Natural Peptides with Antimicrobial Activity

Ralph W. Jack(*) and Günther Jung(**) *

Abstract. Antibiosis is a mechanism by which many cell types control potential environmental takeover or pathogenicity by microbes, especially bacteria. It is now clear that a large proportion of the antimicrobial compounds produced by various cells and organisms including bacteria, fungi, plants, insects, amphibia and mammals are antibiotics based around peptides. These antimicrobial peptides can be broadly subdivided into two groups: those which are produced by multienzyme complexes and those which are encoded by a structural gene, the transcription and translation of which results in a peptide template which may (in some cases) undergo further enzymatic modifications. In this context, the chemistry, biosynthesis and functional aspects of a broad range of bioactive peptides from both subgroups will be briefly discussed, including: gramicidin, bacitracin, vancomycin, lamethicin and polymyxin B from the first group, and defensins (and related peptides), bacteriocins, lantibiotics and microcins from the second subgroup. From a biotechnological perspective, the peptide antibiotics produced by multienzyme complexes are open to a variety of manipulations to produce novel antibiotic compounds, whilst the ribosomally synthesised peptides may be specifically altered by site-directed mutagenesis. Furthermore, the enzymes capable of transforming some ribosomally synthesised peptide antibiotics may be of particular interest to the biotechnologist, because of the novel transformations of peptide substrates which they are able to perform.

1. Introduction

Bacteria represent some of the smallest life forms known; some can be our best friends, others our most deadly enemies. For example, bacterial metabolism is responsible for the production of many foods we take for granted (e.g., yoghurt, cheese and salami), but it is also responsible for some of the most serious diseases (e.g., botulism and typhoid fever). Because bacteria are prolific, they necessarily interact with almost all other cell types in some way or another, at some time or another. Therefore, many nonbacterial cells, as well as many bacteria themselves, have developed ways of combatting a bacterial 'take-over' of their environment. One of the many ways they achieve this is through the production of specific antimicrobial substances or antibiotics. Historically, it was the famous French microbiologist Louis Pasteur who first reported antibiotic and, more than a century ago, realised the potential of antibiotics for the development of therapeutics. Unfortunately, to cover in such a short space all that has been reported about antibiotics would be an injustice; here we will limit ourselves to a brief discussion of antimicrobial peptides from a variety of sources, particularly those with proven or potential applications in various areas of biotechnology.

From a chemical and structural viewpoint, the broad group of substances which can be called peptide antibiotics includes a large variety of compounds, many of which are still relatively uncharacterised. However, it is evident that they exhibit a high degree of structural diversity: some are simple linear peptides, whilst others are heterocyclic, some contain only representatives of the 20 proteinogenic amino acids (i.e., amino acids for which there is a genetic code), whilst others are heavily modified, and some are very short, while others consist of relatively long polypeptide chains. The variety of forms is also reflected in the myriad biological activities; some form pores or ion channels in membrane bilayers, others inhibit specific enzyme reactions, while still others act as ion-transfer shuttles or as ion pumps.

Principally, peptide antibiotics can be divided into two broad subgroups, depending on how they are produced within the cell: those that are produced by the action of multienzyme complexes and those that are synthesised on the ribosome as a precursor peptide which may (in some cases) undergo further, extensive modification [1]. The principal differences between these groups then is that the template of a peptide antibiotic which is ribosomally synthesised is encoded by a specific (structural) gene, whilst peptide antibiotics synthesised by multienzyme complexes are not encoded by a specific gene.

2. Antibiotic Peptides Produced by Non-Ribosomal Biosynthesis

The mechanism(s) which give rise to the formation of peptide antibiotics produced by non-ribosomal synthesis have been well studied; many are formed by the so-called 'thiotemplate mechanism' [1a-d]. This is a particularly interesting biosynthetic mechanism, because it involves some exceptionally large enzymes, each containing many different enzyme domains capable of carrying out one or more of the steps in the biosynthesis of the respective peptide antibiotic. After each step, the growing peptide antibiotic is passed along to the next domain for further synthetic steps [1c,d][2]. Since it has been demonstrated that the domains can function on their own and that they are separated from each other by nonfunctional spacer regions, it does not seem unreasonable to expect that molecular biology could be used to 'mix-and-match' enzyme domains and create an entire enzyme capable of producing almost any peptide-antibiotic sequence. The biotechnological applications in this area are substantial, particularly as many of these enzyme domains have specificities for the coupling of highly unusual, modified amino acids, amino acid analogues and derivatives. In addition, a number of these peptide antibiotics have been applied in therapeutic preparations, particularly in topical creams for use against localised infections and acne as well as in the treatment of bacterial throat infections (pharyngitis).

2.1. Gramicidin S

The gramicidin peptides represent the best studied examples of non-ribosomally
synthesised peptide antibiotics; they are produced by strains of the bacterium *Bacillus*. Gramicidin S (Fig. 1, A) is a dimer of two identical pentapeptides linked head-to-tail to form a cyclic structure; the sequence of the monomer is D-Phe-L-Pro-L-Val-L-Orn-L-Leu. A related family of peptide antibiotics called tyrocidins A, B, C and D are formed in a similar way, except that the sequence of the second half of the dimer is different and they are therefore asymmetric. As might be expected, the large enzyme complex responsible for their syntheses contains additional domains to that involved in gramicidin S formation; the additional domains contribute the additional specificities necessary for the extra amino acids. Thus, this mechanism for peptide-antibiotic manufacture seems to be both quite widespread and flexible with respect to the incorporation of novel amino acids. Gramicidin has found some application and is used mainly in the topical treatment of infections, e.g., pharyngitis [1a–d].

2.2. Alamethicin

The peptaiabol alamethicin (Fig. 1, B) is produced by the fungus *Trichoderma vireide*. The acylated 20-amino-acid peptide is typical of the peptaiabol group, which all contain the non-proteinogenic amino acid α-aminoisobutyric acid as well as a C-terminal amino alcohol [3]. Alamethicin is of particular biophysical importance because it has been used as a model peptide in the quest to understand how peptides are able to form pores or channels in membrane bilayers. Alamethicin is amphiphilic, very hydrophobic and adopts an essentially helical structure, although it is quite flexible in the central region. Physically, the peptide possesses an extremely high dipole moment; the strength of which is affected by its shape; when alamethicin is elongated, the dipole moment is ca. 140 Debye, but when it is kinked, this is reduced to ca. half that value. It inserts in a single-step process into bilayers and is then thought to undergo conformational alteration and to arrange into multimeric aggregates which subsequently form pores (a single peptide is insufficient for pore formation). One model of alamethicin pore formation suggests that the peptide aggregates are formed by antiparallel stacking of the peptides and that the addition of a voltage across the membrane is necessary for the peptide to reorganise (flip around to a parallel stacking arrangement). However, it has also been suggested that such a mechanism would be energetically unfavourable.

2.3. Bacitracin

The rather 'loose' affinities displayed by the domains involved in thiopeptide biosynthesis of peptide antibiotics often mean that a particular antibiotic is a family of related peptides, rather than a single, homogeneous form, and bacitracin is no exception; the major form of the antibiotic is shown in Fig. 1, C [1a–d]. Bacitracin is produced by the bacterium *Bacillus licheniformis* and is cyclic; cyclisation is attained through the coupling of the ε-amino function of the Lys residue at position 5 to the penultimate Asp residue. A second cycle is also formed within this peptide antibiotic when the two N-terminal amino acids (Ile-Cys) form a thiazoline ring; the thiol group of Cys is added to the Ile backbone.

Bacitracin does not form ion channels or shuttles like the membrane-active peptide antibiotics; instead, it is an effective inhibitor of bacterial cell-wall biosynthesis. It does this by blocking recycling of the membrane-bound carrier of many cell-wall subunits, undecaprenyldiphosphate. In addition, bacitracin is one of the most successful discoveries amongst the peptide antibiotics formed by non-ribosomal biosyntheses, since it has found wide application in medicine. Like gramicidin, it is used as an antiseptic for local application. However, it is also included in a number of microbiological agars and other diagnostic test systems to prevent the growth of certain bacteria and allow others to be more accurately enumerated [1a–d].
In addition, bacitracin acts as an efficient inhibitor of a number of proteases; the structure of bacitracin complexed to a number of different peptidases has recently been reported [4].

2.4. Valinomycin
The cyclic depsipeptide antibiotic valinomycin (Fig. 1, D) acts as an ion carrier. However, rather than forming a channel in the membrane bilayer and allowing release of ions through the pore lumen, this peptide antibiotic shuttles ions across the membrane, continually moving from a loaded to an unloaded state and back again. This mechanism is much slower (ca. 1000-fold) than formation of ion channels in achieving an ion efflux from the affected cells. The peptide has a preferential affinity for potassium ions, which it coordinates with its six valine carboxyl O-atoms. The amino acids of valinomycin are extremely hydrophobic, a feature which allows it to move easily through the hydrophobic interior of the membrane as it shuttles from side to side. The structure and mechanism of this particular peptide ionophore have been exhaustively studied [1-6][5].

2.5. Polymixin B
The 10-amino-acid peptide antibiotic polymyxin B is produced by Bacillus polymyxma and has the sequence Dab-Thr-Dab-Dab-Dab-D-Phe-Leu-Dab-Dab-Thr (where Dab is diaminobutyric acid). The peptide is cyclised from a coupling of the C-terminus with Dab5, and a number of analogues are found with either the Thr2, Phe6 or Leu7 being substituted by other amino acids. In addition, the peptides have an extremely amphipathic nature, since the N-terminus is acylated (e.g. with an isoctanoyl moiety). Polymyxin B is able to kill some gram-negative bacteria by disrupting the outer membrane and has therefore been used to some extent in the treatment of intestinal infections. In addition, the structure of the peptide and the molecular mechanism by which it is able to disrupt the outer membrane of gram-negative bacteria (leading to their death) have been extensively studied [1a-d][6].

3. Antibiotic Peptides Produced by Ribosomal Biosynthesis
In contrast to the antimicrobial peptides described above, there are also a number of natural peptide antibiotics which are produced by posttranslational modification of a ribosomally synthesised precursor peptide. The principal difference here is that the template for subsequent reactions, the pre-peptide, is encoded by a specific gene. Thus, this precursor peptide may only contain proteinogenic amino acids. Not all peptide antibiotics produced in this way are further posttranslationally modified; in those that are, all of the non-proteinogenic amino acids then arise by enzymatic processes from amino acids incorporated in the precursor peptide. For example, the lantibiotics (discussed below) contain α,β-dehydrated Ser and Thr residues. Hydroxyaminoacid dehydratases are not uncommon in nature. However, known dehydratases cannot act on a Ser or Thr inserted into a nascent peptide chain, since they utilise a pyridoxyl-phosphate-dependent Schiff's base mechanism. Thus, the enzymes responsible for this transformation in the lantibiotics are novel and are therefore of biotechnological importance.

3.1. Defensins and Related Peptides
The α-defensins (Fig. 2) have now been isolated from a variety of sources, including human, pig and rat neutrophils and various mucosal sites of mammals [7]. Since the neutrophils are such an important part of the immune system, it is thought that these peptides form the front-line strategy in the immune responses to infection by bacteria. Similarly, their presence in the mucosa suggests that they may be an important component of the innate immune system. The peptides of this group generally share substantial degrees of homology, contain a large number of Arg residues (but not Lys) and the disulfide-bond arrangement is uniformly conserved; disruption of the disulfide bonds results in loss of antimicrobial activity. The defensins are produced as prepro-peptides, and the leader- and pro-segments are probably involved in directing their transport to storage vesicles to await use. The β-defensin shown in Fig. 2 (TAP) was isolated from bovine mucosa; β-defensins are larger than the α-defensins and have a different disulfide arrangement. In recent times, a large number of different defensin peptides and peptide families differing in their Arg/Lys content, number and arrangement of disulfide bonds and of differing overall length have been isolated. A close relative of HNP-1 called HNP-3 has been crystallised and analysed by X-ray diffraction. The peptide forms a rigid β-sheet structure, stabilised by a salt bridge and a turn induced by a Gly residue at position 24. The tachyplesins are 18-amino-acid antimicrobial peptides isolated from the horseshoe crab. The peptide shown in Fig. 2 is part of a family of tachyplesins whose primary sequence varies slightly, but whose disulfide-bond arrangement and secondary structure remains conserved [7]. The peptides are produced as longer (77 amino acids) prepro-sequences which consist of a pre-peptide at the N-terminal side of the mature segment and a pro-peptide on the C-terminal side. The N-terminal end of the pro-segment contains an amidation motif; tachyplesins are peptide amides. The peptides form rigid secondary structures, consisting of two antiparallel β-sheets separated by a β-turn and stabilised by the two disulfide bonds (Fig. 2).

A great number of defensin-like peptides have now been described, having been isolated from a variety of sources including immune cells of mammals, nucosa, insect haemolymph, crustacea and plants and their seeds. The vast distribution of these peptides and peptide families underlines their mechanistic importance to the defence of eucaryotic cells against
bacterial infection. Some of them may also have biotechnological applications. For example, the magainins (Fig. 2) are a group of peptides isolated from the skin of amphibia and which apparently form an essential part of the primitive immune system, defending against infection [7a–b]. The peptides are 20–25 amino acids in length and form amphiphilic, helical structures, although they generally contain no cycles. Currently, a synthetic peptide, derived from a consensus sequence of the magainin family, has reached phase Ib of clinical trials for application as a topical agent against bacterial infection.

3.2. Non-Modified Bacteriocins

A further group of antimicrobial peptides have also been isolated from the microbial world. These peptides probably serve a number of functions to the bacteria which produce them: they may give the bacteria an ecological advantage by killing off competitors and they may also act (in some cases) as signals to tell the producing bacteria something of the state of their local environment and population density (so-called 'quorum-sensing'). Bacteriocins, or bacterial-derived antibacterial peptides, are produced by many different bacterial species. The structures vary considerably: some contain disulfide bonds (Fig. 3), some contain free cysteine, some contain neither cysteine nor cystine and a fourth group consists of two peptides whose complementary presence is required for antimicrobial activity [8]. However, regardless of their structural characteristics, they all act by forming hydrophilic pores in the cytoplasmic membrane of susceptible bacteria. These pores or channels depolarise the cytoplasmic membrane, disrupting energy transduction and ATP production; these cells then 'starve' for want of an energy source [1f][8].

All bacteriocins are produced on the ribosome as a precursor peptide which is later processed to release the active moiety; often (but not always), this occurs during transport out of the cell. The leader peptide which is removed during this processing probably keeps the bacteriocin inactive inside the cell and helps to direct its transport to the outside. In addition, because bacteriocins are toxic to the producing cells as well as to other bacterial types, the producing cells also produce specific self-protection mechanisms to resist the action of their own bacteriocin; however, just how these mechanisms function at the molecular level is not well understood [1e–h][8].

One well-characterised bacteriocin is pediocin PA-1 (Fig. 3), produced by Pediococcus acidilactici PAC1.0 [9]. The bacteriocin contains no posttranslational modifications, but does contain two essential disulfide bonds and a N-terminal sequence which is homologous with a number of other bacteriocins; the C-terminal sequences of these homologous peptides differ to a much greater extent. Although this particular N-terminal sequence is often reported as a specific motif, its relevance (the reason(s) for its conservation) is not understood; such conservation may well represent an evolutionary phenomenon. The mode of action of pediocin PA-1 has been extensively studied [9e, d], and the peptide is active against a number of foodborne bacterial pathogens of humans, including Listeria monocytogenes. Hence, it has been proposed that this bacteriocin might prove useful as a novel food preservative. Other non-modified bacteriocins have also been suggested to have similar potential applications in the food industry [9e].

3.3. Lantibiotics

Lantibiotics [1e–h][10] are also bacteriocins and therefore they are ribosomally synthesised as precursor peptides. However, unlike the bacteriocins described above, the lantibiotics contain a large number of posttranslational modifications; the name lantibiotic is derived from their content in the thioether amino acids lanthionine and 3-methyllanthionine. These amino acids arise through a multistep process; firstly, β-hydroxy-α-amino acids in...
the pro-peptide portion of the precursor are enzymatically dehydrated to their respective \(\alpha,\beta\)-unsaturated equivalents and the thiol group of a neighbouring Cys residue is then added to the double bond in a protein-mediated reaction to generate the corresponding thioether (Scheme 1). At the present time, more than 25 lantibiotics have been characterised [1h]. Most lantibiotics are highly modified, many containing 50% (or more) non-proteinogenic \(\alpha\)-amino acids.

In addition to the thioether amino acids, the lantibiotics contain a variety of other modified amino acids including 2,3-didehydroalanine and -butyryne, lysinoalanine, 2-aminovinyl-D-cysteine, hydroxy-aspatic acid, a number of N-terminal modifications and D-Ala [1e–h]. Not all lantibiotics contain each of these modified amino acids; in some cases, they are widespread, whilst in others they are specific to only one lantibiotic. For example, to date D-Ala residues are found in only the lantibiotic lactocin S [11]; however, they are of particular interest since the enzymes responsible for stereoconversion could prove useful for the introduction of D-Ala into peptides and peptide mimetics of biotechnological interest. Although the enzyme(s) responsible has not yet been isolated, the mechanism of stereoconversion has been proposed; Ser residues in the pre-peptide are enzymatically dehydrated to 2,3-didehydroalanine, and the \(\alpha,\beta\)-unsaturated amino acid then undergoes stereospecific enzymatic hydrogenation to produce D-Ala (Scheme 2).

Because the lantibiotics are highly modified bacteriocins, there are necessarily a number of proteins involved in their biosynthesis, proteins which may have biotechnological uses. The general biosynthesis of lantibiotics involves a pre-peptide as a template, an enzyme for the site-specific dehydration of \(\beta\)-hydroxy-\(\alpha\)-amino acids, a thioether-formation-assisting protein, a protease able to process the

Scheme 2. The Proposed Mechanism for the Formation of a D-Ala Residue at Position 7 in the Lantibiotic Lactocin S [11]

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\[ \text{\textit{\textbf{Lactocin S (1-6) \rightarrow Lactocin S (8-37)}}} \]

\[ \text{\textit{\textbf{\(-H_2O\)}}} \]

\[ \text{\textit{\textbf{Site-specific dehydration (enzyme-mediated)}}} \]

\[ \text{\textit{\textbf{\(+2H^+\)}}} \]

\[ \text{\textit{\textbf{Stereospecific hydrogenation (enzyme-mediated)}}} \]

\[ \text{\textit{\textbf{\[(D)-Ala7\]}}} \]
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Scheme 3. The Proposed Mechanism for the Formation of an Enethiolate Anion from the C-Terminal Cys Residues of Synthetic Peptides Treated with the Flavoprotein Enzyme EpiD [12]

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\[ \text{\textit{\textbf{\[\text{Synthetic peptide}\]}}} \]

\[ \text{\textit{\textbf{\[-H_2O\]}}} \]

\[ \text{\textit{\textbf{Oxidation - catalysed by the flavoprotein EpiD}}} \]

\[ \text{\textit{\textbf{\[\text{Synthetic peptide}\]}}} \]

\[ \text{\textit{\textbf{\[-CO_2\]}}} \]

\[ \text{\textit{\textbf{Decarboxylation}}} \]

\[ \text{\textit{\textbf{\[\text{Synthetic peptide}\]}}} \]

\[ \text{\textit{\textbf{\[-H_+\]}}} \]

\[ \text{\textit{\textbf{\[\text{Synthetic peptide}\]}}} \]
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The proposed mechanism for the formation of a D-Ala residue at position 7 in the lantibiotic lactocin S [11] involves the dehydration of the pre-peptide to 2,3-didehydroalanine, followed by stereospecific hydrogenation to produce D-Ala. The mechanism for the formation of an enethiolate anion from the C-terminal Cys residues of synthetic peptides treated with the flavoprotein enzyme EpiD [12] involves oxidation catalysed by the flavoprotein EpiD, followed by decarboxylation and protonation to produce the enethiolate anion.
Fig. 4. The structures of the type-A lantibiotics nisin and gallidermin and the type-B lantibiotics cinnamycin and actagardine [1e-h]. Abu, α-amino butyric acid; Ala-S-Ala, la nthionine; Abu-S-Ala, 3-methyl lanthionine; AspOH, hydroxy aspartic acid; Dha, 2,3-didehydroalanine; Dhb, 2,3-didehydrobutyryl e; Lys-NH-Ala, (2S,8S)-lysino alanine; the remaining amino acids are indicated in standard three-letter code.

Type A

Nisin

Gallidermin

Type B

Duramycin B

Actagardine

pre-peptide, transporter(s) for translocation, regulatory elements and self-protection factor(s). Furthermore, other enzymes, such as those involved in the formation of D-Ala and S-aminovinyl-D-cysteine, are also required in specific cases to introduce these particular modifications [1e-h]. These enzymes are also of interest to biotechnologists because of the novel transformations which they are able to carry out. Their application in biotechnology could allow the introduction of further modifications, reactive groups or diversity into compounds of economic and commercial interest. Indeed, it has recently been shown that EpipD, an enzyme capable of catalysing the oxidative decarboxylation of the C-terminal Cys residue of epidermin (necessary in the formation of S-aminovinyl-D-cysteine), is also able to act on synthetic test peptides of a variety of sequences, thereby generating an highly reactive enethiolate anion at their C-terminus (Scheme 3) [12]. This finding reinforces the fact that the novel activities of these enzymes really could be harnessed for use in biotechnology [1g-h][13].

Other applications of lantibiotics have also been demonstrated. In particular, the lantibiotic nisin (Fig. 4) has proven valuable in the food industry as a natural food preservative. Nisin has been used in this way for the last ca. 40 years [1e-h]. Nisin is particularly practical because the bacterium that produces it, Lactococcus lactis, is also the major organism utilised in the manufacture of cheese. Thus, it is possible to use nisin-producing strains to make cheese and the nisin left after the fermentation protects the cheese from spoilage by unwanted bacteria. Alternatively, bulk nisin may be added after cheese production in order to increase the product’s shelf-life. Nisin is similarly useful in the prevention of spoilage of canned foods (especially fish and vegetables). What makes the use of nisin so appealing is that it is perceived to be ‘natural’, is not toxic, is poorly immunogenic or allergenic, is heat- and acid-stable and is very effective against the kinds of organisms most likely to cause spoilage and food-poisoning outbreaks.

The potential uses of lantibiotics such as nisin lead to more detailed analyses of how the lantibiotics function (i.e., the mechanism of their antibiotic activities), and the biological mode of action is also very clearly related to structural features of the peptides. Thus, a subdivision of the lantibiotics has been proposed [1e]. Type-A lantibiotics are elongated, helix-like peptides (Fig. 4) which form pores in energised membrane bilayers. Thus, lantibiotics such as nisin and gallidermin are able to kill susceptible cells by the formation of nonspecific, transient channels in the cytoplasmic membrane [1e-h]. Type-B lantibiotics are globular, constrained peptides (Fig. 4) which affect specific enzyme function [1e-h]. Thus, lantibiotics such as cinnamycin are able to bind the phospholipid phosphatidyl ethanolamine and render it unavailable as a substrate for phospholipase A2 [14]; this enzyme is important in the biosynthesis of many immune factors such as prostaglandins and leukotrienes, and cinnamycin may therefore function as an immunopotentiator. Alternatively, the type-B lantibiotics actagardine (Fig. 4) and mersacidin are able to inhibit cell-wall biosynthesis in susceptible bacteria [1e-h][15].

Therapeutic applications of lantibiotics have also been suggested. The lantibiotic gallidermin (Fig. 4) is active against Propionibacterium acnes, a causative fac-
tor in juvenile acne. Thus, gallidermin has been tested as an alternative application to current acne treatment regimens [1e]. The lantibiotics actagardine and mersacidin act against a number of pathogenic bacteria which are of particular importance because they resist many (in some cases, all) of the currently available antibiotics [15]. The mechanism by which they kill these bacteria involves specific inhibition of cell-wall biosynthesis and is similar to, but different from the glycopeptide vancomycin. Thus, at the very least, these two lantibiotics appear to be lead structures, identifying a novel target for future antibiotics. It is interesting to note that the structures of the two peptides share a highly conserved ring. Since three-dimensional structures for these peptides are now available [16], it is to be hoped that they can now be studied complexed to their respective targets, in the same way as has been done for cinnamycin [14d]. This may give new clues as to exactly how these peptides function at the molecular level.

3.4. Microcin B17

Microcin B17 (Fig. 5) is a peptide antibiotic produced by *Escherichia coli*. The peptide is characterised by its content of some 70% glycine residues and by its content of oxazole and thiazole rings [17]. These heterocycles arise from backbone modification of Gly and Ser and Gly and Cys residues, respectively. Recently, the biosynthetic mechanism of microcin B17 biosynthesis has been studied extensively and much light has been shed on its production. A schematic depiction of the synthesis and formation of the ring structures is shown in Scheme 4. Like the lantibiotics, microcin B17 is produced as a pre-peptide which is modified and processed to give the mature form, which is then exported into the surrounding medium; the reactions generating these modifications are carried out by a complex of three different enzymes [17c]. The leader peptide has been shown to be essential for correct modification of the pre-peptide [17d]; it appears to bind to the synthetase complex and direct the modification reactions. Furthermore, the structure of active microcin B17 has been confirmed by its total synthesis [18], opening the way to the synthesis of analogues of the peptide and further lead structures.

The mode of action of microcin B17 has also been studied [19]. The peptide appears to interact specifically with the B subunit of DNA gyrase. This enzyme is responsible for several reactions involved in DNA biosynthesis. Microcin B17 blocks the function of the enzyme, apparently when it is complexed with DNA, thereby generating breaks in the DNA. Microcin

![Fig. 5. The primary structure of microcin B17](image_url)

The oxazole and thiazole rings are indicated as structures and the remaining amino acids are indicated in standard single-letter code.

![Scheme 4. The Proposed Mechanism of Oxazole and Thiazole Formation in Microcin B17](image_url)

The cyclisation and dehydration reactions involve FMN and FMN-H₂, while the dehydrogenation reactions involve oxygen and water.
B17 action shares many of the characteristics shown by another antibiotic class, namely the quinolones. Because these are particularly important antibiotics for therapeutic use against many gram-negative bacterial infections, microcin B17 may represent a lead structure identifying new targets in these organisms for future antibiotic applications. With respect to the biological activity of microcin B17, it is interesting to note that a number of other compounds containing oxazole and thiazole rings have been isolated from nature and most have proven to be either antibiotics or anti-tumour agents [17c]. Thus, the search for novel antimicrobial compounds based around the lead structure of microcin B17 would not be unreasonable.

4. Conclusions

There is a vast array of different peptide-based compounds which have been isolated from natural sources and which display antimicrobial activity. The structures of the peptides are highly diverse; some are relatively simple, others highly complex. Moreover, the mechanism(s) by which they function is as different as their structural formulae. While not all of these peptide antibiotics are suitable for immediate therapeutic application, these compounds represent the most obvious lead structures for the development of the antibiotics of tomorrow. With the increasing development of multidrug-resistant pathogenic bacterial strains, the search for new antibiotics takes on a new priority. It is becoming increasingly necessary to identify new targets for future antibiosis.

However, many of these peptide antibiotics also have other applications. Some can be applied to research tasks, such as model peptides for understanding pore formation in biological membranes or as ion transporters helping fathom the mysteries of cellular metabolism. Alternatively, regardless of whether the peptide antibiotic is ribosomally synthesised or not, the enzymes responsible for their production are of great interest. In some cases, there is the possibility to 'mix-and-match' enzyme domains, allowing new substrate specificities and thereby 'designing' new antibiotic compounds. In other peptide-antibiotic systems, we have the possibility to use the isolated transformation enzymes to carry out novel reactions and introduce new functional groups into peptide-based compounds of commercial interest. These researches do not have to be restricted to antibiotics and related structures.

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[1] a) H. Kleinkauf, H. von Döhren, Ann. Rev. Microbiol. 1987, 41, 259, and ref. cit. therein; b) M.M. Nakano, P. Zuber, Crit. Rev. Biotechnol. 1990, 10, 223; c) H. Kleinkauf, H. von Döhren, Eur. J. Biochem. 1990, 192, 1; d) H. Kleinkauf, H. von Döhren, ibid. 1996, 236, 335, and ref. cit. therein; e) G. Jung, Angew. Chem., Int. Ed. Engl. 1991, 30, 1051; f) H.-G. Sahli, R.W. Jack, G. Bierbaum, Eur. J. Biochem. 1995, 230, 827, and ref. cit. therein; g) R.W. Jack, F. Götz, G. Jung, in 'Biotechnology', Eds. H. Kleinkauf and H. von Döhren, VCH, Weinheim, 1997, Vol. 7, p. 323–368, and ref. cit. therein; h) R.W. Jack, G. Bierbaum, H.-G. Sahli, in 'Lantibiotics and related peptides', R.G. Laiders, Austin, 1997, in press.

[2] a) T. Stachelhaus, M.A. Marahiel, J. Biol. Chem. 1995, 270, 6163; b) T. Stein, J. Vater, V. Kruft, A. Otto, B. Wittmann-Liebold, P. Franke, M. Pancio, R. McDowell, H.R. Morris, ibid. 1996, 271, 15424.

[3] a) M.S.P. Sommer, Eur. J. Biochem. 1993, 22, 105, and ref. cit. therein; b) H. Schmitt, G. Jung, Liebigs Ann. Chem. 1985, 321; c) G. Boheim, W. Hanke, G. Jung, Biophys. Struct. Mech. 1983, 9, 181; d) G. Schwarz, S. Stankowski, V. Rizzo, Biochim. Biophys. Acta 1986, 863, 141; e) V. Rizzo, S. Stankowski, G. Schwarz, Biochemistry 1987, 26, 2751; f) S. Stankowski, U.D. Schwarz, G. Schwarz, Biochim. Biophys. Acta 1988, 941, 11; g) H. Vogel, L. Nilson, R. Rigler, S. Meder, G. Boheim, W. Beck, H.-H. Kurth, G. Jung, Eur. J. Biochem. 1993, 212, 305.

[4] S. Pfeffer-Hennig, Z. Dauter, M. Henning, W. Hohe, K. Wilson, C. Betzel, Adv. Exp. Med. Biol. 1996, 379, 29.

[5] a) D.A. Langs, R.H. Blessing, W.L. Duax, Int. J. Pept. Protein Res. 1992, 39, 291; b) R. Bassalle, A. Gorea, I. Shalit, J.W. Mettger, C. Zat, D.M. Desiderio, M. Fridkin, J. Med. Chem. 1993, 36, 1203; c) M. Jackson, H.H. Mantis, Biochemistry 1991, 31, 3205; d) W.L. Duax, J.F. Griffin, D.A. Langs, G.D. Desiderio, J. Grodzki, V. Pietrov, V. Ivanov, ibid. 1996, 40, 141; e) T.R. Forester, W. Smith, J.H. Clarke, Biophys. J. 1997, 71, 544.

[6] a) G. Schröder, K. Brandenburg, U. Seydel, Biochemistry 1992, 31, 631; b) Y. Kimura, H. Matsunaga, M. Vaara, J. Antibiot. Tokyo 1992, 45, 742; c) S. Fukuoka, I. Karube, Appl. Biochem. Biotechnol. 1994, 49, 1; d) S.Y. Liao, G.T. Ong, K.T. Wang, S.H. Wu, Biochim. Biophys. Acta 1995, 1232, 312.

[7] a) H.G. Boman, S. Sand, J. Immunol. 1996, 153, 475; b) H.G. Boman, Ann. Rev. Immunol. 1995, 13, 61, and ref. cit. therein; c) R.I. Lehrer, A.K. Lichtenstein, T. Ganz, ibid. 1993, 11, 105, and ref. cit. therein; d) E. Martin, T. Ganz, R.I. Lehrer, J. Leuko cyt Biochem. 1995, 60, 128; e) R.I. Lehrer, T. Ganz, Ann. N. Y. Acad. Sci. 1996, 797, 228.

[8] R.W. Jack, J.R. Tagg, B. Ray, Appl. Environ. Microbiol. 1995, 59, 171, and ref. cit. therein.

[9] a) J.T. Henderson, A.L. Chopko, P.D. van Wassenhove, Arch. Biochem. Biophys. 1992, 295, 5; b) J.D. Marugg, C.F. Gonzales, B.S.