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# Posttranslationally Modified Bacteriocins—The Lantibiotics

***Abstract:*** *Lantibiotics are a subgroup of bacteriocins that are characterized by the presence of the unusual thioether amino acids lanthionine and 3-methyllanthionine generated through posttranslational modification. The biosynthesis of lantibiotics follows a defined pathway comprising modifications of the prepeptide, proteolytic activation, and export. The genes encoding the biosynthesis apparatus and the lantibiotic prepeptide are organized in clusters, which also include information for proteins involved in regulation and producer self-protection. The elongated cationic lantibiotics primarily act through the formation of pores and recent progress with nisin and epidermin has shown that specific docking molecules such as lipid II play an essential role in this mechanism. Mersacidin and actagardine inhibit cell wall biosynthesis by complexing the precursor lipid II, whereas the cinnamycin-like peptides bind to phosphoethanolamine thus inhibiting phospholipase A2.* © 2000 John Wiley & Sons, Inc. Biopoly 55: 62–73, 2000

***Keywords:*** *lantibiotics; bacteriocins; posttranslational modification*

## INTRODUCTION

The interactions between microbes in an ecosytem are manifold and among those interactions primarily antagonistic, bacteriocins are supposed to be of major importance. Bacteriocins are protein toxins elaborated by bacteria and designed to specifically kill other bacteria. The prototype bacteriocins of gram negative bacteria are the colicins, comparatively large protein toxins (approximately 30–70 kD in molecular mass) with defined domains for the interaction with specific receptors on the cell surface, for translocation through the cell envelop and for the toxic activity. In contrast, bacteriocins of gram-positive bacteria are usually small peptides (2–5 kD in mass), which may be a consequence of the different envelop architecture of gram-positive bacteria consisting largely of the dense peptidoglycan network that is permeable to peptides but not to proteins. Most of the bacteriocins from gram-positive bacteria are produced as unmodified peptides (see contribution of I. Nes elsewhere in this volume); however, a distinct group of these bacteriocins (summarized in Table I) undergoes posttranslational side-chain modifications resulting in unusual amino acids not known to occur in other peptides or proteins. The unique structural features present in

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**Table I Known Lantibiotic Peptides and Their Structural Features**

| Lantibiotic | Molecular Mass (Da) | Producer Species | Modified Residues | | | | Ref. |
|---|---|---|---|---|---|---|---|
| | | | Lan | MeLan | Dha | Dhb | |
| Nisin Group | | | | | | | |
| Nisin A | 3353 | *Lactococcus lactis* | 1 | 4 | 2 | 1 | 4,5 |
| Nisin Z | 3330 | *Lactococcus lactis* | 1 | 4 | 2 | 1 | 4,5 |
| Subtilin | 3317 | *Bacillus subtilis* | 1 | 4 | 2 | 1 | 4,5 |
| Epidermin group | | | | | | | |
| Epidermin | 2164 | *Staphylococcus epidermidis* | 2 | 1 | 0 | 1 | 4,5 |
| [Val1-Leu6]-epidermin[a] | 2151 | *Staphylococcus epidermidis* | 2 | 1 | 0 | 1 | 4,5 |
| Gallidermin | 2164 | *Staphylococcus epidermidis* | 2 | 1 | 0 | 1 | 4,5 |
| Mutacin B-Ny266[a] | 2270 | *Streptococcus mutans* | 2 | 1 | 1 | 1 | 4,5 |
| Mutacin 1140[a] | 2263 | *Streptococcus mutans* | 2 | 1 | 1 | 1 | 84 |
| Mutacin III[a] | 2266 | *Streptococcus mutans* | 2 | 1 | 1 | 1 | 85 |
| Pep5 group | | | | | | | |
| Pep5 | 3488 | *Staphylococcus epidermidis* | 2 | 1 | 0 | 2 | 4,5 |
| Epilancin K7 | 3032 | *Staphylococcus epidermidis* | 2 | 1 | 2 | 2 | 4,5 |
| Epicidin 280[a] | 3133 | *Staphylococcus epidermidis* | 1 | 2 | 0 | 1 | 4,5 |
| Lacticin 481 group | | | | | | | |
| Lacticin 481 | 2901 | *Lactococcus lactis* | 2 | 1 | 0 | 1 | 4,5 |
| Streptococcin A-FF22[a] | 2795 | *Streptococcus pyogenes* | 1 | 2 | 0 | 1 | 4,5 |
| Butyrivibriocin OR79A[a] | ? | *Butyrivibrio fibrisolvens* | 1 | 2 | 0 | 1 | 86 |
| Salivaricin A[a] | 2315 | *Streptococcus salivarius* | 1 | 2 | 0 | 0 | 4,5 |
| [Lys2, Phe7]-salivaricin A[a] | 2321 | *Streptococcus salivarius* | 1 | 2 | 0 | 0 | 4,5 |
| Variacin[a] | 2658 | *Micrococcus varians* | 2 | 1 | 0 | 1 | 4,5 |
| Lactocin S | 3764 | *Lactobacillus sake* | 2 | 0 | 0 | 1 | 4,5 |
| Cypemycin | 2094 | *Streptomyces* ssp | 0 | 0 | 0 | 4 | 4,5 |
| Plantaricin C | 2880 | *Lactobacillus plantarum* | 1 | 3 | 1 | 0 | 87 |
| Mersacidin group | | | | | | | |
| Mersacidin | 1825 | *Bacillus* ssp. | 0 | 3 | 1 | 0 | 4,5 |
| Actagardine | 1890 | *Actinoplanes liguriae* | 1 | 2 | 0 | 0 | 4,5 |
| Ala(0)-actagardine[a] | 1961 | *Actinoplanes liguriae* | 1 | 2 | 0 | 0 | 88 |
| Cinnamycin group | | | | | | | |
| Cinnamycin | 2042 | *Streptomyces cinnamoneus* | 1 | 2 | 0 | 0 | 4,5 |
| Duramycin | 2014 | *Streptomyces cinnamoneus* | 1 | 2 | 0 | 0 | 4,5 |
| Duramycin B | 1951 | *Streptoverticillium* sp. | 1 | 2 | 0 | 0 | 4,5 |
| Duramycin C | 2008 | *Streptomyces griseoluteus* | 1 | 2 | 0 | 0 | 4,5 |
| Ancovenin | 1959 | *Streptomyces* ssp. | 1 | 2 | 1 | 0 | 4,5 |
| Structures incomplete | | | | | | | |
| Sublancin 168 | 3877 | *Bacillus subtilis* | | | | | 89 |
| Mutacin II | 3245 | *Streptococcus mutans* | | | | | 90 |
| Carnocin UI 49 | 4635 | *Carnobacterium piscicola* | | | | | 4,5 |
| Nukacin ISK-1 | 2960 | *Staphylococcus* ssp. | | | | | [b] |
| Structures incomplete/two-component lantibiotics | | | | | | | |
| Cytolysin A1 | 4164 | *Enterococcus faecalis* | | | | | 13 |
| Cytolysin A2 | 2631 | | | | | | |
| Staphylococcin C55$\alpha$ | 3339 | *Staphylococcus aureus* C55 | | | | | 14 |
| Staphylococcin C55$\beta$ | 2993 | | | | | | |
| Lacticin 3147 A | 3322 | *Lactococcus lactis* DPC3147 | | | | | 15 |
| Lacticin 3147 B | 2847 | | | | | | |

[a] Bridging patterns are not proven but assumed to be identical to those of the related peptides in the respective group. References 4 and 5 contain detailed literature on structures identified before 1995 or 1998.

[b] Sonomoto, K. pers. communication.

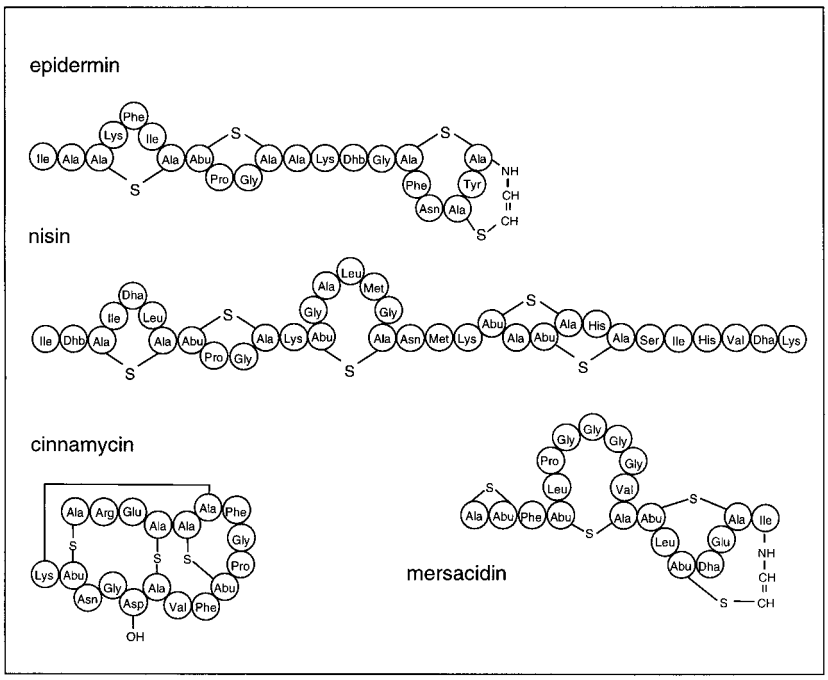


**FIGURE 1** Primary structure of the lantibiotics epidermin, nisin, cinnamycin and mersacidin. Dha: 2,3-didehydroalanine; Dhb: 2,3-didehydrobutyrine; Abu: $\alpha$-aminobutyric acid; Ala-S-Ala: lanthionine; Abu-S-Ala: 3-methyllanthionine

these bacteriocins are the intramolecular rings formed by the thioether amino acids lanthionine (Lan) and methyllanthionine (MeLan), which led to the designation lantibiotics (*lan*thionine-containing an*tibiotic* peptides).[1] Additionally, the dehydrated amino acids 2,3-didehydroalanine (Dha) and 2,3-didehydrobutyrine (Dhb) are common in lantibiotics, whereas modified residues such as S-aminovinyl-D-cysteine, S-aminovinyl-D-methylcysteine, lysinoalanine, or erythro-3-hydroxyaspartic acid are only found in a few. The synthesis from ribosomally made prepeptides clearly distinguishes lantibiotics from classical peptide antibiotics produced through multienzyme complexes.[2,3] In recent years, interest in lantibiotics has continuously increased, mainly due to their potential to serve as natural food preservative that might replace harmful chemical agents. This article focuses on the main aspects of structure, biosynthesis, and mode of action of lantibiotics. For more detailed information the reader is referred to different reviews and books.[4–7]

## PRIMARY STRUCTURES

Lantibiotics do not form a homogeneous group regarding size, structure, or mode of action. Based on chemical and structural features, Jung suggested a subdivision of the lantibiotics into type A and type B groups.[8] Following this proposal, type A lantibiotics are elongated, flexible molecules that are positively charged and act on bacterial membranes by the formation of pores. The most prominent member of this group is nisin, which was already identified in 1928. However, it was not before 1970 that the complete structure of nisin was elucidated (Figure 1).[9] In contrast, type B lantibiotics such as ancovenin, duramycin A, B, C, and cinnamycin (Figure 1) have globular structures due to their characteristic head-to-tail cross-linkage.[10] These peptides form a group of natural variants that carry a negative or no net charge and interfere with various enzyme functions. Compared to the cinnamycin-like type B lantibiotics, mersacidin (Figure 1) and actagardine show a much higher degree of antimicrobial activity. Although they lack the typical head-to-tail structure,[11,12] they are regarded as a subgroup of the type B lantibiotics mainly due to their overall similarity of their leader peptides. The recent characterization of new lantibiotics with intermediate properties or novel features (e.g., the two-component lantibiotics requiring the synergistic interaction of two structurally different peptides[13–15]) makes their classification into type A and type B categories difficult. Nevertheless, groups of related peptides probably

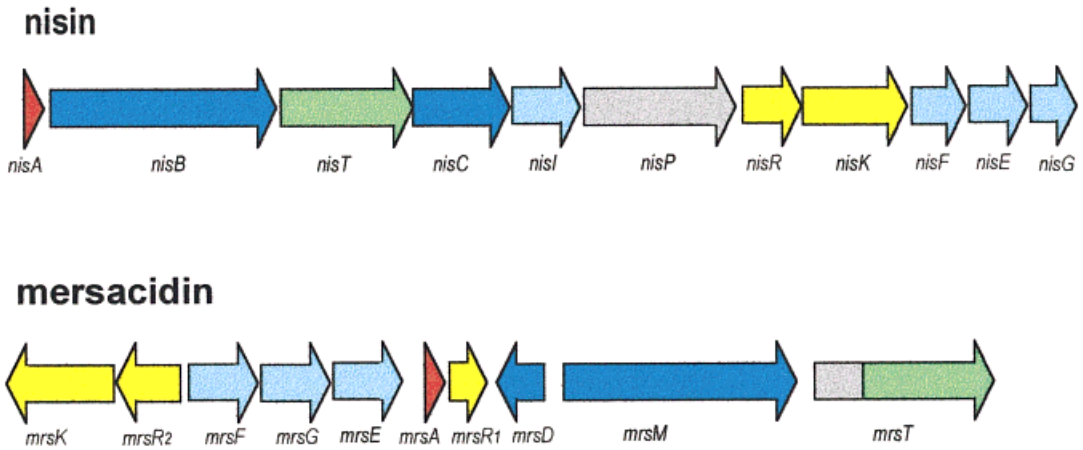


**FIGURE 2** Biosynthetic gene clusters of nisin and mersacidin. The structural genes are marked in red, genes necessary for modification in dark blue and for export in green. Genes involved in regulation are in yellow and genes for producer self-protection in light blue. Genes (or gene segments in the case of the hybrid transporter) encoding the processing protease are shaded in grey.

originating from a common ancestor peptide can be clearly distinguished (Table I). The content of modified amino acids generally causes serious problems for structure elucidation of lantibiotics. Dehydrated residues located at the N-terminus of the peptide or exposed during Edman degradation are highly unstable and spontaneously deaminate resulting in sequencing block.[16] Meyer et al.[17] developed a method allowing direct sequencing of dehydroamino acids and thioethers after treatment with thiol compounds under alkaline conditions. While this procedure can be used to elucidate the primary structure, the bridging pattern must be determined using additional methods such as two-dimensional (2D) nmr spectroscopy. Within a group of natural variants it may also be deduced from patterns of known structures.

## BIOSYNTHETIC GENE CLUSTERS AND BIOSYNTHESIS

Lantibiotics are encoded by the structural genes *lanA* (the abbreviation *lan* is used for homologous genes of different lantibiotic gene clusters) and produced as prepeptides consisting of an N-terminal leader peptide and a C-terminal propeptide part. Ser, Thr, and Cys residues in the propeptide part are subject to the modification reactions (see below). The genes responsible for modification (*lan*B, *lan*C, *lan*M, and *lan*D), for proteolytic processing (*lan*P), transport (*lan*T), immunity (*lan*I, *lan*EFG), and for regulation (*lan*R, *lan*K, *lan*Q) are located in the vincinity of the respective structural gene. Together they form biosynthetic gene clusters with several transcription units that can be located either on the chromosome or on mobile elements such as plasmids or transposons.[6,18] Concerning the biosynthetic pathway, two different classes of lantibiotics can be distinguished. The peptides of class I are modified by LanB and LanC proteins and processed by a serine protease LanP. The export is performed by the ABC-transporter LanT. In comparison, lantibiotics of class II are modified by the action of a single LanM protein, and processing takes place simultaneously with transport by LanT(P), a hybrid ABC-transporter with an additional proteolytic domain. As an example, the gene clusters of nisin (class I)[19] and mersacidin (class II)[20] are shown in Figure 2.

## DEHYDRATATION AND THIOETHER FORMATION

Figure 3 shows a general scheme of the proposed two-step mechanism of Lan/MeLan formation. In the first step the hydroxyl amino acids Ser and Thr are dehydrated yielding the $\alpha,\beta$-unsaturated amino acids 2,3-didehydroalanine (Dha) or 2,3- didehydrobutyrine (Dhb), respectively.[21] While some of the dehydrated amino acids, for lack of Cys residues, are not further modified and represent the Dha and Dhb residues found in the mature peptide, others undergo an intramolecular Michael addition with neighboring Cys residues resulting in the formation of thioether bridged *di*-carboxy-*di*-amino acids. Both steps are catalyzed by specific enzymes; in producers of the

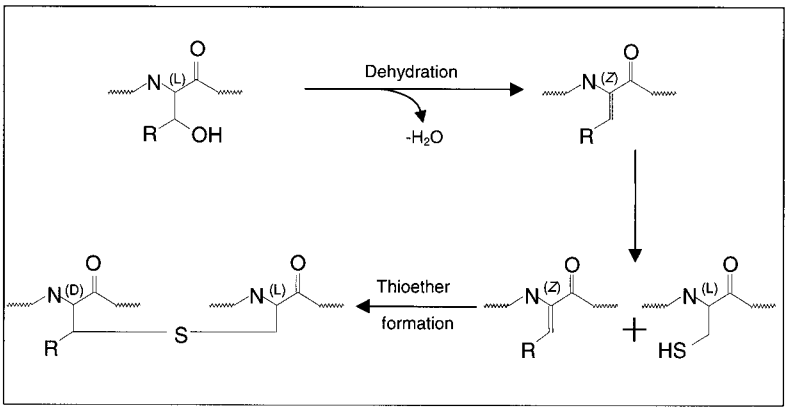


**FIGURE 3** General mechanism of Lan and MeLan formation. Ser (R: H) → Dha → Lan; Thr (R: $CH_3$) → Dhb → MeLan

class I lantibiotics, the dehydration reaction is likely performed by the LanB proteins, while LanC is responsible for thioether formation.[22] The LanB proteins are of about 1000 amino acids in size and most likely associated with the cell membrane.[23–25] LanC proteins are smaller and consist of about 400 amino acids.[23] In producer strains of class II lantibiotics a single modification enzyme of 900–1000 amino acids, LanM, seems to catalyze both reactions. Its C-terminus shares striking similarities to LanC enzymes, whereas the N-terminus does not seem to be related to the LanB proteins. So the possibility of a gene fusion of *lan*B and *lan*C can be excluded.[18] It is proposed that the C-terminus of LanM fulfills the function of LanC while the N-terminus of LanM could be involved in dehydration. However, this proposal remains to be proven.

## OXIDATIVE DECARBOXYLATION

Several lantibiotics with a C-terminal Cys residue are oxidized and decarboxylated before addition to the unsaturated Dha and Dhb yielding S-[(Z)-2-aminovinyl)]-D-cysteine (epidermin and cypemycin) or S-2-aminovinyl-3-methyl-D-cysteine (mersacidin; see Figure 1). Oxidative decarboxylation is performed by an additional modification enzyme, the oxidoreductase LanD. The LanD proteins are small enzymes of 181 (EpiD) or 194 amino acids (MrsD) containing FMN[26] or FAD, respectively. Kupke et al.[27–29] showed that EpiD catalyzes the oxidation reaction resulting in the formation of a double bond and a reduced coenzyme. Subsequently, the oxidized cysteine decarboxylates to yield a (Z)-enethiol compound which most probably converts to an enethiol anion. This anion is thought to react with a 2,3-didehydroalanine residue in position 19 to form the C-terminal S-[(Z)-2-aminovinyl)]-D-cysteine of mature epidermin. MrsD presumably catalyzes the analogous reaction to form S-2-aminovinyl-3-methyl-D-cysteine during mersacidin maturation. However, this is still subject of examination.

## REGULATION OF BIOSYNTHESIS

Biosynthesis of many lantibiotic and nonlantibiotic bacteriocins seems to be regulated by typical bacterial two-component regulatory systems, consisting of a membrane-bound histidine kinase and a response regulator.[30,31] Generally, the effect of two-component systems is triggered by an external signal molecule.[32] The kinase LanK is a protein of 380–480 amino acids that is able to receive the signal molecule with its extracytoplasmic domain, and in response, to autophosphorylate a conserved histidine residue within the intracellular domain. Subsequently, the phosphate group is transferred to a conserved Asp residue of the response regulator LanR. LanR, a small protein of 200–220 amino acids, then undergoes a conformational change that enables the C-terminal domain to bind to the operator region of the respective gene. Consequently, transcription of the regulated genes is activated or repressed. In the case of nisin and subtilin the lantibiotics themselves serve as signals, thus autoregulating their own synthesis and functioning as "quorum sensing" molecules.[33] Apparently, uninduced cells produce small amounts of the bacteriocin up to a threshold concentration recognized by LanK as the input signal. Similar regulation cascades have been described for plantaricin production by *Lactobacillus plantarum* C11[34] and for the competence factor of *Streptococcus pneumoniae*[35]. In contrast to the two-component systems, the production of epider-

min is regulated by a single protein of 205 amino acids, designated as EpiQ. EpiQ shares only limited similarities with response regulator proteins encoded in the gene clusters of nisin and subtilin. As it lacks the conserved Asp residue and an associated kinase, it is assumed that phosphorylation is not necessary for regulatory activity of EpiQ.

## EXPORT AND PROTEOLYTIC PROCESSING

The modified LanA prepeptide needs activation through proteolytic removal of the leader peptide. In the case of class I lantibiotics and lactocin S, this is achieved by dedicated subtilisin-like serine proteases LanP. These proteins can be located inside the cells as in the case of lactocin S, Pep5, and epilancin K7 or outside the cell as for epidermin and the cytolysins. NisP, the protease involved in processing of the nisin prepeptide, is most probably coupled to the peptidoglycan of the bacterial cell wall.[36] Leader peptides cleaved by LanP show a conserved FNLD motif and a conserved Pro in position −2. The location of the respective protease determines the timing of processing; it can take place before or after export from the cell through the dedicated LanT transport proteins.[22,37,38] In almost every gene cluster of the class I lantibiotics a gene encoding such a protein with significant sequence similarities to the group A (i.e., encoded by one gene) ATP-binding-cassette (ABC)-transport protein superfamily was found.[5] These transport proteins of 500–600 amino acids can be functionally divided into two domains. The N-terminus consists of six membrane-spanning helices, while the C-terminal domain contains two conserved ATP-binding motifs (Walker motifs A and B). The latter is located at the inside of the cytoplasmic membrane and responsible for binding and subsequent hydrolysis of ATP, which is the driving force of export. Class II lantibiotics such as lacticin 481 and mutacin II are activated concomitantly with export by the action of an ABC-transport protein with a N-terminal protease domain. This additional domain of 100–200 amino acids is also commonly found in nonlantibiotic bacteriocins.[39] It contains a conserved cysteine residue regarded to be an essential part of the active site. For lactococcin G it has been shown that the first 150 amino acids are sufficient for specific cleavage of the prepeptide.[39] The leader peptides of the modified and unmodified bacteriocins processed by these hybrid proteins are characterized by a conserved "double-glycine" cleavage site with Gly in position −2 and Gly, Ala, or Ser in position −1. Neither the leader peptides of lantibiotics cleaved by LanP nor those cleaved by LanT share any similarity to signal sequences involved in the *sec*-dependent peptide transport. Several functions of the leader peptide are currently discussed; for those lantibiotics processed after export, the presence of the leader peptide most probably keeps the lantibiotic in an inactive state, thereby protecting the producing cell.[36] Furthermore, the conserved motif within the leader peptide of class I lantibiotics might indicate its function as a recognition signal for the modification enzymes.[40,41]

## PRODUCER IMMUNITY

The antibiotic activity of the lantibiotics (see below) forces their producers to elaborate specific self-protection mechanisms. Generally, the proteins conferring immunity to the producer strains of unmodified bacteriocins are relatively small (up to 100 amino acids) and antagonize specifically the bacteriocins. This is also true for the immunity proteins of the lantibiotics Pep5, lactocin S, and epicidin 280.[38,42,43] In contrast, gene clusters of the lantibiotics nisin and subtilin harbor genes encoding larger lipoproteins (NisI 245 amino acids, SpaI 165 amino acids) involved in self-protection.[19,44] Additionally they contain an ABC-transporter that could function by removing bacteriocin molecules inserted in the membrane back to the supernatant and thus keeping the concentration of the bacteriocin under a certain level. As the immunity related transporter proteins are encoded by multiple genes (*lan*EFG, see Figure 2), they belong to the group B ABC-transport protein superfamily. LanE and LanG form membrane-spanning subunits, while LanF contains the ATP-binding site. It has been shown that a nonproducing strain of *Lactococcus lactis*, which was transformed with a plasmid harboring *nis*EFG and the respective genes for the proteins involved in regulation displays immunity against nisin.[45]

## BIOLOGICAL ACTIVITIES

Lantibiotics are primarily active against gram-positive bacteria and exert their mode of action at the cytoplasmic membrane. Resistance of gram-negative bacteria results from the protective effect of the outer membrane that is impenetrable to these peptides. Type A lantibiotics, as defined by Jung[8] and represented by nisin, epidermin, and Pep5 are flexible, elongated, amphipathic molecules and possess an overall positive net charge. Their mode of action is a

rather complex process that results in the formation of pores in the bacterial cytoplasmic membrane and that leads to immediate cell death. Type B lantibiotics such as cinnamycin and mersacidin are comparatively smaller peptides consisting of approximately 20 amino acids only. These peptides are rigid globular molecules with either no or a negative net charge and inhibit enzyme functions by forming complexes with specific integral membrane components.

Cinnamycin and duramycin possess only weak antibacterial activities. Treatment of *Bacillus* cells with these peptides resulted in increased cytoplasmic membrane permeability[46] and interference with several membrane transport mechanisms.[47,48] Additionally, various effects on eucaryotic cells were described, e.g., the hemolysis of erythrocytes[49] and immunomodulating effects through inhibition of phospholipase A2.[10,50] Analysis of a duramycin-resistant *Bacillus* strain revealed that the bulk phospholipid phosphatidylethanolamine in the cytoplasmic membrane had been replaced by its plasmalogen form.[51] In subsequent experiments, the molecular mechanism that leads to the various effects observed was identified. Cinnamycin and duramycin specifically bind to phosphatidylethanolamine,[10,52] thereby blocking phospholipase A2 from binding to its substrate. Mersacidin and actagardine are potent inhibitors of cell wall biosynthesis of bacteria. Treatment of bacterial cells with mersacidin induced slow lysis after one generation time. Electron microscopy revealed that the cell wall was reduced in diameter and de novo synthesis, specially at the septum, was inhibited. As demonstrated by incorporation assays with radiolabeled precursors, cell wall biosynthesis was the only process that was inhibited, while biosynthesis of RNA, DNA and proteins was not affected.[53] Using a cell-free peptidoglycan synthesis assay it was shown that mersacidin and actagardine interfere with cell wall biosynthesis at the level of transglycosylation, e.g., the continuous addition of the disaccharide units of the ultimate membrane bound cell wall precursors (lipid II) to the growing peptidoglycan chain was blocked[54] (Figure 4A). It was demonstrated that both lantibiotics form a tight complex with lipid II (undecaprenyl-diphosphoryl-N-acetylmuramic acid-[pentapeptide]-N-acetylglucosamine) isolated from bacterial membranes.[55] The structural basis for the activity of both lantibiotics seems to be associated with a conserved sequence motif in both peptides that comprises one complete thioether ring system.

Type A peptides show faster killing kinetics than the type B peptides. First experiments were already performed in 1960 by Ramseier,[56] who observed leakage of uv-absorbing intracellular compounds from nisin treated *Clostridia* cells and presumed a detergent-like disruption of the cytoplasmic membrane. Subsequent studies with nisin and other type A lantibiotics demonstrated rapid efflux of ions, solutes, and small metabolites such as amino acids and nucleotides from susceptible cells. The concomitant dissipation of the membrane potential lead to an instant stop of all biosynthetic processes.[57–59] Studies with black lipid membranes revealed the formation of nonselective, voltage-dependent, short-lived transmembrane pores.[60] Nuclear magnetic resonance based structural analysis of nisin in the presence of membrane mimicking micelles indicated that the hydrophilic groups of the peptides interact with the phospholipid head groups. The hydrophobic side chains were immersed in the hydrophobic core of the membrane bilayer such that the molecules adopted a rod-like conformation.[61,62] These data and experiments with artificial membrane systems were summarized to the following model for the pore formation process.[63,64] The positively charged peptide side chains bind to the phospholipid head groups of the target membrane via ionic forces. Since the peptides are too small to span the bilayer more than once, several molecules are presumed to preaggregate for pore formation. Upon wedge-like insertion into the membrane, the molecules remain surface-bound, thus causing local perturbation, which finally creates short-lived pores.

Although this model is able to explain all results obtained from experiments with model membrane systems, various activity data of type A lantibiotics in vivo do not correlate with a simple poration model. For example, much higher peptide concentrations were found to be necessary for pore formation in model membrane systems than for killing in vivo and striking differences in the sensibility of bacteria even of closely related strains were observed. First hints for the existence of a specific factor that could play a role in the pore formation process and for the identity of this factor were obtained from early experiments of Linnett and Strominger,[65] who demonstrated the inhibition of cell wall biosynthesis by nisin in in vitro systems. As shown later, this inhibition is due to binding of nisin to the cell wall precursors, lipid I and lipid II.[66] Studies with whole cells demonstrated that indeed the availability of lipid II is relevant for the sensitivity of bacteria. Membrane poration by nisin and epidermin could be prevented by preincubation of *Micrococcus luteus* or *Staphylococcus simulans* with ramoplanin, a lipopeptide which is known to also bind to lipid II,[68] thus competing with the lantibiotics for specific binding sites.[67] Additionally, cytoplasmic membrane vesicles of *Micrococcus flavus* became

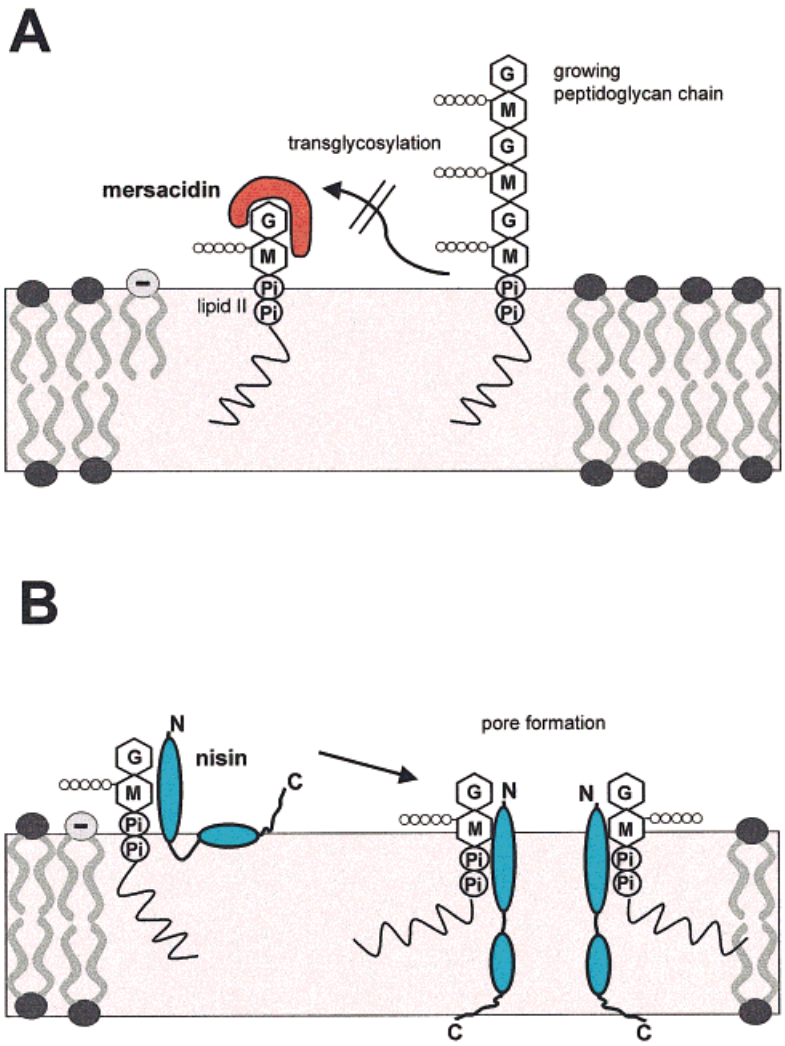


**FIGURE 4** Mode of action of mersacidin (A) and nisin (B). During peptidoglycan synthesis the cell wall precursor lipid II is transferred to the outside of the membrane and becomes accessible for the lantibiotics. Mersacidin (red) complexes the disaccharide moiety thereby blocking its incorporation into the growing peptidoglycan chain. Nisin (green) binds to the cell wall precursor and primarily use it as a docking molecule for pore formation.

more sensitive to nisin upon increasing the lipid II content.[69] In artificial membranes it was shown that upon incorporation of purified lipid II nisin activity increased by three orders of magnitude, e.g., from the $\mu M$ to the n$M$ concentration range.[70] Binding of nisin and epidermin to lipid I and lipid II, which differ only by the presence of the N-acetylglucosamine residue in lipid II, was demonstrated in a cell free peptidoglycan synthesis assay. Other pore forming type A lantibiotics, e.g., Pep5 have no affinity to the cell wall precursor and might target another yet unidentified membrane molecule.[67]

Defined structural elements of nisin for binding to lipid II and for pore formation activity were identified in a study with nisin mutant peptides. These studies also revealed that, through the interaction with lipid II, nisin indeed is a dual function antibiotic. It efficiently blocks peptidoglycan biosynthesis in a way similar to mersacidin and simultaneously uses lipid II for targeted pore formation (Figure 4B). The N-terminal part of nisin appears to be necessary for initial binding to lipid II. Mutations that affect the conformation of the N-terminal rings (e.g., S3T, which contains an additional methyl group in the first lanthionine ring) reduced the affinity for lipid II and consequently reduce both the ability to block peptidoglycan biosynthesis and to form pores. Mutations that affect the flexible hinge region in the center of the molecule [e.g., ΔN20/ΔM21, see Figure 1] only knock out pore formation, but do not affect binding of lipid II and

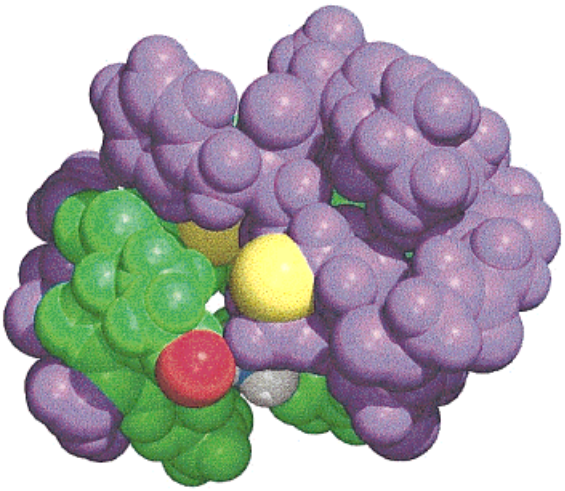

**FIGURE 5** 3 D-structure of mersacidin as elucidated by Prasch et al[91]. Coordinates were kindly provided by T. Prasch and C. Griesinger. Sulfur atoms are highlighted in yellow. The oxigen of Glu17 is in red and the ring structure that is conserved in mersacidin and actagardine is illustrated in green. Reprinted from Chemistry & Biology, **3,** 548 (1996) with permission from Elsevier Science.

inhibition of cell wall biosynthesis. Thus, the combination of two killing mechanisms in one molecule potentiates the antibiotic activity and results in nanomolar minimal inhibitory concentrations (MIC values). It is interesting to point out that what first appeared to be different modes of action with fundamentally different consequences for the cell, i.e., inhibition of cell wall biosynthesis and slow lysis (mersacidin) and fast killing through pore formation (nisin), is based on the same type of molecular interaction, which is complex formation with lipid II. Pore formation is a fast killing process that overrides the slow lytic effect induced by peptidoglycan biosynthesis inhibition. However, the latter mechanism could be important for cells that survive pore formation, a well-known but poorly understood phenomena, and can explain the low MIC values of nisin observed for a number of gram-positive bacteria.

The antibacterial activity of this lantibiotics is of special interest with respect to the increasing resistance of bacteria to current antibiotics. In contrast to the glycopeptide vancomycin, which binds to lipid II via the C-terminal D-Ala-D-Ala of the pentapeptide side chain (see Figure 4), complex formation of the lantibiotics involves the sugar–pyrophosphate moiety. Nisin and epidermin were shown to interact with both lipid I and lipid II, which only differs in the N-acetylglucosamine. Mersacidin and actagardine specifically bind only to lipid II and it is likely that the disaccharide moiety is the site of complex formation. Since lantibiotics can be modified by site-directed mutagenesis, the identification of defined structural features targeting new binding sites at the cell wall precursor can lead to the development of future potent antibiotic peptides.

## APPLICATIONS

Nisin is successfully used as a food preservative in more than 50 countries, mainly in cheese, canned vegetables, various pasteurized dairy, liquid egg products, and salad dressings. Its proteinaceous nature and lack of toxicity make nisin ideally suited for the food industry. Its comparatively broad antimicrobial spectrum includes many gram-positive bacteria and important endospore-forming food pathogens such as *Clostridium botulinum*. The combination of heat treatment and addition of nisin has been shown very effective in controlling spoilage. Recent experiments indicate that nisin could also be useful in therapy of peptic ulcer caused by *Helicobacter pylori*.[71] Because of its stability toward acidic conditions and pepsin, nisin maintains its antimicrobial activity in the stomach and is finally inactivated by pancreatic enzymes leaving the flora of the intestine unaffected. Moreover, mersacidin has shown promising in vivo activity against methicillin-resistant Staphylococcus aureus (MRSA), which represent a threatening clinical problem.[72] Infections with MRSA are currently treated with gycopeptide antibiotics vancomycin and teicoplanin; however, emergence of vancomycin resistant *S. aureus* strains has already been reported.[73] The efficiency against MRSA and its novel mode of action make mersacidin an interesting lead structure for the development of a new class of antibiotics.

## PEPTIDE EGINEERING

Gene-encoded peptides such as the lantibiotics can be modified through site-directed mutagenesis, which is a useful tool for research focusing on structure–function relationships as well as for optimizing chemical and physical properties. The posttranslational modification of lantibiotics requires that the production of mutated genes is performed in the wild-type producer or in a closely related strain harboring the respective biosynthetic apparatus. Such systems have been successfully developed for nisin,[74–76] epidermin,[77] subtilin,[78] Pep5,[79] and mutacin II,[80] enabling the exchange of certain amino acids or even the introduction of new modified residues with effects on peptide properties like stability, solubility, or antimicrobial activity. In the case of nisin Z it has been shown, for example, that an increase of solubility in water could be achieved by replacement of the amino

acids in position 27 or 31 for Lys (N27K and H31K nisin Z) without altering antimicrobial activity and spectrum.[81] The variant peptide T2S nisin Z displays a higher activity than wild-type nisin Z toward individual indicator strains, while the mutation S3T results in a dramatic loss of activity.[82] Exchanging the Dha of nisin Z in position 5 for Dhb reduces activity 5–10-fold,[76] while replacement for Ala in nisin has no effect on pore formation.[75,83] Similar results were obtained by site-directed mutagenesis of other lantibiotics.[82] Although the effects of amino acid exchanges are difficult to predict, it has been shown for several lantibiotics that mutations affecting the residues involved in thioether formation usually result in a large decrease of activity or even in a complete loss of production like in the case of S19A epidermin or S23A nisin. So far, it was not possible to substantially broaden the spectrum of activity or to construct mutant peptides with increased bactericidal activity against all indicator strains tested.

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