM dehydrated Ser6, it did not catalyze the formation of Lal. A small protein encoded by *cinorf7* is critical for the formation of the crosslink between Lys19 and dehydroalanine 6.