5.08 Biosynthesis and Mode of Action of Lantibiotics

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5.08.1 Introduction

Lantibiotics are peptide-derived antimicrobial agents that are ribosomally synthesized and posttranslationally modified to their biologically active forms. Their name was introduced in 1988 as an abbreviation for lanthionine-containing antibiotic peptides.[1] Lanthionines consist of two alanine residues that are linked at their β-carbons by a thioether bridge (**Figure 1**). In lantibiotics, these lanthionines are imbedded within cyclic peptides. All lantibiotics that have been characterized with respect to the stereochemistry of the thioether linkage contain (2*S*,6*R*)-lanthionines (Lan), with many family members also containing (2*S*,3*S*,6*R*)-3-methyllanthionines (MeLan; **Figure 1**). In addition, they typically (but not always) contain the unsaturated amino acids 2,3-dehydroalanine (Dha) and (*Z*)-2,3-dehydrobutyrine (Dhb). In all, no less than 15 different posttranslational modifications have been identified in lantibiotics (**Figure 1**),[2,3] and it is likely that other modifications remain to be discovered. These modifications release the peptides from the structural and functional constraints typically imposed on naturally occurring ribosomal peptides.

Lantibiotics are produced by both low and high G + C Gram-positive bacteria including the lactic acid bacteria (LAB), *Bacillus*, *Enterococcus*, *Micrococcus*, *Streptococcus*, *Staphylococcus*, and actinomycetes, and recent genome database searches show that their gene clusters are also present in select Gram-negative bacteria. Nisin, the most-studied lantibiotic, is produced by *Lactococcus lactis* and has been used extensively as a preservative against foodborne pathogens without substantial development of bacterial resistance.[4] Nisin was discovered in 1928,[5,6] and is one of the oldest known antibacterial agents; however, its structure was not determined until 1971 (**Figure 2**).[7] In 1969, nisin was conferred generally recognized as safe (GRAS) status by the World Health Organization. Since then, a steady stream of lantibiotics has been reported, each varying in size, structure, and biological activity, and with the advent of complete genome sequences, it has become clear that the lantibiotic

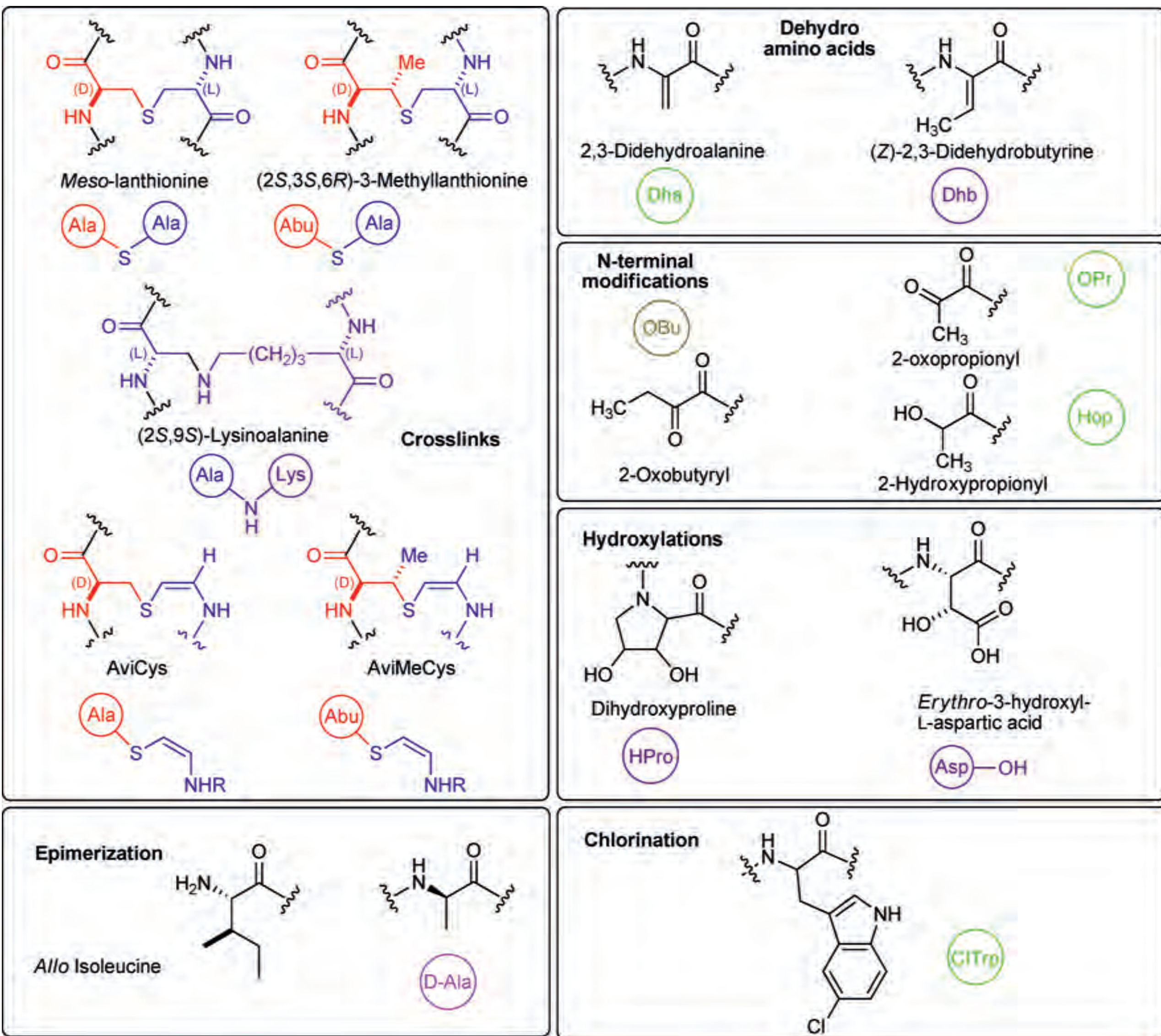

Figure 1 Posttranslational modifications that have been reported to date for the lantibiotic family. The shorthand notation that will be used in other figures in this review is listed below each structure.

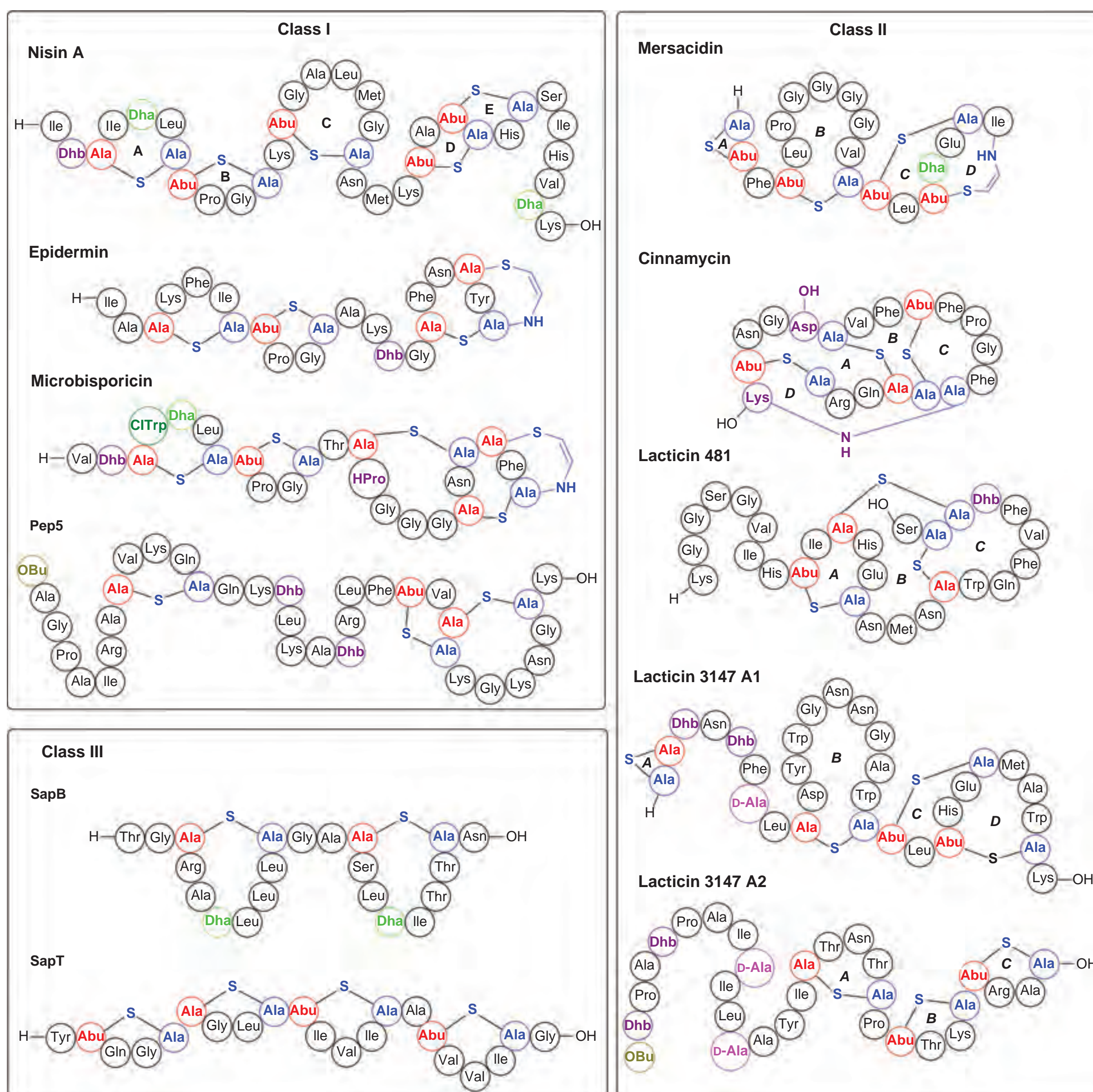

Figure 2 Representative examples of the three classes of lantibiotics. The same color-coding and shorthand notation is used as defined in **Figure 1**. For Lan and MeLan structures, the segments derived from Ser/Thr are in red whereas those derived from Cys are in blue. The ring numbering is shown for some members and is typically alphabetical from the N- to C-terminus.

gene clusters are ubiquitous and widespread. For instance, polymerase chain reaction (PCR) analysis of actinomycetes not known to produce lantibiotics revealed a large fraction of strains with the genetic capability to produce these compounds.[8] Furthermore, lantibiotics, which were originally believed to be limited to bactericidal activity against closely related species, have been shown recently to have a wide spectrum of biological activities, including immunomodulatory[9,10] and morphogenetic functions.[11]

The lantibiotics are ribosomally synthesized as precursor peptides (prepeptides) with an N-terminal leader peptide and the C-terminal structural peptide (also called propeptide) that undergoes processing to the mature active compound. As such, the lantibiotics are examples of a growing group of natural products including microcins (See Chapters 2.16 and 7.19),[12–16] patellamides (see Chapter 2.16),[17,18] and conotoxins[19] (see Chapter 5.10) for which a leader peptide appears to direct the maturation process. This chapter will focus on the structures, biosynthetic mechanisms, and biological activities of lantibiotics. For in-depth coverage of their gene clusters, regulation, applications, and self-immunity, the reader is referred to a number of reviews.[2,3,9,10]

5.08.2 Overview of Lantibiotic Structures

At present, more than 50 different lantibiotics are known displaying a striking diversity in structure, size, ring topology, and mode of action. A representative collection is depicted in **Figure 2** illustrating the high level of posttranslational modifications that typically amount to structural changes to about one-third of all amino acids in the peptide. The conformational constraints induced by lanthionines are essential for nisin's antimicrobial activity through binding to the cell wall biosynthetic intermediate lipid II[20,21] and forming pores in the bacterial cell membrane[22] (see Section 5.08.4). These multiple modes of bactericidal action are believed to account for the observed high efficacy of nisin (minimum inhibitory concentration (MIC) in the range of nanomolar) as well as the slow emergence of resistance.[23] Other lantibiotics with entirely different primary and three-dimensional structures such as mersacidin and cinnamycin also recognize with high affinity their targets, lipid II[24] and phosphatidylethanolamine[25] (PE), respectively, suggesting that the lanthionine motif is a naturally privileged architecture for constraining peptides into a bioactive conformation.[2] In addition, the lanthionine moiety provides improved chemical, proteolytic, and metabolic stability. For the majority of currently known lantibiotics, the targets are unknown, but based on the current knowledge on the biological activities of nisin, mersacidin, cinnamycin, lacticin 3147, and their structural analogues, it is highly likely that most, if not all, lantibiotics recognize specific targets with high affinity.

As depicted in **Figure 1**, a wide variety of posttranslational modifications are introduced into individual lantibiotics. In addition to the characteristic thioether cross-links Lan and MeLan, some lantibiotics, for example, epidermin and mersacidin (**Figures 1** and **2**), contain other cross-links such as *S*-aminovinyl-D-cysteine (AviCys) or *S*-aminovinyl-3-methyl-D-cysteine (AviMeCys). Yet another type of cross-link is found in cinnamycin in which a Lys residue is connected through its side chain ε-amino group to an Ala (lysinoalanine). In addition to these modifications that result in cyclic structures, a large number of structural modifications occur to amino acids that are not involved in ring formation. Among these are hydroxylations of Asp in cinnamycin and the duramycins,[26,27] hydroxylation of Pro and chlorination of Trp in microbisporicin,[28] and epimerizations resulting in *allo*-Ile in cypemycin[29] and D-Ala in lacticin 3147[30] and lactocin S.[31] Furthermore, Dha and Dhb residues that are N-terminally exposed after leader peptide processing spontaneously hydrolyze to yield a 2-oxopropionyl (OPr) moiety present in lactocin S,[31] and a 2-oxobutyryl (OBu) group present in Pep5.[32] OPr may be further modified by reduction to a 2-hydroxypropionyl (Hop) residue found in epilancin 15X,[33] epilancin K7,[34] and epicidin 280.[35]

The presence of posttranslational modifications has required the development of specialized methods for the structural determination of lantibiotics. Edman degradation is typically ineffective and therefore chemical fragmentation and derivatization techniques have been employed. Cyanogen bromide (CNBr) digestion followed by fast atom bombardment mass spectrometry (FAB-MS) was used to determine the thioether bridging pattern in lacticin 481.[36] The CNBr cleaves peptide bonds C-terminal to Met residues, resulting in the N-terminal portion of the peptide terminating with a homoserine lactone in place of Met. Additional chemical derivatization techniques focused on disruption of Lan/MeLan rings such that standard Edman degradation protocols could be utilized. Removal of an N-terminal OBu group has been achieved efficiently and successfully for Pep5 and lacticin 3147 A2 by reaction with 1,2-diaminobenzene in aqueous acetic acid.[37–39] Treatment of lantibiotics with an alkaline ethanethiol solution results in elimination reactions of the thioethers and addition of ethanethiol to the originally bridging residues.[38] However, because ethanethiol also adds to the nonbridging Dha/Dhb residues, it is not possible to distinguish Dha/Dhb from the thioether rings in the original structure. A more recently developed approach overcomes this drawback.[39] The protocol, involving the desulfurization of Lan/MeLan rings and the simultaneous reduction of Dha/Dhb using nickel boride (Ni_2B) with sodium borodeuteride ($NaBD_4$) in deuterium-labeled methanol/water (CD_3OD/D_2O, 1:1), was used for the structural determination of both peptides of lacticin 3147. A single deuterium atom was incorporated at the β-carbon of each residue that was linked in a Lan/MeLan whereas a deuterium atom was introduced at both the α- and β-carbon of former Dha/Dhb residues. Although bridging patterns are not revealed by this technique, it facilitates rapid differentiation between Dha/Dhb and Lan/MeLan