ACCOUNTS
of chemical research

Biosynthesis of Aminovinyl-Cysteine-Containing Peptides and Its Application in the Production of Potential Drug Candidates

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RECEIVED ON OCTOBER 14, 2010

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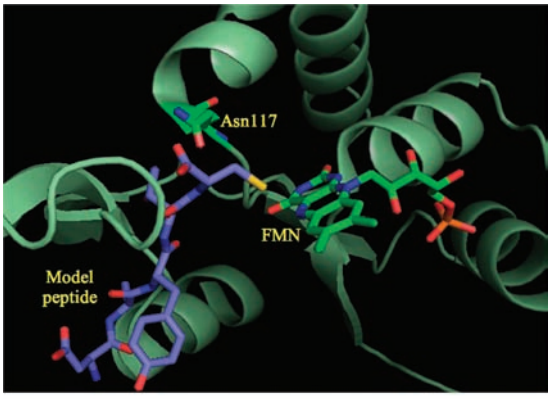


Bacteria produce a wide array of metabolites to protect themselves from competing microbes. These antimicrobial compounds include peptides with an *S*-[(*Z*)-2-aminovinyl]-D-cysteine (AviCys) or *S*-[(*Z*)-2-aminovinyl]-(3*S*)-3-methyl-D-cysteine (AviMeCys) residue, which have been isolated from several different bacterial species. The peptides are structurally diverse: some feature polycyclic backbones, such as the lantibiotic epidermin, and others feature a mostly linear structure, such as cypemycin. Each of the AviCys-containing peptides characterized to date exhibit highly potent biological activities, ranging from antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) to anticancer activity against mouse leukemia cells. The AviCys-containing peptides gallidermin and mutacin 1140 have been suggested as possible treatments of acne and of throat infections, respectively.

Unfortunately, their low production yield in fermentation (typically only 10−200 mg/L) remains a major hindrance to the widespread use and clinical testing of AviCys-containing peptides for human therapeutics. Although scientists have made great strides in the total chemical synthesis of polycyclic peptides on solid support, an efficient method to form the AviCys ring has yet to be developed. In light of these difficulties, it may be possible to draw inspiration from the natural biosynthesis of AviCys-containing peptides within the producer organisms. In this Account, we examine the characteristics of the enzymes responsible for constructing AviCys to evaluate possibilities for generating high yields of bioactive AviCys- or AviMeCys-containing peptides for research and clinical use.

The gene cluster for the biosynthesis of epidermin has been studied in depth, leading to the proposal for a mechanism of AviCys formation. First, a serine residue upstream of the C-terminus is enzymatically dehydrated to form a dehydroalanine residue. Then, the C-terminal cysteine residue is oxidatively decarboxylated to form an enethiolate, which subsequently cyclizes onto the dehydroalanine to give the AviCys ring. Extensive research on EpiD, the enzyme responsible for the oxidative decarboxylation reaction, has led to its purification and cocrystallization with a model substrate peptide, yielding an X-ray crystal structure. An *in vitro* assay of the enzyme with a library of synthetic heptapeptides has resulted in the discovery that EpiD has low absolute substrate specificity and can oxidatively decarboxylate a wide variety of C-terminal cysteine-containing peptides.

Recently, the gene cluster for the biosynthesis of cypemycin was also identified. Despite certain structural similarities between cypemycin and the lantibiotic peptides, analysis of the biosynthetic genes suggests that cypemycin production is quite different from that of the lantibiotics. In particular, the AviCys residue in cypemycin is formed from two cysteine residues instead of one serine and one cysteine, and the CypD enzyme that catalyzes the oxidative decarboxylation of the C-terminal cysteine shows little homology to EpiD.

The knowledge accrued from studying EpiD and CypD could be used to develop a semisynthetic methodology to produce AviCys-containing peptides. In particular, suitable precursor peptides could be synthesized on solid support before being fed to either of these enzymes *in vitro* to generate the C-terminal AviCys moiety. Exploring the potential of this methodology could lead to the efficient production of epidermin, cypemycin, and analogues thereof.

Published on the Web 03/02/2011 www.pubs.acs.org/accounts
10.1021/ar1001395

Introduction

Bacteria are known to produce an enormous variety of metabolites, likely for the purposes of self-protection and survival.[1] Although researchers predict that the vast majority of these metabolites have yet to be characterized, the array of molecules that have already been isolated are thought to be involved in defense mechanisms, gene expression, and intercellular communication, among other functions.[2−4]

The metabolites involved in bacterial defense systems include a group of antimicrobial compounds that are peptide-based.[5,6] These peptides can be further divided into two categories: the bacteriocins, which are produced by ribosomal synthesis often followed by post-translational modification,[3,4,7,8] and the nonribosomal peptides,[9] which are produced by nonribosomal peptide synthases (NRPS). Due to their unique features, both the bacteriocins and the nonribosomal peptides have captured the attention of scientists from many different disciplines.

From a chemical and structural perspective, most bacteriocins and nonribosomal peptides are interesting due to the unusual amino acid residues incorporated into their sequences. The lantibiotics, a well-studied class of bacteriocins, feature (2*S*,6*R*)-lanthionine (Lan) and (2*S*,3*S*,6*R*)-3-methyllanthionine (MeLan) residues (Figure 1) alongside other post-translational modifications in their structures.[3,7] Indeed, the word "lantibiotic" serves as an abbreviation for the term, "lanthionine-containing antibiotic."[10] These lanthionine bridges form rings along sections of the peptide backbone, giving the molecules added stability and resistance to pH and temperature variation, while governing the binding specificity of the peptides for their targets. Although lanthionines have only been found in the lantibiotics, another set of unusual amino acid residues, the *S*-[(*Z*)-2-aminovinyl]-D-cysteine (AviCys) and *S*-[(*Z*)-2-aminovinyl]-(3*S*)-3-methyl-D-cysteine (AviMeCys) residues (Figure 1), are thought to exist in both lantibiotics and nonribosomal peptides.[3,11−14]

Specific examples of lantibiotics that contain AviCys or AviMeCys residues include gallidermin, which is produced by *Staphylococcus gallinarum* Tü 3928,[11] and mersacidin, which is produced by *Bacillus* sp. strain HIL Y-85,54728[14] (Figure 2). The nonlantibiotic thioviridamide, which is produced by *Streptomyces olivoviridis* NA05001,[13] also contains an AviCys residue but is proposed to be of NRPS origin due to the presence of multiple thioamides in the peptide's backbone (Figure 2).

From a biosynthetic and ecological perspective, the AviCys and AviMeCys containing peptides generate curiosity with

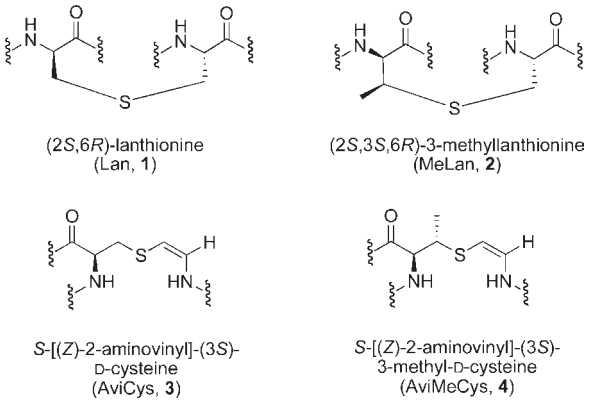


FIGURE 1. Structures of Lan, MeLan, AviCys, and AviMeCys residues found in lantibiotic peptides.

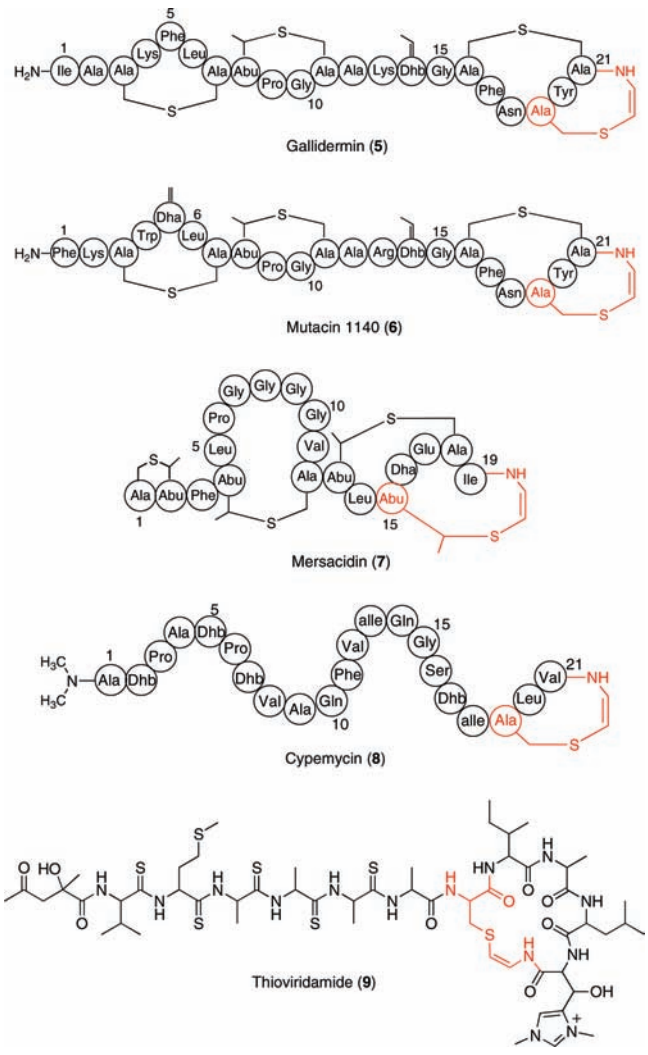


FIGURE 2. Structures of natural peptides that contain an AviCys or AviMeCys residues.

regards to how these compounds are produced. Specifically, questions have arisen as to which enzymes are involved in the biosynthesis of AviCys-type residues, as well as what

mechanisms of action they employ. For the biosynthesis of ribosomal peptides such as mersacidin, specific enzymes have been investigated for their role in the post-translational modification of the parent peptide to generate an AviCys or AviMeCys at the C-terminal end of the mature peptide.[15,16] On the other hand, the enzymes responsible for synthesizing the AviCys moiety in the nonribosomal peptide thioviridamide are unknown. It is plausible that AviCys biosynthesis in an NRPS system could be achieved by post-NRPS modification of the C-terminal cysteine in a fashion similar to the post-translational modification of the ribosomal peptides. However, much research still needs to be done before this hypothesis can be tested. First, the biosynthetic genes for thioviridamide need to be identified and sequenced to confirm that the peptide is indeed produced by an NRPS. Afterward, the enzyme or enzymes responsible for AviCys formation need to be characterized and compared with the enzymes involved in AviCys biosynthesis in ribosomal peptides. The degree of sequence homology between these enzymes will help establish whether this biosynthetic machinery is preserved from a common ancestor or whether the machinery evolved from distinct enzymes. In turn, such knowledge may offer insight into why AviCys and AviMeCys are recurring structural motifs in both ribosomal and nonribosomal peptides.

From a medical perspective, the AviCys-containing peptides represent a group of compounds with potent bioactivity. *In vitro* assays of the AviCys-containing lantibiotics suggest that these peptides can inhibit the growth of sensitive bacterial strains at nanomolar concentrations while showing little toxicity toward human epithelial cells.[17] Gallidermin and one of its naturally occurring structural analogues, epidermin, are both active against pathogenic strains of staphylococci and streptococci.[18] In addition, the two peptides are active against *Propionibacterium acnes*, and this property has led to the proposal of gallidermin as a potential acne treatment for humans.[18] Mutacin 1140 (Mu1140, Figure 2),[19] another structural analogue of gallidermin, exhibits activity against a spectrum of Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and, consequently, has been suggested for the treatment of throat infections and the prophylaxis of dental caries.[18] Mersacidin, a shorter lantibiotic that features AviMeCys in its structure, is likewise active against MRSA, with *in vivo* studies demonstrating that it is equipotent to vancomycin in treating MRSA infections in mice.[18] Aside from possessing antibacterial activity, some AviCys-containing peptides also show anticancer activity. Cypemycin (Figure 2),[20,21] another ribosomally synthesized AviCys-containing peptide that has structural similarities to the lantibiotics but lacks a true lanthionine ring, exhibits potent cytotoxicity against mouse leukemia cells. Interestingly, thioviridamide has been found to induce apoptosis in tumor cells.[22]

Although AviCys-containing peptides show much potential as antimicrobial and anticancer agents, the poor yielding production of these peptides could limit their applications in clinical usage. On average, fermentation of the producer organisms affords between 10 and 200 mg of purified compound per liter of culture but rarely exceeds that amount.[23,24] In a different vein, the total synthesis of mersacidin and other related lantibiotics is being investigated,[25] since effective methodology for the incorporation of lanthionines into peptides has been established.[26−29] However, these efforts have also been hampered, thus far, by an inability to chemically synthesize the AviCys ring. Since the biosynthesis of AviCys has been studied in several different bacteria,[16,21,30,31] a better solution may be to use *in vitro* biosynthesis or a mixture of chemical synthesis and biosynthesis to produce these peptides in higher yields. As well, by harnessing the activity of these biosynthetic enzymes it may be possible to produce AviCys peptide analogues with more desirable characteristics, such as increased activity, reduced toxicity, and better bioavailability. This Account will highlight the characteristics of the enzymes responsible for AviCys biosynthesis in lantibiotics, as well as discuss the biosynthesis of cypemycin, a structurally simpler AviCys-containing peptide. Together, this knowledge will be used to evaluate the possibilities of generating high yields of bioactive AviCys- or AviMeCys-containing peptides.

Overview of AviCys Biosynthesis in Lantibiotics

Of the lantibiotics that have been structurally characterized, numerous peptides have been found to contain AviCys or AviMeCys, including gallidermin, epidermin, mersacidin, microbisporicins A1 and A2, and several different mutacins.[3,11,12,14,32] The study of these peptides' biosyntheses begins with the sequencing of their biosynthetic gene clusters.

To date, the known biosynthetic gene clusters of the lantibiotics follow a general trend. Each cluster contains a structural gene, commonly referred to as *lanA*, that encodes for a prepeptide consisting of an N-terminal leader portion and a C-terminal structural component.[7,8] Two distinct enzymatic activities are then required to post-translationally install lanthionine rings in the prepeptide. The first is a dehydration of serine or threonine to dehydroalanine

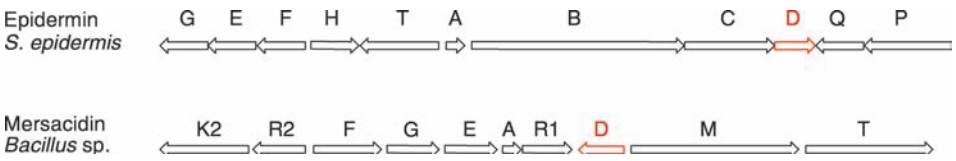


FIGURE 3. Representative biosynthetic gene clusters of the lantibiotics epidermin and mersacidin.

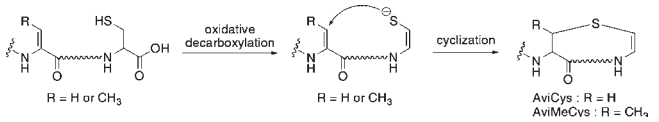


FIGURE 4. Enzymatic formation of the AviCys or AviMeCys residue in lantibiotics.

(Dha) and dehydrobutyrine (Dhb), respectively.[3,7] The second is a Michael addition of cysteine to the Dha or Dhb to form lanthionine or methyllanthionine. In the biosynthetic systems of some lantibiotics, these functions are performed by separate enzymes, namely, the dehydratase LanB and the cyclase LanC.[7,8] In other lantibiotic systems, however, both functions are performed by a single enzyme, LanM.[7] *In vitro* studies on LanM suggest that it requires the presence of the N-terminal leader of the prepeptide to recognize its substrate and perform sequence-specific modifications.[33] Once the post-translational modifications on the structural peptide are complete, the leader portion is cleaved off (sometimes by a dedicated protease, LanP), and the mature structural peptide is transported out of the cell via an ATP-binding cassette (ABC) transport system, LanT (Figure 3).[7,8]

The gene clusters that encode for the biosynthesis of AviCys-containing lantibiotics are distinguished by the presence of the *lanD* gene.[7] The LanD enzyme that results from this gene has been isolated and characterized in depth in two specific instances: EpiD catalyzes the formation of AviCys in epidermin,[30,34] and MrsD catalyzes the formation of AviMeCys in mersacidin.[16] EpiD and MrsD belong to a class of enzymes called the homo-oligomeric flavin-containing cysteine decarboxylases (HFCD). This family of enzymes catalyzes an oxidative decarboxylation of the C-terminal cysteine residue with the assistance of a flavin cofactor, which yields a reactive "thio−enol" intermediate (Figure 4). Interestingly, 4′-phosphopantothenoyl-cysteine (*P*PC) decarboxylases also belong to the HFCD family. *P*PC decarboxylases are enzymes found in eukaryotes and prokaryotes that are involved in the biosynthesis of 4′-phosphopantetheine.[35,36]

Characteristics of EpiD and MrsD

The study of epidermin biosynthesis began with the sequencing of its biosynthetic gene cluster. The first gene identified in the operon was *epiA*, which encoded for a 52 amino acid prepeptide.[10] Other genes that were found included *epiB*, *epiC*, *epiD*, *epiQ*, and *epiP*, all of which were confirmed as necessary elements for the production of mature epidermin through complementation analysis and heterologous gene expression.[37−39] In 1992, Kupke et al. cloned and overexpressed EpiD in *Escherichia coli*, before proceeding to purify EpiD and a maltose binding protein (MBP)−EpiD fusion protein to homogeneity.[34] This represented the first time that a lantibiotic-synthesizing enzyme had been successfully purified.

EpiD was found to be yellow in color, with an absorption spectrum typical of a flavoprotein in its oxidized state. Heat treatment of the protein led to the release of flavin mononucleotide (FMN), a coenzyme that is usually involved in oxidation−reduction reactions. Since AviCys is the only moiety that is oxidized in the peptide, EpiD was established as an oxidoreductase involved in the removal of two reducing equivalents from the C-terminal cysteine of the EpiA prepeptide.[34]

The enzymatic activity of EpiD was further probed by feeding EpiA to EpiD and tracking the reaction outcome using electrospray mass spectrometry. It was determined that EpiA lost 46 Da upon treatment with EpiD, corresponding to oxidation (a loss of 2H) and decarboxylation (a loss of CO_2).[15]

Once the enzymatic activity of EpiD was determined, Kupke et al. investigated the substrate specificity of this enzyme.[30] The study was done using the full length precursor EpiA, the leaderless epidermin propeptide, and several other synthetic heptapeptides. The reaction products were characterized by measuring the absorbance at 260 nm and mass spectrometry.[30] The results indicate that EpiD is able to decarboxylate a wide variety of substrates, each containing a C-terminal cysteine residue. The length of the substrates ranged from 52 amino acids (the prepeptide) to 4 amino acids long (the synthetic peptide library).[30] From this investigation, it was shown that EpiD could decarboxylate most of the peptides containing the following three amino acid sequence at its C-terminus: AA1-AA2-Cys, where AA1 = V/I/L/(M)/F/Y/W and AA2 = A/S/V/T/C/(I/L).[30] Moreover, it

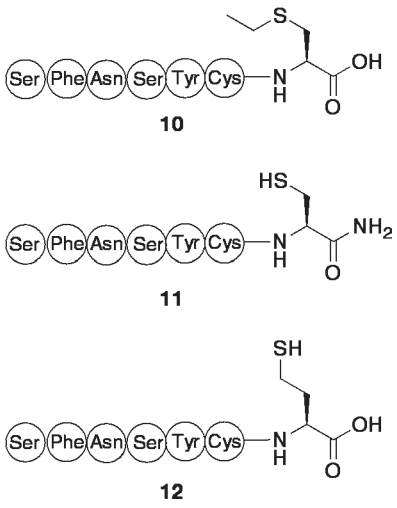


FIGURE 5. Structures of selected model peptides containing a modified C-terminal end that are not substrates for EpiD.

has been shown using peptides containing cysteine derivatives that the substrate needs to have a natural cysteine residue at its C-terminal end for EpiD to accept the substrate. Specifically, the thioether SFNSYCC(Et) (**10**), the amide SFNSYCC-NH_2 (**11**), and a homocysteine (HCy)-containing heptapeptide, SFNSYCHCy (**12**) (Figure 5) could not be modified by EpiD, indicating that the free thiol and carboxyl group of the C-terminal cysteine are crucial for the proper binding of the substrate and the function of EpiD.[30] Apart from this requirement, EpiD has no absolute substrate specificity. The fact that EpiD is not able to decarboxylate SFNSYCC(Et) is interesting from a mechanistic standpoint, because it suggests that decarboxylation takes place first before the C-terminal thioether ring is formed.

Although these initial studies established that EpiD catalyzes an oxidative decarboxylation of the C-terminal cysteine, the structure of the resulting intermediate had not been determined. It was speculated that the decarboxylation yields an enethiolate moiety, which can then cyclize onto a Dha to form the C-terminal ring of epidermin.

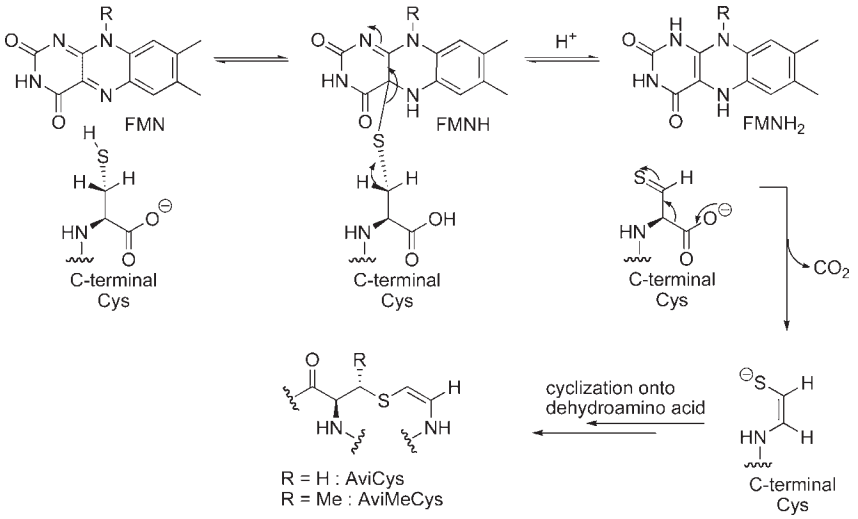
A later study was reported by Kempter et al., where NMR experiments were used to determine the structure of the EpiD reaction product, using the model peptide KKSFNSYTC, where the C-terminal cysteine was ^{13}C-labeled at its β-position.[31] After mixing of the substrate peptide and EpiD together in an NMR tube, the progress of reaction was monitored by HSQC experiments at different time points.[31] The starting peptide, KKSFNSYTC showed a cross peak at $\delta(^1H)$ 2.83 ppm and $\delta(^{13}C)$ 29.1 ppm, corresponding to the β-proton and ^{13}C-labeled β-carbon of the C-terminal cysteine. During the course of the NMR experiment, a new cross peak emerged at $\delta(^1H)$ 6.01 ppm and $\delta(^{13}C)$ 120.5 ppm, and the intensity of this signal increased over time. This signal was assigned as the correlation between the β-proton and β-carbon of an oxidatively decarboxylated C-terminal cysteine. After 16 h, a combination of 1D and 2D NMR experiments, including HSQC−TOCSY, 1D proton, and 1D carbon NMR experiments were conducted to assign α-proton, β-proton, and β-carbon signals of the enethiolate obtained from the EpiD reaction. The chemical shifts observed for the α-proton (δ = 6.50 ppm), β-proton (δ = 6.01 ppm), and β-carbon (δ = 120.5 ppm) are characteristic values of an alkene functionality. In addition, the signal for the β-proton was a doublet of doublets ($^1J_{H-C}$ = 170 Hz and $^3J_{H-H}$ = 7.3 Hz), suggesting that the double bond has a (*Z*) configuration.[31]

This finding led Kupke et al. to propose a mechanistic model (Scheme 1) for EpiD, whereby the enzyme uses FMN to oxidize the C-terminal cysteine of EpiA, leading to the formation of a thioaldehyde. Subsequent decarboxylation and rearrangement generates an enethiolate ion that presumably closes onto a preformed Dha at position 19.[40,41]

Crystal structures (Figure 6) of EpiD and the inactive mutant enzyme EpiD H67N, which is complexed with a pentapeptide substrate, have been reported.[42] In the crystal structure of EpiD H67N, the side chain amide of Asn117 appears in close proximity to one of the β-protons from the C-terminal cysteine of the substrate. Blaesse et al. suggest that the carbonyl of this amide could assist in abstracting the β-proton of the activated Cys, arguing for the importance of Asn117 in the mechanism of action of EpiD.[42] Furthermore, this residue is conserved in both EpiD and MrsD. The active site His67 appears to be important for enzymatic activity, but its exact role during the catalysis is still unclear. It has been proposed that His67 may be assisting in the oxidation of the thiol group of the C-terminal cysteine to a thioaldehyde by activating the thiol group, thus promoting the removal of a β-proton from the cysteine by Asn117.[42] Further mutational studies are needed to probe the exact mechanism of this reaction and the role of each active site residue.

MrsD is a second example of a LanD enzyme that has been characterized.[16,43] MrsD catalyzes the generation of the enethiolate that forms the AviMeCys moiety during the biosynthesis of mersacidin. In contrast to EpiD, MrsD uses flavin adenine dinucleotide (FAD) as the redox cofactor.[16] From the crystal structure of MrsD, an active site Asn125 and the hydrophobic residue Val76 are identified as important residues that may play a role in substrate binding and catalysis.[43] These two residues are similar to those found in the active site of EpiD (Asn117 and Ile68),[42] suggesting that the mechanism

SCHEME 1. Proposed Mechanism for the Formation of the AviCys or AviMeCys Residues



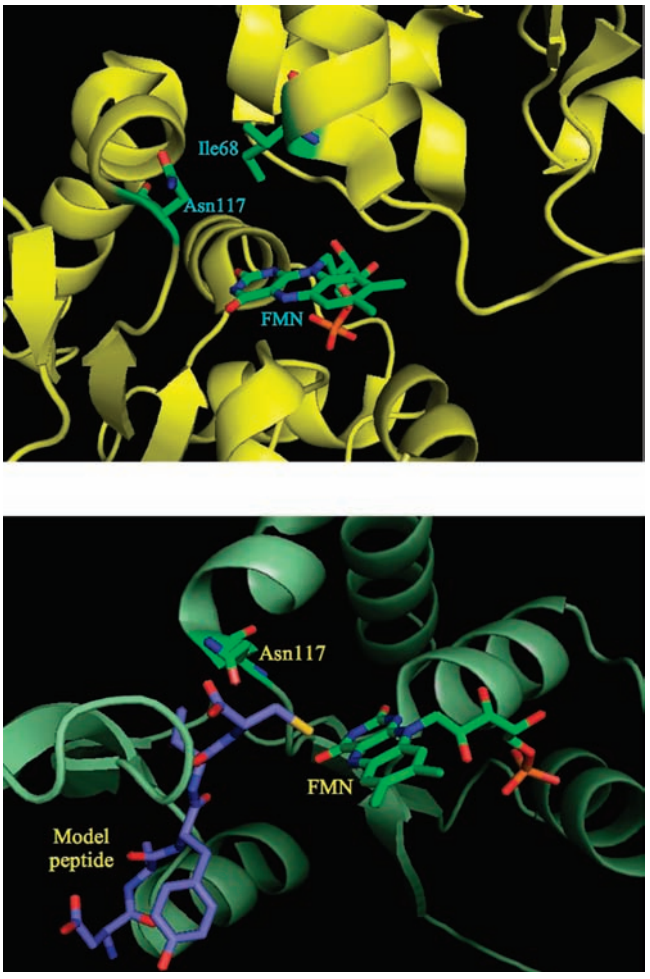


FIGURE 6. Crystal structures of EpiD and EpiD bound to a substrate peptide.

of formation of AviMeCys in mersacidin biosynthesis may be similar to that of epidermin biosynthesis. Although both EpiD and MrsD have similar substrate binding clamps in the active site, they have very different substrate specificities.[16] There has only been one study reported on the investigation of substrate specificity of MrsD, where epidermin precursor peptide EpiA and mersacidin precursor peptide MrsA are treated with MrsD. MrsD is only able to decarboxylate MrsA, suggesting that the enzyme is less promiscuous compared with EpiD. At this time, there is little else known about the substrate specificity of MrsD.[16] Further investigation using alternate substrates such as smaller synthetic peptides need to be done to test the ability of MrsD to perform the decarboxylation reaction on substrates other than MrsA.

Cypemycin biosynthesis

As introduced earlier, cypemycin is a ribosomally synthesized peptide that features an AviCys moiety at its C-terminal end. Produced by *Streptomyces sp.* OH-4156, cypemycin was identified and isolated based on its cytotoxic activity against mouse leukemia cells.[20] Minami et al. characterized the structure of cypemycin using mass spectrometry and NMR spectroscopy.[44] They determined that, in addition to AviCys, the 22-amino acid peptide features Dhb, *N*,*N*-dimethylalanine (Me_2-Ala), and L-*allo*-isoleucine (*a*-Ile) residues (Figure 2). Although AviCys and Dhb residues are commonly found in lantibiotics, cypemycin lacks a true lanthionine or methyllanthionine ring, arguing against its classification as a lantibiotic.

Recently, Claesen and Bibb identified and analyzed the gene cluster for cypemycin biosynthesis (Figure 7).[21] Through genome scanning and heterologous expression, they were able to confirm that nine genes contained in a single operon were required for cypemycin production. One of these genes

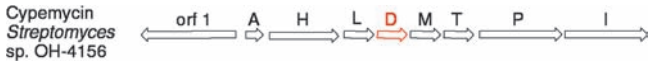


FIGURE 7. Schematic representation of the biosynthetic gene cluster of cypemycin.

is *cypA*, which encodes for a cypemycin prepeptide consisting of a 42 amino acid leader and a 22 amino acid structural region. As predicted, four threonine residues serve as the precursors for the Dhb residues at positions 2, 5, 7, and 17 of the mature peptide. Unexpectedly, however, none of the genes in the operon share a significant amount of homology with the characteristic dehydratases found in other lantibiotic systems. Instead, mutational analysis, where each gene is selectively deleted and the resulting mutant evaluated for cypemycin production, was needed to identify *cypH* and *cypL* as the two genes required for Dhb synthesis. In fact, when either of these genes was deleted, no cypemycin was detected by mass spectrometry or antimicrobial activity against *Micrococcus luteus*.[21]

More surprising was the discovery that the AviCys moiety is derived from two cysteine residues rather than one serine and one cysteine, as normally observed in epidermin and other AviCys-containing lantibiotics. Because CypA features a cysteine at position 19, the authors propose that this residue is dethiolated to form a Dha by CypH or CypL or through the activity of both enzymes. Oxidative decarboxylation of Cys22 and subsequent closure of the enethiolate onto the Dha forms the AviCys ring. Support for this mechanism was drawn from the isolation of a modified cypemycin peptide from a Δ*cypD* mutant, which lacks the ability to oxidize and decarboxylate Cys22. Since CypH and CypL activity were still intact in the Δ*cypD* mutant, the presence of a Dha residue at position 19 of the modified peptide could be confirmed by tandem mass spectrometry sequencing (MS/MS sequencing). Notably, the *cypD* gene has little sequence homology to the LanD enzymes, although *cypD* does contain the partial sequence of a *P*PC decarboxylase and, consequently, belongs to the HFCD family of proteins.[21]

The formation of Me_2-Ala and *a*-Ile, both of which have only been observed in cypemycin to date, was also investigated. It was found that CypM, an *S*-adenosyl methionine (SAM)-dependent methyltransferase, methylates the N-terminal alanine twice to form Me_2-Ala. Since the remainder of the gene products from the operon had already been ascribed roles, including the ABC transporter CypT and the transmembrane protein CypP, CypI was proposed as the enzyme responsible for the isomerization of isoleucine side chains to generate *a*-Ile. Interestingly, the *Streptomyces* sp. OH-4156 Δ*cypI* mutant showed the same anti-*M. luteus* phenotype as the wildtype, suggesting that if CypI truly converts isoleucine to *a*-Ile, then the *a*-Ile residues are not required for cypemycin's antibacterial activity.[21]

Taking into consideration the different enzymatic activities and biosynthetic pathways involved in the production of cypemycin, it should be classified as a ribosomal peptide distinct from the lantibiotics. After Claesen and Bibb conducted a search of the National Center for Biotechnology Information (NCBI) sequence database, they identified nine bacteria and one archaea species with *cypL* and *cypH* homologues. The authors propose that the peptides resulting from these organisms should be grouped together and called the "linaridins", since they are predicted to be mostly linear (lin) or noncyclized and to contain dehydrated (arid) amino acids.[21]

Potential Use of EpiD and CypD Enzymes in the Synthesis of AviCys-Containing Peptides

As mentioned earlier, most of the known AviCys-containing peptides exhibit interesting biological activities yet suffer from low-yielding production by fermentation. Efforts toward the total synthesis of these peptides have seen significant advances, especially with the development of efficient approaches to the solid phase peptide synthesis (SPPS) of lantibiotics.[28,29] However, a method for synthesizing the AviCys moiety remains elusive.

From the knowledge accrued through the study of EpiD and CypD, it may be possible to make use of these enzymes in a semisynthesis approach to generate epidermin and cypemycin, as well as analogues of these peptides. The use of a LanM enzyme, LtcM, for the synthesis of the lantibiotic lacticin 481 *in vitro* has been done by van der Donk and co-workers,[33] and a similar application could be envisioned for the LanD enzymes. Since both EpiD and CypD can be heterologously expressed as MBP fusions and purified to homogeneity, their enzymatic functions can be exploited for the *in vitro* biosynthesis of the AviCys motif.[21,34] In particular, EpiD may be able to oxidatively decarboxylate the C-terminal cysteines of a wide variety of peptides, from long substrates that mimic EpiA to short leaderless peptides.[30] The versatility of EpiD lends itself to the possibility of first synthesizing epidermin's lantibiotic backbone, via SPPS and on-resin cyclization of orthogonally protected Lan and MeLan precursors, before feeding the synthetic substrate to EpiD to form the AviCys ring in the last step. Conversely, a tetrapeptide substrate with a Dha residue at the −3 position from the C-terminal Cys could be fed to

EpiD to form the AviCys ring first, before coupling it to the N-terminal lantibiotic backbone fragment of epidermin. Similarly, linear analogues of cypemycin containing Me_2-Ala, *a*-Ile, Dhb, and Dha residues at the desired positions could be synthesized before being fed to CypD *in vitro*.

Exploration of these semisynthetic methods could lead to the production of highly varied libraries of AviCys-containing peptides, all of which can be screened for enhanced antimicrobial or anticancer activity, as well as improved pharmacological properties. As scientists learn more about the mechanism and scope of activity of the LanD or HFCD enzymes, the potential uses for these enzymes will increase.

The authors thank the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Research Chair in Bioorganic & Medicinal Chemistry for financial support.

FOOTNOTES

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REFERENCES

1 Gulder, T. A. M.; Moore, B. S. Chasing the treasures of the sea - bacterial marine natural products. *Curr. Opin. Microbiol.* **2009**, *12*, 252–260.

2 Antunes, L. C. M.; Ferreira, R. B. R.; Buckner, M. M. C.; Finlay, B. B. Quorum sensing in bacterial virulence. *Microbiology* **2010**, *156*, 2271–2282.

3 Willey, J. M.; van der Donk, W. A. Lantibiotics: Peptides of diverse structure and function. *Annu. Rev. Microbiol.* **2007**, *61*, 477–501.

4 van Belkum, M. J.; Stiles, M. E. Nonlantibiotic antibacterial peptides from lactic acid bacteria. *Nat. Prod. Rep.* **2000**, *17*, 323–335.

5 Cotter, P. D.; Hill, C.; Ross, R. P. Bacteriocins: Developing innate immunity for food. *Nat. Rev. Microbiol.* **2005**, *3*, 777–788.

6 McIntosh, J. A.; Donia, M. S.; Schmidt, E. W. Ribosomal peptide natural products: Bridging the ribosomal and nonribosomal worlds. *Nat. Prod. Rep.* **2009**, *26*, 537–559.

7 Chatterjee, C.; Paul, M.; Xie, L. L.; van der Donk, W. A. Biosynthesis and mode of action of lantibiotics. *Chem. Rev.* **2005**, *105*, 633–683.

8 Bierbaum, G.; Sahl, H. G. Lantibiotics: Mode of action, biosynthesis and bioengineering. *Curr. Pharm. Biotechnol.* **2009**, *10*, 2–18.

9 Finking, R.; Marahiel, M. A. Biosynthesis of nonribosomal peptides. *Annu. Rev. Microbiol.* **2004**, *58*, 453–488.

10 Schnell, N.; Entian, K. D.; Schneider, U.; Götz, F.; Zähner, H.; Kellner, R.; Jung, G. Prepeptide sequence of epidermin, a ribosomally synthesized antibiotic with 4 sulfide-rings. *Nature* **1988**, *333*, 276–278.

11 Kellner, R.; Jung, G.; Horner, T.; Zähner, H.; Schnell, N.; Entian, K. D.; Götz, F. Gallidermin - a new lanthionine-containing polypeptide antibiotic. *Eur. J. Biochem.* **1988**, *177*, 53–59.

12 Allgaier, H.; Jung, G.; Werner, R. G.; Schneider, U.; Zähner, H. Epidermin - sequencing of a heterodet tetracyclic 21-peptide amide antibiotic. *Eur. J. Biochem.* **1986**, *160*, 9–22.

13 Hayakawa, Y.; Sasaki, K.; Nagai, K.; Shin-ya, K.; Furihata, K. Structure of thioviridamide, a novel apoptosis inducer from Streptomyces olivoviridis. *J. Antibiot.* **2006**, *59*, 6–10.

14 Chatterjee, S.; Chatterjee, S.; Lad, S. J.; Phansalkar, M. S.; Rupp, R. H.; Ganguli, B. N.; Fehlhaber, H. W.; Kogler, H. Mersacidin, a new antibiotic from Bacillus fermentation, isolation, purification and chemical characterization. *J. Antibiot.* **1992**, *45*, 832–838.

15 Kupke, T.; Kempter, C.; Gnau, V.; Jung, G.; Götz, F. Mass spectroscopic analysis of a novel enzymatic-reaction - oxidative decarboxylation of the lantibiotic precursor peptide EpiA catalyzed by the flavoprotein EpiD. *J. Biol. Chem.* **1994**, *269*, 5653–5659.

16 Majer, F.; Schmid, D. G.; Altena, K.; Bierbaum, G.; Kupke, T. The flavoprotein MrsD catalyzes the oxidative decarboxylation reaction involved in formation of the peptidoglycan biosynthesis inhibitor mersacidin. *J. Bacteriol.* **2002**, *184*, 1234–1243.

17 Maher, S.; McClean, S. Investigation of the cytotoxicity of eukaryotic and prokaryotic antimicrobial peptides in intestinal epithelial cells in vitro. *Biochem. Pharmacol.* **2006**, *71*, 1289–1298.

18 Cotter, P. D.; Hill, C.; Ross, R. P. Bacterial lantibiotics: Strategies to improve therapeutic potential. *Curr. Protein Pept. Sci.* **2005**, *6*, 61–75.

19 Smith, L.; Zachariah, C.; Thirumoorthy, R.; Rocca, J.; Novak, J.; Hillman, J. D.; Edison, A. S. Structure and dynamics of the lantibiotic mutacin 1140. *Biochemistry* **2003**, *42*, 10372–10384.

20 Komiyama, K.; Otoguro, K.; Segawa, T.; Shiomi, K.; Yang, H.; Takahashi, Y.; Hayashi, M.; Otani, T.; Omura, S. A new antibiotic, cypemycin - taxonomy, fermentation, isolation and biological characteristics. *J. Antibiot.* **1993**, *46*, 1666–1671.

21 Claesen, J.; Bibb, M. Genome mining and genetic analysis of cypemycin biosynthesis reveal an unusual class of posttranslationally modified peptides. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 16297–16302.

22 Hayakawa, Y.; Sasaki, K.; Adachi, H.; Furihata, K.; Nagai, K.; Shin-ya, K. Thioviridamide, a novel apoptosis inducer in transformed cells from Streptomyces olivoviridis. *J. Antibiot.* **2006**, *59*, 1–5.

23 Kim, W. S.; Hall, R. J.; Dunn, N. W. The effect of nisin concentration and nutrient depletion on nisin production of Lactococcus lactis. *Appl. Microbiol. Biotechnol.* **1997**, *48*, 449–453.

24 Horner, T.; Ungermann, V.; Zähner, H.; Fiedler, H. P.; Utz, R.; Kellner, R.; Jung, G. Comparative-studies on the fermentative production of lantibiotics by staphylococci. *Appl. Microbiol. Biotechnol.* **1990**, *32*, 511–517.

25 Narayan, R. S.; VanNieuwenhze, M. S. Versatile and stereloselective syntheses of orthogonally protected β-methylcysteine and β-methyllanthionine. *Org. Lett.* **2005**, *7*, 2655–2658.

26 Bregant, S.; Tabor, A. B. Orthogonally protected lanthionines: Synthesis and use for the solid-phase synthesis of an analogue of nisin ring C. *J. Org. Chem.* **2005**, *70*, 2430–2438.

27 Paul, M.; van der Donk, W. A. Chemical and enzymatic synthesis of lanthionines. *Mini-Rev. Org. Chem.* **2005**, *2*, 23–37.

28 Pattabiraman, V. R.; McKinnie, S. M. K.; Vederas, J. C. Solid-supported synthesis and biological evaluation of the lantibiotic bis(desmethyl) lacticin 3147 A2. *Angew. Chem., Int. Ed.* **2008**, *47*, 9472–9475.

29 Ross, A. C.; Liu, H. Q.; Pattabiraman, V. R.; Vederas, J. C. Synthesis of the lantibiotic lactocin S using peptide cyclizations on solid phase. *J. Am. Chem. Soc.* **2010**, *132*, 462–463.

30 Kupke, T.; Kempter, C.; Jung, G.; Götz, F. Oxidative decarboxylation of peptides catalyzed by flavoprotein EpiD - determination of substrate-specificity using peptide libraries and neutral loss mass-spectrometry. *J. Biol. Chem.* **1995**, *270*, 11282–11289.

31 Kempter, C.; Kupke, T.; Kaiser, D.; Metzger, J. W.; Jung, G. Thioenols from peptidyl cysteines: Oxidative decarboxylation of a C-13-labeled substrate. *Angew. Chem., Int. Ed.* **1996**, *35*, 2104–2107.

32 Foulston, L. C.; Bibb, M. J. Microbisporicin gene cluster reveals unusual features of lantibiotic biosynthesis in actinomycetes. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 13461–13466.

33 Levengood, M. R.; Knerr, P. J.; Oman, T. J.; van der Donk, W. A. In vitro mutasynthesis of lantibiotic analogues containing nonproteinogenic amino acids. *J. Am. Chem. Soc.* **2009**, *131*, 12024–12025.

34 Kupke, T.; Stevanovic, S.; Sahl, H. G.; Götz, F. Purification and characterization of EpiD, a flavoprotein involved in the biosynthesis of the lantibiotic epidermin. *J. Bacteriol.* **1992**, *174*, 5354–5361.

35 Hernandez-Acosta, P.; Schmid, D. G.; Jung, G.; Culianez-Macia, F. A.; Kupke, T. Molecular characterization of the Arabidopsis thaliana flavoprotein AtHAL3a reveals the general reaction mechanism of 4′-phosphopantothenoylcysteine decarboxylases. *J. Biol. Chem.* **2002**, *277*, 20490–20498.

36 Kupke, T. Molecular characterization of the 4′-phosphopantothenoylcysteine decarboxylase domain of bacterial Dfp flavoproteins. *J. Biol. Chem.* **2001**, *276*, 27597–27604.

37 Schnell, N.; Engelke, G.; Augustin, J.; Rosenstein, R.; Ungermann, V.; Götz, F.; Entian, K. D. Analysis of genes involved in the biosynthesis of lantibiotic epidermin. *Eur. J. Biochem.* **1992**, *204*, 57–68.

38 Augustin, J.; Rosenstein, R.; Wieland, B.; Schneider, U.; Schnell, N.; Engelke, G.; Entian, K. D.; Götz, F. Genetic-analysis of epidermin biosynthetic genes and epidermin-negative mutants of Staphylococcus epidermidis. *Eur. J. Biochem.* **1992**, *204*, 1149–1154.

39 Augustin, J.; Götz, F. Transformation of Staphylococcus epidermidis and other staphylococcal species with plasmid DNA by electroporation. *FEMS Microbiol. Lett.* **1990**, *66*, 203–207.

40 Steinbacher, S.; Hernandez-Acosta, P.; Bieseler, B.; Blaesse, M.; Huber, R.; Culianez-Macia, F. A.; Kupke, T. Crystal structure of the plant PPC decarboxylase AtHAL3a complexed with an ene-thiol reaction intermediate. *J. Mol. Biol.* **2003**, *327*, 193–202.

41 Strauss, E.; Zhai, H.; Brand, L. A.; McLafferty, F. W.; Begley, T. P. Mechanistic studies on phosphopantothenoylcysteine decarboxylase: Trapping of an enethiolate intermediate with a mechanism-based inactivating agent. *Biochemistry* **2004**, *43*, 15520–15533.

42 Blaesse, M.; Kupke, T.; Huber, R.; Steinbacher, S. Crystal structure of the peptidyl-cysteine decarboxylase EpiD complexed with a pentapeptide substrate. *EMBO J.* **2000**, *19*, 6299–6310.

43 Blaesse, M.; Kupke, T.; Huber, R.; Steinbacher, S. Structure of MrsD, an FAD-binding protein of the HFCD family. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2003**, *59*, 1414–1421.

44 Minami, Y.; Yoshida, K.; Azuma, R.; Urakawa, A.; Kawauchi, T.; Otani, T.; Komiyama, K.; Omura, S. Structure of cypemycin, a new peptide antibiotic. *Tetrahedron Lett.* **1994**, *35*, 8001–8004.