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Published in:
Biochemistry

DOI:
10.1021/bi050081h

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Rink, R., Kuipers, A., de Boef, E., Leenhouts, K.J., Driessen, AJM., Moll, GN., Kuipers, OP., & Leenhouts, K. J. (2005). Lantibiotic structures as guidelines for the design of peptides that can be modified by lantibiotic enzymes. Biochemistry, 44(24), 8873-8882. https://doi.org/10.1021/bi050081h
Lantibiotic Structures as Guidelines for the Design of Peptides That Can Be Modified by Lantibiotic Enzymes

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Received January 14, 2005; Revised Manuscript Received April 21, 2005

ABSTRACT: Lantibiotics are (methyl)lanthionine-containing bacterial peptides. (Methyl)lanthionines are posttranslationally introduced into the prepropeptides by biosynthetic enzymes that dehydrate serines and threonines and couple these dehydrated residues to cysteine residues. Thirty seven lantibiotic primary structures have been proposed to date, but little is known about the substrate specificity of the lantibiotic modifying enzymes. To define rules for the rational design of modified peptides, we compared all known lantibiotic structures by in silico analysis. Although no strict sequence motifs can be defined that govern the modification, statistical analysis demonstrates that dehydratable serines and threonines are more often flanked by hydrophobic than by hydrophilic amino acids. Serine residues escape dehydration more often than threonines. With these rules, novel hexapeptides were designed that either were predicted to become modified or will escape modification. The hexapeptides were fused to the nisin leader and expressed in a Lactococcus lactis strain containing the nisin modifying and export enzymes. The excreted peptides were analyzed by mass spectrometry. All designed fusion peptides were produced, and the presence or absence of modifications was found to be in full agreement with the predictions based on the statistical analysis. These findings demonstrate the feasibility of the rational design of a wide range of novel peptides with dehydrated amino acid residues.

Peptides and proteins can be modified in various ways in order to improve their therapeutic use, for instance, by coupling to poly(ethylene glycol) or by glycosylation, amidation, and backbone cyclization. Local backbone cyclization limits the conformational freedom of a (poly)peptide, which allows modulation of its activity and specificity (1). In addition, the constrained cyclized structure protects (poly)peptides against proteolytic action (1–3). Stabilization of therapeutic peptides by cyclization may allow longer storage, lower doses, and less frequent administration to patients. The potential impact of cyclization is, for instance, illustrated by the fact that thioether-containing enkephalin showed no proteolytic degradation in rat brain homogenate, whereas Leu-enkephalin without the thioether ring is degraded for 50% in less than 7 min (4). Thioether enkephalin is in vivo extremely effective (5).

Therapeutic peptides of modest size have been chemically cyclized, which enhanced or modulated their activity (1, 5). Chemical methods, however, face several drawbacks, such as lack of stereo- and regiospecificity, multimerization instead of cyclization, and inefficient multistep synthesis involving the use of protecting groups. Chemical approaches are meeting limits especially when applied for the synthesis of larger peptides with multiple ring structures.

Enzymatic cyclization overcomes several of the challenges for chemical synthesis and occurs during the synthesis of, for instance, the cyclic antibiotics, tyrocidine and gramicidin S, and of the lipopeptapeptide surfactin A (6, 7). Isolated thioesterase domains of the nonribosomal peptide synthetases of the above antibiotics retain their activity. These domains cyclize the peptides head to tail or head to side chain.

Peptide backbone modifications, comprising head to tail cyclization but also thiazole and oxazole cyclizations, occur in posttranslationally modified microcins, a group of antimicrobial peptides produced by, and active against, Gram-negative bacteria (8). In these peptides the modification reactions are dependent on the leader peptide. Cyclization is also known for certain bacteriocins, e.g., circularin A (9) and enterocin AS-48 (10), produced by Gram-positive bacteria.

The present study concerns a special group of biologically, or in vitro (11), synthesized dehydro residue- and (methyl)-lanthionine-containing bacterial peptides. In these so-called lantibiotics dehydroalanine and dehydrobutyryne result from enzyme-mediated dehydration of serines and threonines. Enzyme-catalyzed coupling of cysteines to dehydro residues leads to thioether cyclizations. Instead of one head to tail cyclization found in nonribosomal peptides, one to five ring-like structures occur per lantibiotic (12–14). In naturally occurring lantibiotics, these ring-like structures comprise a thioether bond between neighboring amino acids or spaced up to 19 amino acids. The position of these rings depends on the positions of the dehydratable serines/threonines and

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cysteines in the peptide and on the cyclase-mediated region and stereospecific ring closure. Thioether ring formation in lantibiotics depends on the leader peptide (11). With respect to the homology of the SapB modifying enzyme with serine/threonine kinases (15), enzyme-mediated dehydration possibly occurs via phosphorylation of serines or threonines. A mechanism for enzymatic thioether ring formation has been proposed (16).

Lantibiotics are produced by and inhibit the growth of Gram-positive bacteria (12, 14, 17–19). Nisin is the best known and characterized lantibiotic (20, 21). It is widely applied as a food additive (22). Previously, lantibiotics were subdivided in elongated “type A” and globular “type B” peptides (23). In type A lantibiotics the thioether ring is formed between a serine or threonine with a more C-terminally located cysteine. In type B lantibiotics also the inverse orientation of the thioether ring occurs (14). Further subgroups have been distinguished: among others, a nisin subgroup, a lactacin 481 subgroup, a mersacidin subgroup, and a cinnamycin subgroup (14, 24). When a dehydrated amino acid occurs in position +1, spontaneous deamination takes place, resulting in an oxobutyryl residue. In epilancin K7 (Figure 1D) this residue is reduced, forming a hydroxypropionyl residue (15). When a dehydrated propionyl residue (15) in the Thr(15) of a type A lantibiotic is replaced by an amino acid occurs in position +1, spontaneous deamination takes place, resulting in an oxoacetic acid; Maldi TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

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Recently, we showed that the nisin—enzyme complex can be dissected into active subcomplexes and that nonlantibiotic peptides can also be modified and exported via the nisin enzymes NisB and NisT. This enabled systematic studies on the specificity of the lantibiotic enzymes. Despite the existing data on lantibiotic structures until now no rules have been defined that govern the posttranslational modifications. Such rules might be extracted from the various known lantibiotic structures to yield a predictive model that is disfavored in close proximity of the residues to be modified. There is a likelihood that certain residues are favored or disfavored in close proximity of the residues to be modified.

The purpose of this study was to extract information from existing lantibiotic structures to yield a predictive model that can be used to design novel modified peptides. This information was used to design a series of hexapeptides of which the dehydration by NisB was experimentally investigated.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids. Lactococcus lactis NZ9700 and NZ9000 (27) were used as hosts for expression of peptides. Plasmids encoding leader peptide fusions, pTP-

1 Abbreviations: NisB, the nisin dehydratase; NisT, the nisin transporter; NisC, the nisin cyclase; LanB, lantibiotic dehydratases; LanM, lantibiotic enzymes that dehydrate and cyclize; TFA, trifluoroacetic acid; Maldi TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
rylated downstream sense primer and an upstream antisense primer with a (nonannealing) peptide-encoding tail. The resulting linear peptide-encoding plasmid was self-ligated and transformed to _L. lactis_ NZ9000. DNA amplification was carried out using Expand polymerase (Invitrogen) or, in the case of large constructs, Phusion DNA polymerase (Finnzymes). Ligation was carried out with T4 DNA ligase (Roche). Electrosorption of _L. lactis_ was carried out as previously described (30) using a Bio-Rad gene pulser (Bio-Rad, Richmond, CA). Nucleotide sequence analysis was performed by BaseClear (Leiden, The Netherlands).

**Culturing.** _L. lactis_ was grown in M17 broth (31) supplemented with 0.5% glucose (GM17) or minimal medium with or without chloramphenicol (5 μg/mL) and/or erythromycin (5 μg/mL). The concentration of the antibiotics was reduced to 4 μg/mL when both were present simultaneously. Minimal medium was prepared by adding 20 mL of solution A [per 200 mL: 2.0 g of (NH₄)₂SO₄, 6.0 g of Na₂HPO₄, 3.0 g of KH₂PO₄, 1.0 g of NaCl] to 80 mL of solution B [per 800 mL: 10.0 g of casamino acids, 2.0 g of NaAc, 0.08 g of asparagine, 0.2 g of MgCl₂, 0.01 g of CaCl₂, 0.6 mg of FeCl₃(7H₂O), after which 2.5 mL of 20% glucose and 100 μL of vitamin mix (per 100 mL: 0.01 g of biotin, 0.1 g of folic acid, 0.1 g of riboflavin, 0.1 g of nicotinic acid, 0.1 g of pantotheic acid, 0.2 g of pyridoxal) were added]. Prior to mass spectrometry, cells were cultured and samples were prepared as follows. Overnight cultures of _L. lactis_ NZ9000 containing pIL2angBTC or pIL3BTC and a second, nisin leader fusion-peptide-encoding plasmid, grown in GM17 broth and 4 μg/mL antibiotics, were diluted 1/100. At an optical density at 600 nm of 0.4, cells were centrifuged and the medium was replaced by minimal medium supplemented with 1/1000 volume of filtered (0.45 μm) overnight _L. lactis_ NZ9700 culture medium containing nisin. Incubation was continued overnight. Prior to peptide analysis on gel, cells were cultured in minimal medium containing 0.12 M 3-(N-morpholino)propanesulfonic acid (MOPS), pH 7.

**Gel Electrophoresis.** Protein determinations were performed using the DC protein assay of Bio-Rad. Peptides were analyzed on tricine gels (32) and stained with Coomassie. Samples were prepared as follows: 25 mL of culture supernatant was filtered via 0.45 μm filters, freeze-dried, resuspended in 2.5 mL of distilled water, and passed on a PD10 column (Amersham), eluted with 3.5 mL of distilled water and freeze-dried, and the equivalent of 3.3 mL of culture supernatant was loaded on gel.

**Peptide Modifications.** Hydrogen additions to dehydro residues in peptides were obtained as follows. Dried samples were incubated overnight at room temperature with a 20 μg/mL NaBH₄ solution in 50 mM Tris-HCl, pH 7. After subsequent acidification to pH 2 with 10% TFA solution and purification by ziptip (Millipore), samples were dried, followed by ethanethiol treatment (33) and ziptip purification. The treatment was performed by using a mixture of 80 μL of ethanol, 65 μL of 5 M NaOH, and 60 μL of ethanethiol in 400 μL of milliQ water to yield ethanethiol addition to both rings and dehydro residues or by using a mixture of 80 μL of ethanol, 65 μL of 0.1 M NaOH, and 60 μL of ethanethiol in 400 μL of milliQ water to effect ethanethiol addition to only the dehydro residues. Trypsin treatment of peptide, obtained by TCA precipitation of 20 mL of supernatant, was performed in 10 μL of 0.1 M Tris-HCl, pH 8.0, by incubation for 10 min with 0.02 mg/mL trypsin.

**Mass Spectrometry.** Peptides were isolated from culture supernatants in a single step by applying the ziptip procedure (C18 ziptip; Millipore). Ziptips were wetted and equilibrated with 50% acetonitrile followed by demineralized water. Then peptides from the medium were bound by subjecting 100 μL or more to ziptip treatment, washed with 0.1% TFA, eluted with a solution of 0.1% TFA with 50% acetonitrile, vacuum-dried, and stored at −20°C until analysis. The dried ziptip eluent was resuspended in 5 μL of 50% acetonitrile containing 0.1% (v/v) TFA, and 1 μL was applied to the target. Subsequently, 1 μL of matrix [10 mg/mL α-cyano-4-hydroxycinnamic acid in 50% acetonitrile containing 0.1% (v/v) TFA] was added to the target and allowed to dry.

Mass spectra were recorded with a Bruker Biflex III MALDI-time-of-flight mass spectrometer. To maintain high sensitivity, an external calibration was applied. The calibration was verified internally by the mass of the fusion peptide, MSTKDFFNLVVSVSKKDGSAPRNRSYICP, which was coexpressed in and secreted by the NZ9000 strain containing the pIL2angBTC plasmid.

**In Silico Analyses.** Alignments were performed using the Align X program of vector NTI. When determining the abundance of modified or unmodified serines or threonines or the abundance of specific residues N- or C-terminally from serines or threonines, the following procedure was applied. When two or more lantibiotics had all of the thioether rings in an identical pattern, then overestimation of the occurrence of specific residues was avoided by applying a weight factor. In those cases identical unmodified residues, serines or threonines, in homologous positions were counted as one and identical flanking residues in homologous positions were counted as one.

**RESULTS**

**Flanking Amino Acids of Dehydrated Serines and Threonines.** We have compared lantibiotic structures with respect to the occurrence of flanking residues of dehydrated serine and threonine residues and of modified cysteine residues (Figure 1). Alignment of unmodified lantibiotic propeptides yielded three groups of which the structures with modifications are shown in Figure 1A–C. These groups differed in their thioether ring patterns. One group shared the two most N-terminal rings of epidermin (Figure 1A). A second group shared the most N-terminal ring of variacin (Figure 1B), while the third group shared all rings of ancoevenin (Figure 1C). The remaining group (Figure 1D) is composed of lantibiotics that, according to the alignment, have a different structure from the lantibiotics classified to the first three groups.

Serines and threonines in the leader peptides are never dehydrated, but this may relate to the substrate recognition mechanism that may involve binding of the leader peptide at a binding site remote from the active site. Remarkably, in the lantibiotics considered here, serines escaped dehydration nearly twice as often as threonines (Table 1). For instance, in nisin Ser29 remains unmodified and Ser33 sometimes escapes modification (62). To extract general rules about sequence motifs that favor dehydration of serine and threo-
A

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B

C

D

E

F

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R

S

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W

X

Y

Z
nine residues, we have analyzed the flanking amino acids of these residues. In general, there is no strict sequence motif that governs these modification reactions. Rather, there is a preference for certain amino acids as flanking residues of the modified serine and threonine residues. Hydrophobic, especially nonaromatic, amino acids occurred more often as flanking residues than hydrophilic, especially negatively charged, amino acids (Figure 2). As a control, flanking residues of serines and threonines in the enzymes LanB and LanC were found to be more often hydrophilic than hydrophobic. Taking into account that in some lantibiotics unmodified serines and threonines are sometimes flanked by a hydrophobic amino acid, no strict rule seems to exist. Rather it seems that in lantibiotic propeptides hydrophobic, nonaromatic, residues in the proximity of serines or threonines are favorable for dehydration.

Ring Formation. Flanking residues of modified cysteines were less hydrophobic than those of the converted serines and threonine residues. Hydrophobic, especially nonaromatic, amino acids occurred more often as flanking residues than hydrophilic, especially negatively charged, amino acids (Figure 2). As a control, flanking residues of serines and threonines in the enzymes LanB and LanC were found to be more often hydrophilic than hydrophobic. Taking into account that in some lantibiotics unmodified serines and threonines are sometimes flanked by a hydrophobic amino acid, no strict rule seems to exist. Rather it seems that in lantibiotic propeptides hydrophobic, nonaromatic, residues in the proximity of serines or threonines are favorable for dehydration.

Dehydration of Serines and Threonines in Model Peptides. The flanking residues of the eight dehydratable serines and threonines in prenisin vary, which indicates that the substrate specificity of NisB is not strict. Indeed, we recently demonstrated that also a nonlantibiotic peptide could be dehydrated via NisB (28). To experimentally validate the potential substrate preferences of LanB/LanM (Figure 2), we designed a series of model peptides. According to our analysis of lantibiotic structures, nonaromatic hydrophobic amino acids, such as alanine and valine, are abundantly found N- and C-terminally of dehydratable serines/threonines. Therefore, NisB-mediated dehydration of serine/threonine is expected to occur in the peptides A(S/T)VECK and A(S/T)VWCE when these are fused to the nisin leader. The cysteine in A(S/T)VECK is flanked by glutamate and lysine, amino acids that frequently occur as respectively N- and C-terminal flanking of cysteine in lantibiotics (Figure 2). On the other hand, the cysteine in A(S/T)VWCE is flanked by residues which rarely or not occur in these positions. In the peptide ITRICK, the threonine is flanked by an isoleucine, which frequently flanks dehydrated residues, and arginine, which less frequently flanks dehydrated residues. The peptide ITPGCK contains TPGCK, which is identical to unmodified residues 31–35 of prenisin. This sequence is expected to be fully modified because of its similarity with nisin’s ring B and the presence of the isoleucine that N-terminally flanks the threonine. As a control of the ITPGCK peptide, an ICPGTK peptide was fused to the nisin leader peptide. Hydrophilic and charged amino acids less frequently flank the dehydratable serines/threonines. Aspartate and arginine rarely occur respectively N- and C-terminally of the dehydrated residue. Therefore, no dehydration of the serine/
threonine is expected in the peptides D(S/T)RWCE, D(S/T)RECK, and DTRICK. Another peptide was accidentally obtained with an out of frame mutation, yielding the leader peptide fused with DSRWARVALIDSQKAAVDKAITDIAEKL. Because of their flanking amino acids, the serine residues in this peptide are not expected to be dehydrated, whereas the threonine might be dehydrated. The isoleucine at the N-side of the threonine may favor dehydration, whereas the asparatate at the C-side makes dehydration less likely.

L. lactis NZ9000 containing pIL3BTC and a fusion peptide-encoding plasmid produced all peptides in the culture medium (Figure 3). Differences between the production levels of the different peptides were measured; all levels were much higher than 1 mg/L (data not shown). The secreted peptides were analyzed by mass spectrometry (Table 2). They all contained the leader peptide which lacked the N-terminal methionine. In some cases additional N-terminal amino acids were missing as, for instance, MS, MST, MSTK, and MSTKD (Figure 4). Apparently, the leader peptide was subject to N-terminal proteolytic degradation. The masses of the observed designed peptides in which the threonine was surrounded by at least one hydrophobic amino acid were consistent with a single dehydration: leader-ATVECK, leader-ATVWCE, leader-ITRICK, and leader-ITPGCK. For leader-ASVECK and leader-ASVWCE next to the peaks corresponding to one time dehydration small peaks of unmodified peptide were observed. For leader-ICPGTK a heterogeneous product was obtained with either dehydrated threonine or no dehydration at all. In contrast, no dehydration was observed with leader-D(S/T)RWCE, leader-D(S/T)-RECK, and leader-DTRICK. Since dehydration of the threonine in ITRICK occurs but not in DTRICK, the lack of dehydration can be ascribed to the N-terminal presence of the aspartate. Cells containing pl.lang2BTC and a plasmid encoding leader-DSRAWVALIDSQKAAVDKAITDIAEKL produced both an unmodified and a dehydrated form of this longer peptide. Cells containing pIL3BTC and a plasmid encoding this longer peptide produced a single dehydrated peptide. Mass spectrometry of this peptide after trypsin cleavage showed that all fragments comprising the threonine position were dehydrated and could be modified by ethane-thiol, leading to a mass increase of 62 Da. After trypsin cleavage a mass peak of 1441 Da was observed, which corresponds to residues 39–51 with one dehydration. This peak shifted to 1503 Da after ethanethiol treatment, which confirmed the dehydration. These data indicated that Thr45 was dehydrated and that neither Ser25 nor Ser35 were dehydrated. This was confirmed by making three mutants: Ser25Gly, Ser35Gly, and Thr45Gly. In the case of NZ9000 cells containing pIL3BTC and a plasmid, encoding either the Ser25Gly or the Ser35Gly mutant, mass peaks of 5389

| position | I   | F | V | L | Dhb | M | Dha | A | G | I | P | T | S | C | E | Q | D | K | R | N |
|----------|-----|---|---|---|-----|---|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N-of S*T* | I | F | V | L | T | A | G | C | * | P | . | . | . | Q | K | N |
| C-of S*T* | I | F | V | L | Dhb | M | Dha | A | G | I | P | T | S | H | E | D | K | R | N |
| N-of C*  | I | V | L | T | T | A | G | C | * | P | T | S | H | E | Q | D | K | R | N |
| C-of C*  | I | F | V | L | Dhb | M | Dha | A | G | I | P | T | S | H | E | Q | D | K | R | N |

**Figure 2:** N- and C-terminal flankings of converted serines, threonines, and cysteines. Abbreviations: S*: dehydroalanine/o-alanine; T*, dehydrobutyryl/o-aminobutyric acid; C*, cysteine-derived part of (methyl)lanthionine/vinylcysteine. The size of the letters corresponds to the abundance in the indicated position.

**Figure 3:** Production of hexapeptides fused to the nisin leader. L. lactis containing pIL3BTC and a fusion peptide-encoding plasmid was cultured, and peptides were collected from the culture supernatant as described in the Experimental Procedures section. Peptides were composed of the nisin leader and the following (hexa)peptides: ATVECK (1), ASVECK (2), ATVWCE (3), ASVWCE (4), ITPGCK (5), ICPGTK (6), ITRICK (7), DTRICK (8), DTRECK (9), DSRECK (10), DSRWCE (11), DSRWARVALIDSQKAAVDKAITDIAEKL (13).
Masses correspond to the nisin leader peptide fused to dehydrated and pTP-ITPGCK was subjected to mass spectrometry analysis. A supernatant of the N-terminal leader peptide is present. All peptide dehydration findings were confirmed as previously for the angiotensin variant (28) by treatment with ethanethiol, which reacts with dehydro residues and gives a mass increase of 62 Da.

Intramolecular coupling of cysteine to a dehydro residue does not result in a change in mass. To discriminate between peptides with dehydro residues and peptides with thioether rings, peptides were subjected to chemical modifications, followed by mass spectrometry. In one approach, the treatment with borohydride caused hydrogen addition to dehydro residues while it does not result in a mass change in the case of a thioether ring. On the other hand, ethanethiol is unable to react with dehydro residues that have undergone hydrogen addition, whereas, at high NaOH concentration, ethanethiol addition to peptide with the thioether ring still takes place. In a second approach, peptides were treated with ethanethiol at low NaOH concentration. Under these mild reaction conditions, ethanethiol only reacts with occurring dehydro residues. With this method, the presence of three dehydro residues in nisin could be measured (data not shown). Both methods indicated the presence of thioether rings in the peptides ATVECK and ITPGCK but did not yield conclusive results for other peptides (data not shown). These results therefore indicate that a novel thioether ring, comprising the combination of a valine and a glutamate, has been generated in a model peptide.

### DISCUSSION

Thioether rings are essential for lantibiotic activity (68, 69) and stability (1–3). Dehydro residues (70) and thiols (69) have been engineered in nisin, while in Pep5 dehydro residues and one new thioether ring have been introduced (2). Recently, we have demonstrated that the substrate specificity of the nisin dehydrating and transport enzymes is not restricted to nisin alone but that the system can also handle nonlantibiotic peptides. This indicates that lantibiotic enzymes can be used for the synthesis of a wide range of peptides with new bioactivities and of biostable analogues.

Here, we compared lantibiotic structures with the purpose of deducing information on the substrate specificity of the lantibiotic-modifying enzymes. This information can be used for designing novel peptides. We hypothesized that the amino acids that directly flank serines and threonines will affect the dehydratase activity. Comparison of the structures suggests that hydrophobic residues, mainly nonaromatic ones, around the dehydratable amino acid may favor dehydration. On the other hand, when polar and, in particular, negatively charged amino acids are present, no dehydration is observed. Therefore, the in silico data suggest that the vicinity of hydrophobic amino acids next to serines or threonines would be one of the circumstances that might promote dehydratase activity.

It should be taken into account that the apparent in silico data may not only reflect the substrate specificity of the dehydratase enzyme but also include lantibiotic functioning. For instance, the presence of negatively charged amino acids in lantibiotics interferes with the interaction of the cationic lantibiotic with the anionic target membrane (71, 72). Furthermore, the absence of (full) dehydration might be due to inefficient dehydration rather than an inability of the enzymes to dehydrate this position. In the lantibiotics depicted in Figure 1A,B,D, most thioether ring structures

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<th>theoretical av mass without Met1 [M + H]+ (Da)</th>
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* All serines and threonines are indicated in bold type.
involve two to four additional amino acids within the ring-containing (methyl)lanthionines. This may imply that for some lantibiotic cyclases larger ring structures might be less easily formed. It cannot be excluded that coevolution of the enzymes and their substrates has led to the exclusion of dehydration reactions at positions that are too distant from the cysteines for cyclization. Such positions may also not contribute to lantibiotic functioning.

The theoretical analysis of the lantibiotic structures was followed by an experimental approach in which the NisB-mediated dehydration of designed peptides was tested. These peptides were designed such that they represented extreme cases with respect to the likelihood of modification as suggested by the in silico analyses. Studies on the substrate specificity of lantibiotic enzymes have been hampered for a long time because of the lack of production or detection of produced peptides. In this study we demonstrate that a series of rationally designed peptides is produced, exported, and modified. Most interestingly, all peptide modifications were found to conform to our theoretical prediction, showing that the dehydration reaction at least follows some guidelines that can be applied in successful production of modified peptides.

Several, but not strictly all, previously reported lantibiotic mutants (21, 73, 74) are in agreement with our predictions. Interestingly, V32E and V32K nisin Z (71) share the presence of an unmodified serine at position 33. Since hydrophobic residues are not preferred around dehydratable serines and threonines, these mutants are indeed predicted not to be dehydrated. In addition, in lantibiotics, glutamate residues are rarely (subtilin and ericin-S) observed N-terminally of dehydratable serine residues. Nisin has a lysine at position 34 following the serine at position 33. With the V32E and V32K mutants, serine 33 is surrounded by charged residues, which is unfavorable for dehydration.

Previously, the export of a nonlantibiotic peptide NRSY-ICP was observed concomitantly with partial dehydration (28). This can be most likely attributed to the presence of Arg N-terminally of Ser. In the designed peptides described here, hydrophobic flankings of dehydratable residues such as alanine and valine allowed full dehydration. Also, the positive control, peptide ITPGCK, which is related to ring B of nisin, was fully dehydrated. In sharp contrast, no dehydration of serines/threonines was observed in five designed peptides when these serines/threonines were N-terminally preceded by an aspartate and C-terminally flanked by an arginine. These data again correspond with the in silico analysis and strongly indicate that known lantibiotic structures can be used to deduce guidelines for the designing of posttranslationally modified peptides. Therefore, peptide design and experimental analyses constitute a valuable approach to study remaining questions about the dehydration and thioether bridge formation by lantibiotic enzymes.

Summarizing, this study for the first time demonstrates that in nonlantibiotic peptides the extent of dehydration can be controlled by the amino acid context of the flanking region of the dehydratable serine and threonine residues. The here used system allows the production of considerable amounts of modified peptides. We are currently investigating whether the flanking residues of cysteines influence NisC-mediated ring formation. Such studies will further contribute to the rational design and enzymatic synthesis of a wide range of new biostable thioether ring-containing peptides.

ACKNOWLEDGMENT

Leon Kluskens and Karin Scholtmeijer are gratefully acknowledged for helpful discussions.

REFERENCES

Design of Lantibiotic Enzyme-Modifiable Peptides


