Extensive Post-translational Modification, Including Serine to D-Alanine Conversion, in the Two-component Lantibiotic, Lacticin 3147*


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Lacticin 3147 is a two-component bacteriocin produced by Lactococcus lactis subspecies lactis DPC3147. In order to further characterize the biochemical nature of the bacteriocin, both peptides were isolated which together are responsible for the antimicrobial activity. The first, LtnA1, is a 3,322 Da 30-amino acid peptide and the second component, LtnA2, is a 29-amino acid peptide with a mass of 2,847 Da. Conventional amino acid analysis revealed that both peptides contain the thioether amino acid, lanthionine, as well as an excess of alanine to that predicted from the genetic sequence of the peptides. Chiral phase gas chromatography coupled with mass spectrometry of amino acid composition indicated that both LtnA1 and LtnA2 contain D-alanine residues and amino acid sequence analysis of LtnA1 confirmed that the D-alanine results from post-translational modification of a serine residue in the primary translation product. Taken together, these results demonstrate that lacticin 3147 is a novel, two-component, D-alanine containing lantibiotic that undergoes extensive post-translational modification which may account for its potent antimicrobial activity against a wide range of Gram-positive bacteria.

The term bacteriocin, particularly when applied to those produced by Gram-positive bacteria, defines a large group of proteinaceous compounds which display an antimicrobial activity directed primarily against other Gram-positive organisms (1). One subclass of these antimicrobial peptides comprises the lantibiotics, which are produced by many different strains of Gram-positive organisms, including the lactic acid bacteria (1, 2). Lantibiotics are distinguished from other bacteriocins in that they undergo extensive post-translational modification, with modifications of serine, threonine, and cysteine residues being particularly common. Typically, the hydroxy amino acids (Ser and Thr) are selectively dehydrated to form dehydroalanine (Dha)1 and dehydrobutyrylne (Dhb), respectively (3). The resultant a,b-unsaturated residues may then undergo Michael addition reactions with the thiol group of specific cysteine residues to form lanthionine (Lan) and b-methyl-lanthionine (MeLan), the characteristic features of these peptides (1). The precise role of these lantibiotic residues remains unclear, but they have been shown to contribute to enhanced stability under extreme temperatures (4) and oxidizing conditions (5) and have also been implicated in increased tolerance to acids (6) as well as in resistance to proteolytic activities (7). The best known lantibiotic is nisin which contains five lanthionine rings. This lantibiotic is well characterized at the biochemical and molecular level and has found widespread application as a biopreservative in the food industry (8).

In addition to lanthionine, other unusual modified amino acids such as 2-oxobutyrate (9), 2S,8S-lysinoalanine (10, 11), and D-alanine (12) have been identified in certain lantibiotic peptides. Identification of D-alanine in the lantibiotic lactocin S is of particular interest in that it was the first reported example of the incorporation of a D-amino acid in a ribosomally synthesized prokaryotic peptide (12). Analysis of the genetic sequence predicted serine residues in certain positions in the prepeptide, whereas D-alanine was detected in the mature peptide, suggesting the conversion was the result of a more complex mechanism than a simple isomerase conversion from L-alanine. Although the exact mechanism remains to be elucidated, the authors proposed a model for stereoinversion based on the ω-epimerization sequence (13) and on the stereoinversion involved in lanthionine formation (14). In this mechanism, the D-alanine residues are thought to be introduced by a two-step ω-carbon stereoconversion in which serine is initially dehydrated in the same manner as in the first step of lanthionine formation. The resultant dehydroalanine residue is then further modified by an unidentified stereospecific hydrogenating enzyme or enzyme system.

This study involved the purification and biochemical characterization of the novel two-component antimicrobial peptide lacticin 3147 (15). The results presented allow the two peptides to be ascribed to two genes, ltnA1 and ltnA2 (previously described as ltnA and ltnB) in a gene cluster contained within a large conjugative plasmid pMRC01 (16). Interestingly, this

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1 The abbreviations used are: Dha, dehydroalanine; Dhb, dehydrobutyrylne; Lan, lanthionine; MeLan, β-methyl-lanthionine; RP, reverse phase; HPLC, high performance liquid chromatography; FPLC, fast protein liquid chromatography; ES-MS, electrospray ionization-mass spectra.
cluster contains a number of open reading frames with homology to genes known to be involved in lantibiotic biosynthesis, including two lanM genes associated with dehydration and lanthionine formation. Moreover, a complex array of post-translational modifications of lacticin 3147 are demonstrated, which includes serine to d-alanine conversion, dehydration of serines and threonines, lanthionine formation, and leader peptide cleavage. Taken together, these results reveal a two-peptide bacteriocin with a highly modified structure, which may account for its potent antimicrobial action against a wide variety of Gram-positive bacteria (17–21).

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Culture Conditions, and Bacteriocin Assays**

The producing strain *L. lactis* DPC3147, as well as the sensitive indicator strains *L. lactis* HP and *L. lactis* AM2 were maintained by weekly subculture in M17 (Difco, Detroit, MI) supplemented with 0.5% lactose (LM17) and all strains were stored at –70 °C in 20% glycerol. For production of bacteriocin activity, a modified tryptone/yeast extract medium (15), was used from which hydrophobic components which interfered in subsequent purification procedures had been removed by adsorption to the chromatographic medium XAD-16 (Sigma-Alrich, Dublin, Ireland).

Bacteriocin activity was assayed after each step using *L. lactis* HP as the indicator strain and the method previously described by McAuliffe et al. (20). In order to locate complementary activity, 10-µl aliquots of putative A1 fractions were cross-tested with isolated peptide A2 and vice versa. To demonstrate complementary activity, 10-µl aliquots of isolated fractions, A1, A1’, and A2 were each dispensed into separate, triangularly arranged agar wells formed in an agar plate which had previously been seeded with the sensitive indicator strain *L. lactis* AM2. Synergistic activity between the peptides was indicated by a zone of inhibition between the wells, while activity from individual peptides was observed as a zone around a well on the side facing away from all other fractions.

**Purification of the Inhibitory Peptides**

An overnight culture of the producing strain, *L. lactis* DPC3147 was inoculated into 8 liters of modified tryptone-yeast broth. The inoculated medium was incubated overnight at 30 °C and then centrifuged at 10,000 × g for 30 min and resuspended in 20 ml of sodium phosphate buffer (20 mM, pH 7). The preparation was desalted on a column (5 × 23 cm) containing a 50-g bed of XAD-16 resin (Sigma-Alrich) at a flow rate of 15 ml/min and the column was then washed with 2 liters of 40% aqueous ethanol (pH 6). The bacteriocin was subsequently eluted with 1 liter of 70% propan-2-ol adjusted to approximately pH 2 by the addition of HCl. The propan-2-ol was removed by rotary evaporation and the resultant bacteriocin preparation was fractionally precipitated with the addition of solid ammonium sulfate to a final saturation of 30% at 4 °C. Following gentle stirring overnight at 4 °C, the precipitated bacteriocin was recovered by centrifugation at 10,000 × g for 15 min. The supernatant was applied to a column (5 × 30 cm) with a bed volume of 25 g of XAD-2 resin (Serva, Heidelberg, Germany) and the bacteriocin was again eluted in 70% propan-2-ol/HCl (pH 2), with subsequent concentration by rotary evaporation yielding approximately 4 ml of crude preparation. Aliquots of 2 ml were then applied to a C18 reverse phase (RP)-HPLC column (Nucleosil ODS II, 4.6 × 250 mm) previously equilibrated with 0.1% aqueous trifluoroacetic acid. The column was subsequently developed in a gradient of 30 to 60% propan-2-ol containing 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. Fractions were collected manually and assayed for activity. The two fractions, A1 and A2, containing the bacteriocin activity were concentrated separately by rotary evaporation and each fraction was then re applied to the C18 RP-HPLC column. A gradient of 40 to 46% propan-2-ol (0.1% trifluoroacetic acid) was applied to further fractionate the A1 component while fraction A2 was developed in a gradient of 44 to 50% propan-2-ol (0.1% trifluoroacetic acid) in 30 min. The procedure was repeated until homogenous, biologically active peaks were obtained.

**Chemical or Enzyme-mediated Peptide Modifications**

Reduction with 1-Propanethiol. To facilitate protein sequencing, peptide samples containing dehydrated amino acids were first modified essentially by the method of Meyer et al. (22). Briefly samples containing sufficient material for sequencing (100 to 200 pmol) were dissolved in 15 µl of a freshly prepared solution containing 280 µl of ethanol, 200 µl of H2O, 65 µl of 5 M aqueous NaOH solution, and 60 µl of 1-propanethiol (all from Merck, Damstadt, Germany). The samples were heated under an inert gas atmosphere (Ar) at 50 °C for 1 h in a heated block, diluted 1:1 in glacial acetic acid, and sequenced (as described below) with further treatment.

**Enzyme-mediated Digestion.** Peptides for digestion were dissolved in 50 µl of a solution of 1% acetic acid adjusted to pH 8.5 with ammonia solution and 1 to 2 µl of enzyme (dissolved in H2O) was added to give a final enzyme to peptide ratio of 1:25. Digestions were incubated for 16 to 18 h at 35 °C in a heated block and the reaction was terminated by the addition of 2 µl of trifluoroacetic acid. Reactions were controlled by simultaneously digesting appropriate synthetic peptides possessing the required sites for digestion (EMC, Tübingen, Germany). All enzymes were obtained from Sigma-Alrich and were of sequencing grade quality.

**Mass Spectrometry**

Electrospray ionization (ES)-mass spectra were accumulated and analyzed on a VG Quattro II triple quadrupole mass spectrometer (MS) using Mass Lynx software (Microssa). Samples were infused in the liquid phase in a continuous stream of 50% aqueous acetonitrile containing 0.05% formic acid at flow rates of 5–30 µl/min, either from a syringe pump (Harvard Apparatus) or a model 232XL autosampler (Gilion-Abimed) connected to a HPLC pump. For HPLC-MS experiments, samples were introduced into the mass spectrometer at a flow rate of 45 µl/min after separation through a HPLC column (100 × 1 mm Nucleosil ODS II; Grom, Herrenberg, Germany) to an Integral microanalytical workstation (PE-Biosystems, Germany); the HPLC separation was achieved using appropriate binary gradients of water and acetonitrile, each containing either 0.1% trifluoroacetic acid or 0.1% formic acid. All mass spectra were collected in the positive mode using cone voltages between 25 and 65 V.

**Amino Acid Sequencing**

Automated Edman degradation-based amino acid sequence analysis was performed using a model 477A pulsed liquid-gas phase protein sequencer coupled on-line to a model 120A phenylthiohydantoin-derivatizing analyzer (both from PE-Biosystems). Samples to be sequenced were applied to a trifluoroacetic acid pretreated glass fiber filter coated with 15 µl of Biobrene Plus (PE-Biosystems) and were both subjected to Edman degradation as well as analyzed using the manufacturers chemical and mass spectrometry protocols and software. For the visualization of propanethiol-modified residues (S-propyl-cysteine and 3-methyl-S-propyl-cysteine), the standard analyzer gradient was elongated for a further 8 min.

**Amino Acid Analysis**

Peptides for amino acid analysis were hydrolyzed in an inert gas atmosphere (N2) in 6 ml at HCl at 110 °C for 16 h in sealed glass vials. Following subsequent removal of HCl under vacuum, the amino acid composition of the samples was determined after *ortho*-phthalaldehyde derivatization by a previously described method specifically optimized for the identification of Lan and MeLan (23). In order to quantitate Pro (for which the above method is unsuitable), hydrolysates prepared as above were analyzed on a model 420 derivatizer connected to an on-line narrow bore mode 130A phenyliodohydrantoin-derivativizing analyzer and model 920A data analysis module (all from PE-Biosystems) using the manufacturers chemicals, protocols, and software. The enantiomeric purity of amino acids was determined by gas chromatography (GC)-MS by a previously described method (24). The peptides were hydrolyzed in DCl/D2O at 110 °C for 24 h prior to derivatization and analysis.

**RESULTS**

**Purification of Lacticin 3147.** We previously reported the partial purification of lacticin 3147, a broad spectrum bacteriocin produced by *L. lactis* DPC3147 (15). The final RP-HPLC step of that procedure allowed the identification of two subunits, both of which were required for full biological activity. In the present study, we applied a modified purification protocol, which was successfully used to separate and isolate the lacticin 3147 subunits. The peptides obtained were subsequently subjected to detailed biochemical characterization.

A 30% saturation ammonium sulfate fractionation proved
particularly valuable to both concentrate and further purify the bacteriocin peptides. A further deviation from the previous purification procedure was the replacement of C18 RP-FPLC with C18 RP-HPLC which provided enhanced resolution of the peptides. LtnA2, the more hydrophobic of the two bacteriocin components eluted at approximately 54% propan-2-ol, whereas LtnA1 was comparatively more hydrophilic and eluted at approximately 44% propan-2-ol (Fig. 1). Concomitant with the separation of the two peptides, a significant loss in activity was observed when each were assayed individually against the indicator strain, *L. lactis* HP. However, as observed previously, complementation of fraction A1 with fraction A2 resulted in recovery of full activity (Fig. 1).

Repeated chromatographic separation by HPLC revealed that LtnA1 activity correlated to two individual peaks as characterized by a variation in their respective retention times. The individual peaks were designated LtnA1 and LtnA1', respectively (Fig. 1). By contrast, LtnA2 activity corresponded to an apparently single homogenous peak. Interestingly, when each of these components were assayed individually against the indicator strain, *L. lactis* HP. However, as observed previously, complementation of fraction A1 with fraction A2 resulted in recovery of full activity (Fig. 1).

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Analysis of Amino Acid Composition—Both forms of LtnA1, as well as LtnA2 were hydrolyzed under vacuum for 16 h in 6 M HCl and then derivatized with ortho-phthaldialdehyde whereupon, they were subjected to amino acid analysis by RP-HPLC (with the exception of the Pro values which were determined by the alternative phenythiocarbamoyl-based procedure). The resulting analyses for LtnA1 (similar results were obtained for LtnA1') and LtnA2 are summarized in Table I. In addition to representatives of the usual amino acids, we detected the modified residues, Lan and MeLan in both peptides. Interestingly, amino acid analyses suggested that both peptides contained significantly more alanine than would be predicted from the translated gene sequences (Fig. 2); LtnA1 contained at least one additional residue while LtnA2 contained two additional residues of alanine.

The configurations of the amino acids detected in the hydrolysates of LtnA1 and LtnA2 were also characterized by chiral-phase GC coupled to MS. Surprisingly, while most of the amino acids were present as the expected L-isomer, a considerable proportion (approximately 33%) of the Ala content of both LtnA1 and LtnA2 was found to be present as the D-isomer (data not shown). From the results, it could be determined that LtnA1 contains one residue of D-alanine while LtnA2 contains two such residues. Control hydrolysates of the lantibiotic galilidermin (25) provided standards for the unusual amino acids Lan and MeLan and demonstrated that Ala was not artifactually undergoing racemization during either hydrolysis or the analysis.

Amino Acid Sequence Analysis—Edman degradation of LtnA1 and LtnA1' revealed several interesting features. Initial attempts to sequence the native peptides failed to yield interpretable results and so purified peptides were subjected to 1-propanethiol derivatization which enabled sequencing through to residues 16 and 18 for LtnA1 and LtnA1' respec-
The results of this analysis yielded the following sequences: XXXNFALDXYWGNN and XXXNFALDXYWGNNAW (for LtnA1 and LtnA1\', respectively), where X represents an unidentified amino acid. From previous experience and utilizing an extended HPLC gradient we were able to detect peaks with retention times corresponding to either S-propylcysteine or 3-methyl-S-propylcysteine for several of these unidentified residues which are consistent with the presence of Lan, MeLan, Dha, or Dhb residues in these positions. Furthermore, at position 7 where either a Ser or Dha residue was expected from the gene sequence (Fig. 2), an Ala residue (Fig. 3) was identified, consistent with the additional Ala residue indicated from the amino acid analyses.

By contrast, sequence analysis of both native and chemically modified LtnA2 failed to yield sequence data, apparently due to the absence of a free N terminus. The predicted LtnA2 prepeptide (Fig. 2) contains a putative "double glycine" cleavage site (P1 = Gly, P2 = Gly) followed by a Thr residue at position P'1, which can be further dehydrated to give Dhb. Since \( \alpha,\beta \)-unsaturated amino acids are unstable when located at the N terminus (1), the sequencing results are consistent with cleavage at the predicted site (Fig. 2), the result of which would be the formation of a LtnA2 peptide with a modified N terminus analogous to that found in other lantibiotics such as Pep5 (9).

In an attempt both to localize the predicted d-Ala residues and to gather sequence data for LtnA2, the peptidase was digested separately with either trypsin or \( \alpha \)-chymotrypsin, however, both enzymes failed to digest the peptide even at enzyme to peptide ratios of 1:5. Since both sides of the trypsin cleavage site are surrounded by potentially modified residues, it is possible the modified peptide is no longer a suitable substrate for trypsin. Similarly, HPLC-purified, fully reduced LtnA2 proved to be an unacceptable substrate for \( \alpha \)-chymotrypsin.

**Mass Spectrometry—** The mass spectra for the purified peptides are shown in Fig. 4. From the multiply charged ions detected, the masses estimated for LtnA1 was 3,322.34 ± 0.80 Da, while the mass of LtnA2 was estimated to be 2,847.47 ± 0.53 Da. As indicated above, two forms of LtnA1 exist which differ slightly in their hydrophobicity. To further investigate this phenomenon, each of the two species, LtnA1 and LtnA1', which differ slightly in their hydrophobicity were studied using an alternative HPLC buffer system (0.1% formic acid) which provided a considerably improved separation as well as increased MS sensitivity. With this system, it was observed that although both LtnA1 and LtnA1' have identical masses, each form appears to vary in its susceptibility to dehydration. From the respective mass chromatograms, we calculated that more than 98% of LtnA1 is 6-fold dehydrated, while approximately 33% of the LtnA1' sample contains an additional dehydration (Fig. 5).

Following the observation that LtnA1 exists in various states of dehydration, we also checked preparations of LtnA2 for this microheterogeneity. Although we could separate a small additional peak with apparent increased hydrophilicity, analysis of the masses of the two forms revealed a difference of 16 Da, both the mass and characteristic earlier elution of this peak suggest oxidation of the peptide. In the example given in Fig. 5, 14.74% of the total peptide was in the oxidized form but unfortunately, it was not possible to separate sufficiently the oxidized form for further biological characterization.

**DISCUSSION**

In this study, we reveal that the two-component antimicrobial peptide lacticin 3147 undergoes a series of complex post-translational modifications including conversion of serine residues to \( \delta \)-alanine and the formation of lanthionine bridges. This is a very significant finding given that this is only the second instance of \( \delta \)-alanine occurring in ribosomally synthesized peptide and the first instance of it in a two-component biologically active peptide. The bacteriocin acts by selectively dissipating the membrane potential of target cells through the formation of pores allowing cell leakage of potassium and inorganic phosphate (15). Based on the amino acid sequence and compositional analysis, in addition to mass spectrometry, the structural genes for lacticin 3147 can now be positively assigned to \( \text{ltnA1} \) and \( \text{ltnA2} \), two small open reading frames contained at the start of the larger of two divergent gene clusters encoded on the conjugative plasmid pMRC01. Interestingly, three larger open reading frames follow these structural genes in the cluster, two of which, \( \text{ltnM1} \) and \( \text{ltnM2} \), share homology to modification genes involved in dehydration/lanthionine formation. These putative modification genes are separated by the open reading frame, \( \text{ltnT} \), which probably encodes an ABC-transporter based on data base homologies. This transporter contains a proteolytic domain which is probably involved in the cleavage of the leader peptides during export. Indeed, the results obtained allow us to assign the cleavage site of the leader peptide in both structural components (Fig. 2).

One striking feature of the structural analyses of LtnA1 and LtnA1' is the identification of an additional Ala, both in the amino acid compositional analysis and during N-terminal sequencing of position 7 of the peptide; the genetic sequence clearly encodes a Ser residue at this position (16). Indeed, this is only the second instance where such a substitution has been reported in a ribosomally synthesized prokaryotic peptide. Analysis of component amino acid chirality demonstrates that one of the three Ala residues is present as the \( \delta \)-isomer, while all other common amino acids are present as the L-isomer. The lantibiotic lactocin S (12) has also been reported to contain \( \delta \)-Ala which has been proposed to arise from the stereospecific hydrogenation of Dha which is formed from the dehydration of a gene-encoded Ser residue, although the mechanism and enzyme(s) responsible are still unknown. Thus, we propose that by analogy to lactocin S, position 7 of LtnA1 is occupied by \( \delta \)-Ala, although the more complicated but unlikely possibility that this residue is in the L-configuration and that one of the additional two gene-encoded L-Ala residues has been converted...
to D-Ala by an unidentified L-Ala isomerase cannot be excluded. While the X residues corresponding to some of the serine and threonine residues have yet to be positively identified, they most likely exist as dehydrated derivatives, given that both lanthionine and D-alanine which have been demonstrated to exist in both peptides, probably require a dehydrated intermediate for their formation.

In addition, the calculated mass for the predicted LtnA1 peptide (3,430.88 Da), assuming that it contains six dehydration (−108.06 Da), four of which are necessary precursors for the formation of Lan/MeLan, and a single residue of alanine formed by the hydrogenation of a Dha (+2 Da) is 3,324.82 Da. This value is in reasonable agreement with the measured mass of 3,322.34 ± 0.80 Da, although not so close that we can rule out the possibility of further minor modifications. A further interesting feature of LtnA1 production is the identification of two species, LtnA1 and LtnA1', which differ slightly in their relative hydrophobicity. In addition, these forms differ with respect to their biological activity, in that while both can be complemented by LtnA2, the LtnA1 form possesses an independent antimicrobial activity, while the purified LtnA1' form is inactive. As observed in Fig. 1, a higher concentration of LtnA1 appears to be required to give inhibition on its own, compared with that required to give complementary activity with LtnA2, as implied by the smaller circle encompassing the zone of inhibition formed by LtnA1 alone. Further analysis of the LtnA1 and LtnA1' fractions also revealed another significant difference in that the LtnA1' peptide is apparently more susceptible to dehydration, with approximately 38% of LtnA1' being 7-fold dehydrated. While, such distinguishing features point to some structural difference between the two forms of LtnA1, it is also important to highlight the similarities between
LtnA1 and LtnA1'. Both forms complement LtnA2, have identical masses and sequence (at least to Gly\textsuperscript{16}) as well as similar amino acid composition (data not shown). One hypothesis which offers an explanation for these results is that a differing thioether bridging pattern exists in the two forms. Such a feature would explain the similarities in mass and sequence between the two peptides, yet could also allow for differences in hydrophobicity between two forms with different tertiary structures. This could also account for their characteristic biological activities.

Analysis of LtnA2 demonstrates that, in addition to Lan/MeLan, it also contains several noteworthy features. First, LtnA2 possesses a blocked N terminus. Since the gene sequence for LtnA2 encodes a Thr residue in the position just after the putative cleavage site, it would appear that this hydroxy amino acid is dehydrated during post-translational modification of LtnA2. After cleavage of the leader peptide, the addition of water followed by deamination should take place, as has been proposed for the N-terminal located $\alpha,\beta$-unsaturated residues resulting in formation of a 2-oxobutyryl group in analogy to that found in lantibiotics such as Pep5 (9). Hence, the inability to generate N-terminal sequence from LtnA2 may be explained by this reaction and tends to confirm the previously postulated cleavage site for the leader peptide. Second, amino

![HPLC-ES-MS analysis of LtnA1, LtnA1', and LtnA2.](image-url)
acid analysis demonstrated that LtnA2, like LtnA1 contains an excess of Ala residues (Ala = 6) over that predicted from the gene sequence (Ala = 4) and chiral phase analysis demonstrated that two of these additional Ala residues are present as the non-natural D-isomers. Unfortunately, due to the difficulties encountered in digesting the peptide, we were unable to further localize the position of the residues.

Furthermore, from the mass determined for the peptide, additional predictions can be made for the structure of LtnA2. The mass determined (2,847.84 ± 0.33 Da) is in extremely close agreement to that of a LtnA2 peptide (predicted mass 2,987.46 Da) which undergoes 7-fold dehydration (−144.08 Da), contains three Lan/MeLan residues (no mass change) and two additional residues of Ala replacing two Dha residues (+4 Da), and possesses a 2-oxobutyryl group at the N terminus (+1 Da) to give a peptide with a calculated mass of 2,848.45 Da. In addition, because of this close agreement between calculated and experimentally determined mass, other modifications are unlikely.

In contrast to LtnA1, careful examination of the peptide in HPLC-ES-MS experiments suggested that LtnA2 is isolated in a single state of dehydration. We did observe a small amount of material (about 15%) which elutes in a manner suggesting that it is more hydrophilic than the main peak. However, unlike LtnA1, this peak has a mass which is 16 Da greater than the main peak, suggesting oxidation of the molecule. Since LtnA2 does not contain Met, the next most likely explanation for this observation would be that there is some limited oxidation of one of the other thioether-containing residues (i.e. Lan or MeLan); the lantibiotic, actagardine contains such a MeLanO residue, the origin of which remains obscure (26).

While lanthione groups have previously been demonstrated in cytolysin (27) and staphylococcin C55 (28, 29), both of which are two-component antimicrobial peptides, this is the first report of serine to d-alanine conversion in such systems. Moreover, chiral phase GC analysis has demonstrated that d-alanine is present in both components of the bacteriocin. The presence of these residues, in addition to the formation of dehydrated residues and lanthionine rings, may account for the broad antimicrobial inhibitory spectrum associated with lacticin 3147. Such modifications are undoubtedly enzymatic and are forming a main focus of our future research into this system. This may uncover unique activities which could be harnessed as tools toward the development of novel biologically active peptides in the future.

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