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Lantibiotics and microcins: Novel posttranslational modifications of polypeptides

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Abstract: Unique peptide modification mechanisms have attracted considerable interest from scientists working in both applied and basic research. The design, genetic engineering and production of unusually modified peptides for use in areas such as biomedical applications and food technology has rapidly become a small, but exciting, new branch of biotechnology. Both lantibiotics and microcins are antimicrobial peptides which contain combinations of thioether bridges, α,β-unsaturated amino acids, D-amino acids, N-, C-terminal and heteroaromatic backbone modifications, all of which arise from posttranslational modifications of Ser, Thr, Cys and Gly residues in a ribosomally-synthesised precursor polypeptide. The structures of many of the lantibiotics have been elucidated and the structure of ^{13}C-,^{15}N-labelled microcin B17 has been solved. In addition, the total synthesis of microcin B17, and a number of analogues, was recently accomplished. The previously undescribed enzymes apparently responsible for dehydration, ring formation, oxidative decarboxylation, hydrogenation, and leader peptide cleavage are currently being characterised.

Introduction

Both the lantibiotics and microcin B17 are microbial-derived, antimicrobial peptides which contain posttranslationally modified amino acids (refs. 1-6), such as oxazole and thiazole rings, thioether-bridged *di*-carboxy, *di*-amino acids, D- and α,β-unsaturated amino acids and a variety of N-and C-terminal modifications (Fig. 1). Indeed the name 'lantibiotic' (ref. 7) is derived from these peptides content of lanthionine (Lan) and 3-methyllanthionine (MeLan).

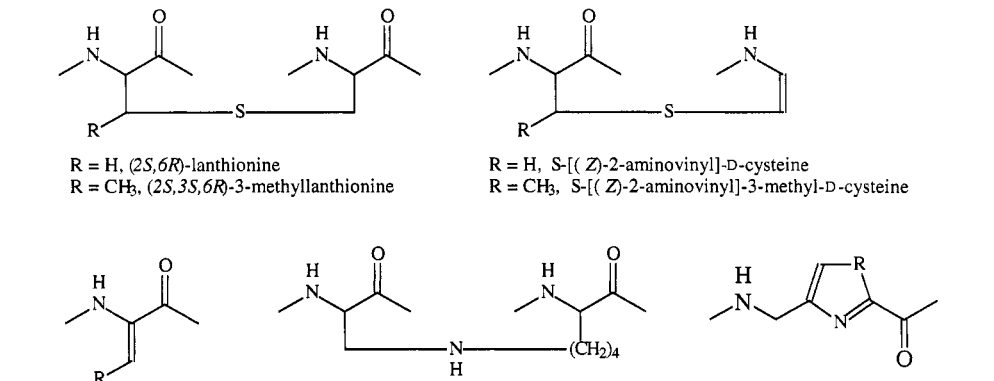

Fig. 1 Structures of some 'unusual' amino acids found in lantibiotics and microcin B17.

Each of the peptides is first synthesised as a polypeptide precursor, which subsequently undergoes specific posttranslational modification reactions, carried out by specific cellular enzymatic machinery, and from which a leader peptide is subsequently cleaved to release the active moiety (Fig. 2). Interestingly, nearly all of the unusual amino acids result from modifications to Ser, Thr, Cys and Gly residues. The leader peptides themselves are not modified, but appear to be necessary for proper transport of the products out of the cell; therefore, they will not be discussed further here.

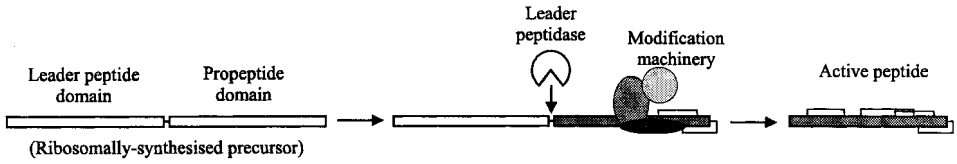

Fig. 2 General biosynthetic steps involved in lantibiotic and microcin formation.

Chemical structures

Lantibiotics

Since a substantial number of lantibiotics have now been described, they have been subdivided into several groups, dependent on their specific chemical characteristics and also on their mode of action (refs. 2,5,6). Type A lantibiotics are longer, elongated, helical peptides which affect energised biological membranes, while the type B lantibiotics are generally smaller, compact, globular peptides which appear to target specific enzyme functions. Table 1 shows those lantibiotics for which structures are available and compares their chemical contents and nature.

TABLE 1. Comparison of the Chemical Nature, Size and Other Distinguishing Characteristics of a Number of Lantibiotics for which Primary Structures are Currently Available[a].

Lantibiotic	Producing Strain	Mass[b]	Length[c]	Charge[d]	Bridges[e]	Dha/Dhb[f]	D-Ala[f]	AviCys[f]	Other[f]
Type A lantibiotics:									
Nisin	*Lactococcus* spp	3353	34	+3	5	3			
Subtilin	*Bacillus* spp	3317	32	+2	5	3			
Lactocin S	*Lactobacillus* spp	3764	39	-1	2	2	2		
Pep5	*Staphylococcus* spp	3488	34	+7	3	2			
Epilancin K7	*Staphylococcus* spp	3032	31	+5	3	4			
Epidermin	*Staphylococcus* spp	2164	22	+3	4	1		1	
Gallidermin	*Staphylococcus* spp	2164	22	+3	4	1		1	
SA-FF22	*Streptococcus* spp	2795	26	+1	3	1			
Lacticin 481	*Lactococcus* spp	2901	27	0	3	1			
Salivaricin A	*Streptococcus* spp	2315	22	0	3	0			
Type B lantibiotics:									
Cinnamycin LysAla	*Streptomyces* spp	2024	19	0	4	0			AspOH,
Duramycin LysAla	*Streptomyces* spp	2014	19	0	4	0			AspOH,
Duramycin B LysAla	*Streptoverticillium* spp	1951	19	0	4	0			AspOH,
Duramycin C LysAla	*Streptomyces* spp	2008	19	-1	4	0			AspOH,
Ancovenin	*Streptomyces* spp	1959	19	0	3	1			
Mersacidin	*Bacillus* spp	1825	20	-1	4	1			3MeAviCys
Actagardine	*Actinoplanes* spp	1890	19	0	4	0			LanO

[a] Taken from Ref. 1

[b] Mass in Da

[c] Number of amino acids, including modified residues. Lanthionine and Methyllanthionine are counted as two amino acid residues

[d] Overall charge, including free N-termini, at pH 7.0

[e] Total number of bridges including Lan, MeLan, LanO, AviCys, 3MeAviCys and LysAla

[f] Dha, 2,3-didehydroalanine; Dhb, 2,3-didehydrobutyrine; D-Ala, D-alanine; AviCys, 2-aminovinyl-D-cysteine; AspOH, 3-*erythro*-hydroxyaspartate; LysAla, (2*S*,8*S*)-lysinoalanine; 3MeAviCys, 2-aminovinyl-3-methyl-D-cysteine; LanO, lanthionine sulphoxide

In general, the type A lantibiotics usually carry an overall positive charge, while the type B lantibiotics are typically uncharged at pH 7. As can be seen by comparing the length of the peptides with the number of modified residues contained, the lantibiotics of both classes generally consist of 30-50% modified residues. The role of the modified residues is not absolutely clear, however it is thought that Lan and MeLan give the peptides stability in solution, since disruption of the rings often leads to rapid proteolytic degradation, either inside the producing cells or in the medium surrounding them (refs. 1,2,5,6). The complete structures of several representative lantibiotics of both subtypes are compared in Fig. 3.

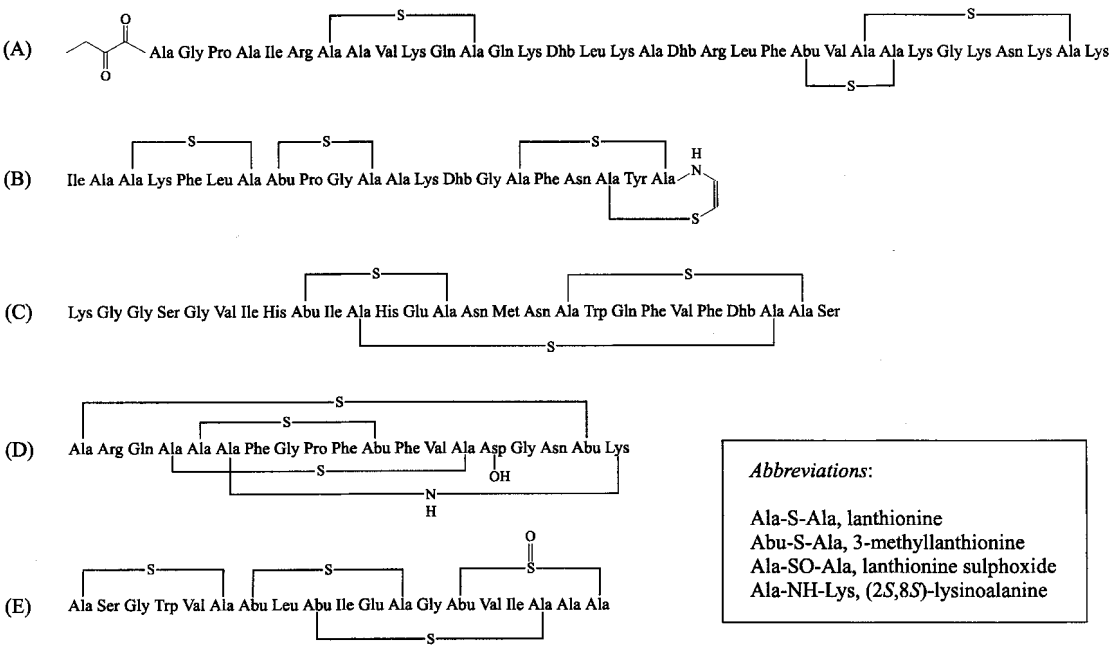

Fig. 3 The structures of the representative lantibiotics (A) Pep5 (ref. 8), (B) gallidermin (ref. 9), (C) lacticin 481 (ref. 10), (D) cinnamycin (ref. 11) and (E) actagardine (ref. 12).

A number of the lantibiotics contain additional modifications not listed above. For example the N-termini of the type A lantibiotics Pep5 (Fig. 3) and epilancin K7 are occupied by 2-oxobutyryl and hydroxypyruvyl groups, respectively (refs. 8,13). These modifications result from the spontaneous oxidative deamination of an N-terminally-located 2,3-unsaturated amino acid (refs. 5,6). However, the hydroxylation of the pyruvyl group in epilancin K7 probably results from a further protein-mediated reaction, suggesting that the producing organism may yet prove to be the source of a novel hydroxylation enzyme (ref. 13).

Microcin B17

Thus far, microcin B17 is the only microcin whose structure has been thoroughly characterised (refs. 14,15). Microcin B17 is 43 amino acid (3093 Da) peptide, which contains both thiazole and oxazole rings (Fig. 1) formed from the modification of specific Ser, Cys and Gly residues in the prepeptide sequence; the structure of microcin B17 is shown in Fig. 4.

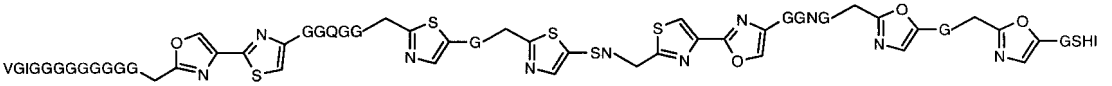

Fig. 4 The structure of the polypeptide antibiotic microcin B17.

Surprisingly, the peptide consists of an amazingly large proportion of Gly residues, more than two-thirds of the total residues.The complete chemical synthesis of both microcin B17 and several analogues of the peptide was recently accomplished (ref. 16). By structural analysis, NMR and biological activity, the fully synthetic microcin was identical to the naturally-isolated form. Because of its extremely hydrophobic nature, the peptide is only very slightly soluble in aqueous solution.

Three-dimensional structures

Determination of the solution structures of peptides such as the lantibiotics and microcin B17 is an important prerequisite to the understanding of how these peptides function and how they are synthesised. A number of the lantibiotics, as well as microcin B17, have had their solution structure elucidated by modern NMR techniques. Here we will discuss three representative structures, those of the type A lantibiotic gallidermin, the type B lantibiotic actagardine and microcin B17. To date x-ray-structures of these peptides have not been published (refs. 1,5,6).

Lantibiotics

The solution structure and a three-dimensional model of gallidermin in solution was first presented in 1991 (ref. 17). Analysis of the structure revealed that gallidermin adopts an amphiphilic, elongated, corkscrew-like conformation, which appears to be hinged in a central, flexible region. The peptide is *ca.* 3 nm in length, has a diameter of *ca.* 1 nm and possesses an extremely high dipole moment of around 75 Debye (Fig. 4). Interestingly, the solution conformation is such that all of the charged amino acids line up on one 'face' of the peptide, while the hydrophobic residue line up on the other. NMR-based analysis of a number of other type A lantibiotics, such as nisin and subtilin (refs. 18-21) has demonstrated that they adopt a similar conformation and possess similar properties.

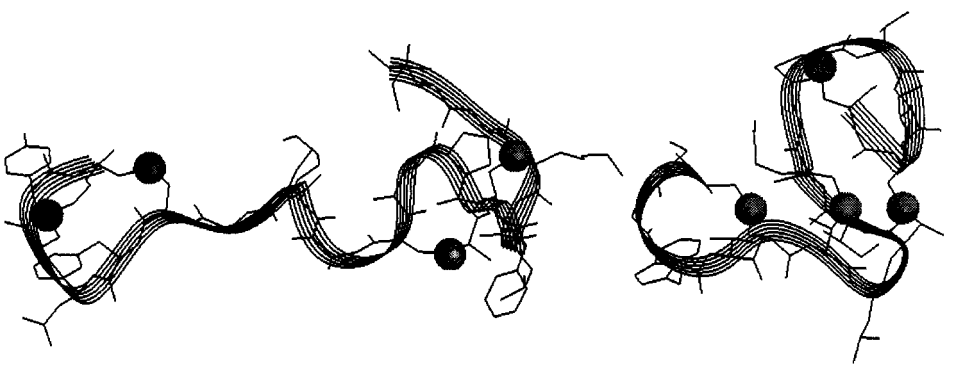

Fig. 5A Solution structure of the lantibiotic gallidermin

Fig. 5B Solution structure of the lantibiotic actagardine

Type B lantibiotics are considerably constrained in their conformational freedom, especially by the presence (in most of them) of head-to-tail bridges (Fig. 2 and Table 1). Thus, the solution structures adopted by type B lantibiotics are considerably more compact and globular. Overall, type B lantibiotics appear to form a bent U-shaped structure, induced by Pro9 and stabilised by the three thioether rings. Like the type A lantibiotics, they are amphiphilic with all of the hydrophobic amino acids located in the U-bend region (ref. 22). Interestingly, even actagardine (ref. 23), which lacks the head to tail bridge found in most other type B structures adopts a similar shape (Fig. 4).

Microcin B17

The structure of ^{13}C, ^{15}N-microcin B17 has also been studied by NMR (ref. 15). Interestingly, the peptide is extremely flexible in solution, adopting essentially no preferred conformation and making it impossible

to produce any reliable model of its solution structure. This is perhaps not so surprising considering its content of Gly and since the peptide does not have any bridges to restrict its conformation. It remains to be seen whether microcin B17 is able to adopt a preferred, or stabilised, conformation upon binding to its target. Such a study may also be important in defining the role of the modified amino acids contained within its structure and determining how they enable it to interact with either its target DNA gyrase or gyrase-bound DNA.

Biosynthetic mechanisms

Since both the lantibiotics and microcin B17 are formed by posttranslational modification of a ribosomally synthesised precursor peptide, it stands to reason that the producing cells contain a complex set of machinery responsible for carrying out the novel transformations encountered (Fig. 2). In addition, the cells also require specific proteases to remove the leader peptide, transporter(s) to get the active product into the surrounding medium and, in many cases, regulatory elements to control when the antimicrobial agents are produced and in what quantity (refs. 1-4). Here we will further discuss only those parts of the machinery specifically involved in amino acid transformations.

Lantibiotics

Lantibiotics are typified by their content of Dha, Dhb, Lan and MeLan (Fig. 1), the formation of which arise following dehydration of specific Ser and Thr residue in the propeptide domain of the prepeptide. Subsequently, some of the Dha and Dhb residues so formed react with neighbouring cysteine residues, undergoing stereospecific addition to produce the thioether-linked Lan and MeLan residues. Analysis of the genes involved in lantibiotic biosynthesis suggests that an enzyme, generally named LanB, is responsible for the dehydration reaction, while a second protein named LanC assists in the stereospecific addition reaction (ref. 24). Interestingly without the LanC protein, only about 10% of the normal amount of Lan and MeLan was formed in the Pep5 producing strain, suggesting that the addition reaction is essentially not spontaneous, as might be thought. In some bacteria, such as the lacticin 481 producing strain, only a single protein (called LanM) appears to be responsible for both these functions and sequence homology suggests it is probably an hybrid of both LanB and LanC (refs. 2,4,25).

The lantibiotic lactocin S contains D-Ala, although the prepeptide sequence contains L-Ser residues at the corresponding positions (ref. 26). Therefore, it is thought that the cells producing lactocin S are able to first dehydrate the Ser residues to Dha and that an as yet unknown enzyme is then responsible for their subsequent stereospecific hydrogenation to give D-Ala. This is the first known case of such a reaction in cellular systems.

Both epidermin and gallidermin possess the C-terminal modification AviCys (Fig. 1), which arises through the oxidative decarboxylation of the C-terminal Cys residue, followed by the electrophilic addition of its thiol group to a neighbouring Dha (refs. 1,5,9). The oxidative decarboxylation is carried out by the flavoprotein enzyme EpiD, which requires the cofactor flavin mononucleotide (FMN) and which has been the subject of a great deal of study (ref. 27). Interestingly, the enzyme EpiD is able to carry out the reaction not only on its natural substrate but also a number of synthetic peptides and peptide libraries (refs. 28,29) and the mechanism of its reactivity have been extensively studied by NMR (ref. 30). These results suggest that it may be practical to apply this enzyme to biotechnological transformations where such a reaction might be required.

Microcin B17

Alternatively, microcin B17 is typified by the heteroaromatic backbone modification it contains (refs. 14,15) in the form of oxazole and thiazole rings (Figs. 1 & 4). As in the case of the lantibiotics, a number of gene products have been shown to be essential in these modifications (ref. 31). The enzymes McbB, McbC and McbD appear to form a synthetase complex (McbBCD) responsible for the modifications observed. In this study it was also shown that, as in the case of the lantibiotics, the synthetase complex recognised only the propeptide domain of the premicrocin, suggesting that the 26 amino acid leader peptide domain plays either a structural role, maintaining a suitable secondary structure for modification

of the propeptide domain, or that it is necessary for the transport of microcin out of the cell. At this stage, it has not been possible to assign specific roles to each component of the synthetase complex.

The microcin B17 prepeptide-converting synthetase complex which has been isolated had at least two cofactor requirements (ref. 31). Firstly, McbBCD required adenine triphosphate (ATP), the normal source of 'energy' in the cell. It is probable that the ATP is used in a phosphorylation reaction to produce a favourable intermediate in the synthesis of the heterocycles. Furthermore, the conversion of each Ser or Cys converted to an heterocycle requires that two electrons must be removed. This observation suggests the need for an external cofactor acting as an electron acceptor; the cofactor identified was FMN, the same cofactor as is required by EpiD.

Biological functions

The mode of action of both the lantibiotics and microcin B17 have been the subject of numerous studies. The type A lantibiotics (such as nisin, Pep5 and epidermin) are able to form pores or channels in bacterial membranes (refs. 32,33). These pores are formed in a voltage-dependent manner, since they require an energised membrane in order to exert their antimicrobial activity. The pores formed allow efflux of essential intracellular components and disrupt energy transduction by disturbing the proton gradient across the cytoplasmic membrane and, thus, disturbing the production of ATP. In addition, some lantibiotics are also able to activate latent cell wall-degrading enzymes in certain sensitive cells (ref. 34). This is a secondary mode of action and, in contrast to pore formation, is not the primary cause of cell death. The activation of autolytic enzymes causes rampant cell wall destruction and, in combination with the influx of water through the membrane pores (which creates markedly increased osmotic stress), results in eventual cell lysis.

By contrast, the type B lantibiotics such as cinnamycin, the duramycins and ancovenin bind to phospholipids (PL), in particular phosphatidylethanolamine (PE), found in the cytoplasmic membrane of the sensitive cells (refs. 35-37). Thus, bacteria which do not contain this PL in their membranes, are essentially immune to these lantibiotics. Binding to PE probably results in disruption of a number of metabolic functions situated at the membrane and is not limited strictly to bacterial cells. In contrast to the type A lantibiotics, these lantibiotics do not require an energised membrane to exert their influence.

A second subgroup of the type B lantibiotics, consisting of mersacidin and actagardine, effect cell wall metabolism in sensitive bacteria (refs. 38,39). The exact mechanisms of action is not yet clear, but they appear to bind to cell wall precursors and act in a similar, but different, manner to the well known glycopeptide antibiotic vancomycin.

Microcin B17 is an inhibitor of DNA gyrases (ref. 40), although the precise interaction between the peptide and the target enzyme is not yet clearly understood. From what is known of analogous substances, it is probable that the oxazole and thiazole rings give microcin B17 the ability to bind to DNA (ref. 31). Certainly the peptide binds to the gyrase B subunit of the enzyme and creates double-stranded breaks in associated DNA. Rampant damage to the cellular DNA rapidly leads to cell death.

Biotechnological potential

The biosynthetic mechanism(s) surrounding the production of both the lantibiotics and microcin B17 are clearly very complex (refs. 1-6). However, several of the transformations have interesting biotechnological potential. The thiazole and oxazole ring-forming mechanisms found in microcin B17-producing bacteria (refs. 14,15,31) could prove to be a novel way of introducing new mimetic structures into peptides for use in the pharmaceutical industry. It is worth noting that a number of other compounds containing such heterocycles have already been described, including the antitumour antibiotic bleomycin, the antibiotic pristinamycin II_A and the anti AIDS-virus compound thiangazole (refs. 31,41,42). These observations suggest that enzymes able to carry out these transformations may prove extremely useful in biotechnology. Moreover, since heterocycle-containing compounds such as microcin B17 are so often found to be antitumour, antiviral or antibiotic in nature, microcin B17 may prove to be a novel lead structure for the further general development of these agents.

Alternatively, the ability to introduce D-amino acids into preformed peptides and/or proteins, such as occurs during lactocin S biosynthesis (ref. 26), could provide a very convenient way of controlling their degradation when used therapeutically. Similarly, thioether bridges apparently give some lantibiotics considerable resistance to proteolytic degradation (ref. 2); thus their introduction into novel peptides and proteins of biotechnological relevance could provide a convenient new method to improve peptide/protein half-life. It has already been demonstrated that EpiD, which carries out the transformation at the C-terminus of epidermin and forms AviCys, can also act on synthetic peptides to generate this novel biotechnological transformation (refs. 27-30). This last result suggests that it may indeed be possible to harness the novel biosynthetic enzymes involved in lantibiotic and microcin B17 biosynthesis to carry out novel biotechnological transformations

Applications

A number of the lantibiotics, as well as microcin B17, could find practical application. Nisin has been used for close to 40 years as a biopreservative (EC 234) and is particularly applicable to increasing the storage life of low pH foods (fermented foods such as salami, cheese, *etc.*), canned fish products, preprepared meats and various cosmetics (refs. 43-45). Nisin has proven to be particularly effective in the area of food biopreservation, since the peptide is extremely stable at low pH, has a lower toxicity than common table salt and is particularly effective against bacterial endospores which are normally difficult to destroy, even when the foodstuff is heated to considerable temperatures.

Alternatively, gallidermin has marked activity against bacteria such as *Propionibacterium acnes*, the causative agent of acnes and it has been suggested that its incorporation into a topical cream preparation could prove a useful alternative to the currently employed erythromycin/vitamin A therapy (refs. 5,6). Similarly, nisin has also been muted to have uses as an antiseptic for use in the dairy industry as well as against *Helicobacter pylori*, a putative causative agent of stomach ulcers in humans (refs. 2,44). The high degree of acid stability of the lantibiotics may make them particularly useful for such applications (ref. 43). Furthermore, the fact that resistance to these agents does not seem to rapidly develop (as is observed with most therapeutic antibiotics) is another point in favour of their further exploration and exploitation.

Amongst the type B lantibiotics, cinnamycin and the duramycins have been shown to be capable of regulating immune function since they inhibit phospholipase A2, an important enzyme involved in leucotriene and prostaglandin biosynthesis (ref. 46). In addition, some of the type B lantibiotics have efficacy in inhibiting the amplification of herpes simplex virus in experimental cell lines (ref. 47). However, perhaps the greatest hopes for the use of a lantibiotic directly as a therapeutic agent lies with mersacidin and actagardine. Both of these lantibiotics could represent an alternative to vancomycin therapy, since they display strong antibiotic activity, even against vancomycin-resistant staphylococci and enterococci (refs. 2,38,39). Such resistant strains are being isolated from infected patients in ever-increasing proportions and their control represents the greatest current challenge in antibiotic therapy. Similarly, microcin B17, which acts primarily against Gram-negative organisms, could prove to be a novel therapeutic agent, although until an exact mechanism of action can be ascribed to the peptide, its uses are probably limited.

Concluding Remarks

Both the lantibiotics and microcins have attracted a great deal of interest in recent times. Their respective structures, containing highly modified amino acids, are naturally-produced peptide mimetics which provide useful lead structures for future biotechnological development. Similarly, the machinery possessed by the cells from which these peptides are produced also has potential application for the biotechnological transformation of synthetic peptides into peptide mimetics with increased stability and/or biological activity; perhaps even with novel biological functions. With continued effort, it is not unreasonable to expect that the large research input already made to study these peptides and the enzymes which produce them, will soon begin to pay off with new, biotechnological applications.

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