

University of Groningen

The structure of the lantibiotic lacticin 481 produced by *Lactococcus lactis*

Hooven, Henno W. van den; Lagerwerf, Fija M.; Heerma, Wigger; Haverkamp, Johan; Piard, Jean-Christophe; Hilbers, Cornelis W.; Siezen, Roland J.; Kuipers, Oscar P.; Rollema, Harry S.

Published in:
 FEBS Letters

DOI:
[10.1016/0014-5793\(96\)00771-5](https://doi.org/10.1016/0014-5793(96)00771-5)

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:
 1996

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Hooven, H. W. V. D., Lagerwerf, F. M., Heerma, W., Haverkamp, J., Piard, J.-C., Hilbers, C. W., Siezen, R. J., Kuipers, O. P., & Rollema, H. S. (1996). The structure of the lantibiotic lacticin 481 produced by *Lactococcus lactis*: location of the thioether bridges. *FEBS Letters*, 391(3). [https://doi.org/10.1016/0014-5793\(96\)00771-5](https://doi.org/10.1016/0014-5793(96)00771-5)

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

The structure of the lantibiotic lacticin 481 produced by *Lactococcus lactis*: location of the thioether bridges

Henno W. van den Hooven^{a,*}, Fija M. Lagerwerf^b, Wigger Heerma^b, Johan Haverkamp^b, Jean-Christophe Piard^{a,**}, Cornelis W. Hilbers^c, Roland J. Siezen^a, Oscar P. Kuipers^a, Harry S. Rollema^a

^aDepartment of Biophysical Chemistry, NIZO (Netherlands Institute for Dairy Research), Kernhemseweg 2, 6718 ZB Ede, The Netherlands

^bBijvoet Center for Biomolecular Research, Mass Spectrometry Group, Utrecht University, 3508 TB Utrecht, The Netherlands

^cNSR Center for Molecular Structure, Design and Synthesis, Laboratory of Biophysical Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

Received 10 June 1996

Abstract The lantibiotic lacticin 481 is a bacteriocin produced by *Lactococcus lactis* ssp. *lactis*. This polypeptide contains 27 amino acids, including the unusual residues dehydrobutyrine and the thioether-bridging lanthionine and 3-methylanthionine. Lacticin 481 belongs to a structurally distinct group of lantibiotics, which also include streptococcin A-FF22, salivaricin A and variacin. Here we report the first complete structure of this type of lantibiotic. The exact location of the thioether bridges in lacticin 481 was determined by a combination of peptide chemistry, mass spectrometry and NMR spectroscopy, showing connections between residues 9 and 14, 11 and 25, and 18 and 26.

Key words: Bacteriocin; Lanthionine-containing polypeptide; Post-translational modification; Cyanogen bromide cleavage; NMR; Mass spectrometry

1. Introduction

Bacteria produce various antagonistic compounds, such as acids, peroxides and bacteriocins, against competing microflora. Of these substances, bacteriocins constitute a large family of polypeptides, which can be subdivided into different classes based on their mode of action and on their structure [1]. One of these classes is formed by the lantibiotics. Of special interest are the lantibiotics produced by lactic acid bacteria, because they can directly be used in the food industry. Examples are nisin and lacticin 481, which are both produced by *Lactococcus lactis* ssp. *lactis*. Nisin has been known since 1928 [2], whereas lacticin 481 has been isolated only recently as a result of screening for bacteriocin production [3,4]. Lacticin 481 exhibits bactericidal activity against a

wide range of Gram-positive bacteria [3,4]. In this respect, its potential use as an agent to prevent late blowing of cheese due to *Clostridium tyrobutyricum* has been proposed [4]. The structural gene of lacticin 481 has been sequenced together with two adjacent genes, believed to be involved in lacticin 481 biosynthesis [5–7]. Lacticin 481 is membrane-active as judged from peptide-induced surface-pressure changes of lipid monolayer films [8].

Lacticin 481 contains the unusual α,β -unsaturated amino acid dehydrobutyrine and the uncommon thioether-bridging residues lanthionine and 3-methylanthionine. Dehydrobutyrine is formed by dehydration of a threonine residue. Lanthionine and 3-methylanthionine are formed by dehydration of a serine and a threonine to form dehydroalanine and dehydrobutyrine, respectively, followed by addition of the thiol group of a cysteine to the α,β -unsaturated residue [9]. Biosynthesis of lantibiotics includes ribosomal synthesis, post-translational modification reactions, transport across the cell membrane and cleavage of the leader peptide. Because of the post-translational modifications, the covalent structure of lacticin 481 cannot be deduced from the gene sequence in a straightforward way. Therefore, to characterize its structure, the location and structure of the unusual residues, the amino-acid sequence and the positions of the thioether bridges of the (3-methyl)lanthionines need to be determined. A number of different techniques have been used to elucidate the structure of lantibiotics. In 1970 the covalent structure of the lantibiotic nisin was determined by chemical and enzymatic methods [10]. Recently, NMR has been brought to bear upon the problem, to arrive at the structure of the lantibiotic epilancin K7 [11,12]. The amino-acid-sequence determination of lantibiotics by Edman degradation is hampered by the presence of unusual residues, but this has been overcome recently by chemical derivatizations prior to Edman degradation [13]. The combination of NMR, MS and this new strategy for direct sequence analysis proved successful for the determination of the structure of the lantibiotic actagardine [14]. For lacticin 481 the amino-acid sequence, including the positions of the post-translationally modified residues, has been determined by amino-acid analysis, NMR and sequencing of the structural gene [5]. In the same study it was also demonstrated that the 3-methylanthionine is formed by residues 9 and 14 (3-methylanthionine^{9,14}). The serine-derived moieties of the two lanthionines occur in positions 11 and 18 and are covalently linked to the cysteine-derived moieties in positions 25 and 26, yielding two possible structures [5] (Fig. 1), both with

*Corresponding author (or O.P. Kuipers). Fax: (31) (318) 650400.

**Present address: INRA, Laboratoire de Génétique Microbienne, 78352 Jouy-en-Josas Cedex, France.

Abbreviations: Ala ζ , 3-methylalanyl moiety of (2S,3S,6R)-3-methyl-lanthionine; Ala δ , D-alanyl moiety of meso-lanthionine; sAla, L-alanyl moiety of meso-lanthionine or of (2S,3S,6R)-3-methylanthionine; 1/2/3D, one-/two-/three-dimensional; Dhb, dehydrobutyrine; DMSO, dimethylsulfoxide; DPC, dodecylphosphocholine; FAB, fast-atom bombardment; Hsl, homoserine lactone; MS, mass spectrometry; NOE, nuclear Overhauser enhancement; NOESY, NOE spectroscopy; SA-FF22, streptococcin A-FF22; TOCSY, total correlated spectroscopy

overlapping ring systems. The bridging pattern of the two lanthionines could not be determined via the NMR approach which was successful for the 3-methylanthionine^{9,14}, because of severe line broadening of resonances of the lanthionine-forming residues.

Here we present the completion of the covalent structure of lactacin 481 by the determination of the location of the thioether bridges formed by the (3-methyl)lanthionines as determined by a combination of peptide chemistry, MS and NMR.

2. Materials and methods

2.1. Cleavage with cyanogen bromide

Lactacin 481 was obtained according to the previously described protocol [15,5]. A solution of 1.9 mg of lactacin 481 in 400 μ l of 70% trifluoroacetic acid (in % by volume) was incubated with 18 mg of cyanogen bromide (CNBr, Sigma) for 17 h in the dark at room temperature.

2.2. Mass spectrometry

Positive-ion FAB mass spectra were recorded on a Jeol JMS-SX/SX102A four-sector instrument of B₁E₁-B₂E₂ geometry operating at an accelerating voltage of 6 kV. Xenon was used as FAB gas; the FAB gun was operated at 6 kV and a 10 mA emission current. The magnet was scanned from *m/z* 10 to 4000 in 30 s and resolution was 1000. Samples were dissolved in methanol/water (1:1, v/v) and loaded into a glycerol matrix. MS/MS spectra were acquired by selecting the desired precursor ion with MS-1 and colliding the ion in the collision cell located in the third field free region. The collision gas (nitrogen) was introduced in the cell so that the intensity of the main beam from MS-1 was reduced to about 50%. The resulting fragment ions were monitored by scanning MS-2.

2.3. NMR spectroscopy

The influence of acetonitrile-d₃ (Wilmad), of dimethylsulfoxide-d₆ (DMSO, Aldrich), and of dodecylphosphocholine-d₃₈ (DPC, Campro Scientific) on the 1D-NMR spectrum of lactacin 481 was examined at 400 MHz on a Bruker AM400 spectrometer, interfaced to an Aspect3000 computer. 1D-NMR spectra were recorded at 5, 25 and 40°C.

1D-NMR spectra, TOCSY spectra [16,17] with a mixing time of 50 ms and NOESY spectra [18,19] with a mixing time of 450 ms were recorded for a sample containing 1.9 mg of the CNBr-treated peptide of lactacin 481 in 0.5 ml H₂O²/H₂O (9:1) pH 3.5 and in 0.5 ml ²H₂O pH 3.5 (pH meter reading) at 400 MHz. Additionally, a NOESY spectrum with a mixing time of 450 ms was recorded for the same polypeptide in 0.5 ml H₂O²/H₂O (9:1) pH 3.5 at 600 MHz on a Bruker AMX2 600 spectrometer, interfaced to an ASPECT station. 2D-NMR experiments were performed at 5°C. The 2D-NMR data were processed using the MNMR program (PRONTO Software Development and Distribution, Copenhagen, Denmark) running on a Silicon Graphics Indigo workstation. Acquisition and processing parameters were essentially the same as those described previously [20,21]. The spectra were referenced to sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS).

3. Results and discussion

The (3-methyl)lanthionine bridging pattern can be established by NMR, provided that the resonances of the (3-methyl)lanthionine moieties and NOEs or heteronuclear multiple-bond correlation (HMBC) cross-peaks over the thioether bridges can be identified. This method was successful for the antibiotics epilancin K7 [11] and actagardine [14]. For lactacin 481 in aqueous solution the NMR approach yielded only the location of the 3-methylanthionine involving residues 9 and 14 [5]. Because of line broadening of some of the resonances (e.g. near 9 ppm, see Fig. 2A), caused by chemical exchange,

the location of the two lanthionine bridges remained unclear. In an attempt to influence this chemical exchange, and thus the NMR line-width, the polypeptide was placed in different environments. It was dissolved in a mixture of acetonitrile and water, and in DMSO (data not shown), and the (supposedly) membrane-interacting molecule was complexed to membrane-mimicking micelles of DPC. In all these systems the NMR line-width remained too large to detect ring-establishing contacts (Fig. 2). The most likely explanation for the observed line broadening is an exchange between at least two different conformations adopted by the molecule in the environments studied. This line broadening severely hampers the determination of the 3D structure of lactacin 481 by high-resolution NMR under the conditions studied.

The key to the determination of the bridging pattern in lactacin 481 was found to be a chemical modification with CNBr. This reagent induces cleavage of the peptide chain on the C-terminal side of a methionine residue, the methionine being converted to a homoserine lactone [22]. Lactacin 481 contains a single methionine residue at position 16. The FAB-MS spectra of untreated lactacin 481 and of its CNBr reaction product are shown in Fig. 3. The protonated mole-

Table 1
Proton resonance assignments of the CNBr-treated lactacin 481 in aqueous solution at 5°C and pH 3.5

Residue	NH	α H	β H	Others
Lys-1	–	4.07	1.93, 1.93	γ CH ₂ 1.48, 1.48, δ CH ₂ 1.71, 1.71 ϵ CH ₂ 3.00, 3.00, ϵ NH ₃ ⁺ 7.61
Gly-2	8.91	4.05, 4.05		
Gly-3	8.55	4.04, 4.04		
Ser-4	8.49	4.48	3.88, 3.88	
Gly-5	8.67	3.95, 3.95		
Val-6	8.05	4.02	1.95	γ CH ₃ 0.87, 0.78
Ile-7	8.36	4.11	1.70	γ CH ₂ 1.41, 1.10, γ CH ₃ 0.80, δ CH ₃ 0.80
His-8	8.74	4.97	3.25, 3.14	2H 8.63, 4H 7.31
Ala ^s -9	8.37	4.61	3.48	γ CH ₃ 1.09
Ile-10	7.94	4.35	1.86	γ CH ₂ 1.38, 1.08, γ CH ₃ 0.88, δ CH ₃ 0.79
Ala ^s -11	8.96	4.45	2.97, 2.97	
His-12	9.15	4.75	3.40, 3.18	2H 8.63, 4H 7.29
Glu-13	8.81	4.15	2.18, 2.08	γ CH ₂ 2.36, 2.36
sAla-14	7.88	4.57	3.12, 2.78	
Asn-15	8.27	4.72	2.79, 2.74	γ NH ₂ 7.67, 6.98
Hsl-16 ^a	8.62	4.64	2.57, 2.32	γ CH ₂ 4.52, 4.36
Asn-17	–	4.25	2.92, 2.92	γ NH ₂ 7.70, 6.99
Ala ^s -18	8.76	4.30	2.68, 2.51	
Trp-19	8.34	4.68	3.32, 3.25	2H 7.21, 4H 7.63, 5H 7.15, 6H 7.22, 7H 7.48, NH 10.20
Gln-20	7.65	4.43	1.77, 1.70	γ CH ₂ 1.95, 1.95, δ NH ₂ 7.37, 6.87
Phe-21	8.73	4.80	2.76, 2.46	2H,6H 6.87, 3H,4H,5H 7.25
Val-22	8.61	4.06	1.91	γ CH ₃ 0.79, 0.79
Phe-23	9.38	4.47	3.38, 3.08	2H,6H 7.24, 3H,5H 7.35, 4H 7.28
Dhb-24	9.46		6.95	γ CH ₃ 1.66
sAla-25	8.10	4.86	3.11, 2.90	
sAla-26	9.21	3.86	2.92, 2.69	
Ser-27	8.42	4.44	3.73, 3.68	

^aHomoserine lactone.

cule ($M+H^+$) of lactacin 481 at m/z 2902.5 disappeared after treatment with CNBr, while an ion corresponding to the CNBr-derived product (m/z 2872.3) appeared in the spectrum.

Besides providing information about the molecular mass, FAB-MS spectra of peptides often contain fragment ions that can be used for the elucidation of the peptide structure [23,24]. The FAB-MS spectra both of lactacin 481 and of CNBr-treated lactacin 481 contained some additional fragment ions corresponding to N-terminal sequence ions (data not shown). However, all ions originated from the linear part of the peptide (Lys-1 to His-8), and fragmentation was not observed in the peptide part containing the thioether bridges. This is in accordance with earlier reported FAB-MS results for other lantibiotics [25,26]. In the spectrum of the CNBr-treated lactacin 481 (Fig. 3B), additional peaks were observed in the mass region between 1200 and 1650. These peaks correspond to the $M+H^+$ ions of the N-terminal peptide (residues 1 to 16, m/z 1587.9 and 1620.1) and the C-terminal peptide (residues 17 to 27, m/z 1253.7 and 1285.7), both with either a Cys or an Ala residue at the position of the original thioether bridge. These peptides are formed in a reduction process of the thioether bridge that connects the N-terminal and the C-terminal peptide. Since either peptide can incorporate the sulfur to form a Cys residue, four different compounds are formed and, consequently, four $M+H^+$ peaks are observed (Fig. 3). The reduction process probably occurred on the FAB probe, since no indication was found in the NMR spectra for the presence of these peptides.

The ion at m/z 1253.7 (Fig. 3) was subjected to MS/MS analysis (data not shown). The mass spectrum showed very limited fragmentation. The most abundant fragment ion (next to the elimination of NH_3 and H_2O) was the N-terminal B_{10} ion (Roepstorff notation, [27]), which results from cleavage of the amide bond between the residues ${}_5Ala$ -26 and Ser-27. The subsequent B_9 ion (elimination of residue 26) was not observed, corroborating the presence of a lanthionine bridge between the residues Ala_5 -18 and ${}_5Ala$ -26 (vide infra). Other ions in the spectrum could be assigned to the loss of CO, which is often observed for cyclic peptides [28], and the loss of a Phe and subsequently a Val residue.

The CNBr peptide of lactacin 481 was studied by NMR without further purification (Figs. 2D and 4). For this modified peptide the line-widths of all its resonances are sufficiently sharp (Fig. 2D) to allow a detailed high-resolution NMR analysis. Patterns of all amino acid residues were identified in TOCSY spectra. The presence of the unusual residues

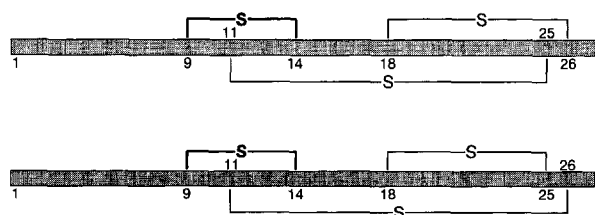


Fig. 1. Schematic representation of the two possible structures for lactacin 481 based on previous work [5]. The known amino-acid sequence is boxed; the thioether bridges are represented by their sulfur atoms and the amino acid residues involved by their number in the sequence. The 3-methylanthionine formed by residues 9 and 14 is, in contrast to the lanthionines, indicated in bold.

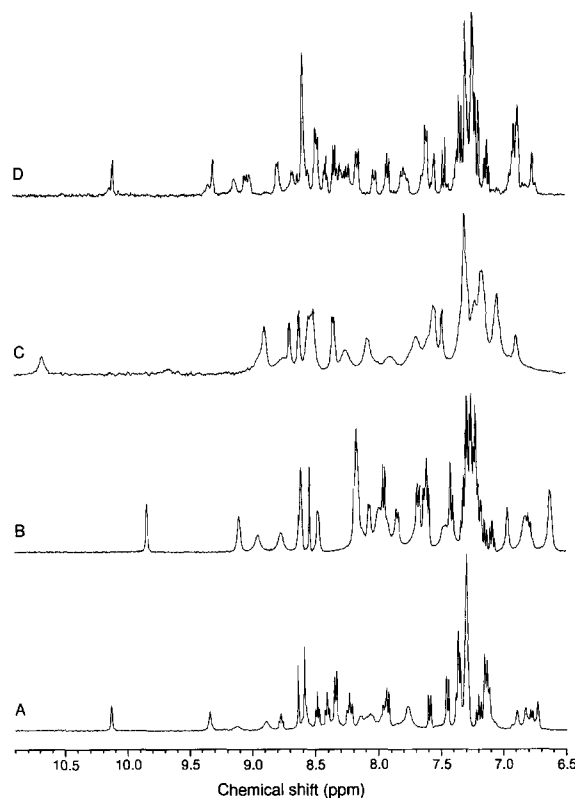


Fig. 2. (A-C) Amide, aromatic and vinyl proton region of the 1H -NMR spectrum of lactacin 481 at room temperature: (A) in aqueous solution at pH 3.5, (B) in 18% water/0.14% trifluoroacetic acid in acetonitrile- d_3 , (C) complexed to DPC- d_{38} micelles (lactacin 481:DPC=1:57) at pH 3.5. (D) 1H -NMR spectrum of the CNBr-treated lactacin 481 in aqueous solution at room temperature and pH 3.5.

was established by observation of their characteristic signals in the NMR spectrum (e.g. [29,11]). Also a homoserine lactone as product of the CNBr treatment was identified. In the NOESY spectra most of the amino acid residues showed connectivities only to a single other residue. Since sequential contacts between neighbouring amino acid residues involve distances which are observable in NOESY spectra, a sequential assignment could be carried out, encompassing two peptide chains involving the residues 1-to-16 and 17-to-27. No NOEs were observed between the residues 16 and 17. The scarcity of long-range NOEs suggests that the CNBr peptide of lactacin 481 does not adopt a single unique conformation, but is al-

Table 2
NOEs used for the determination of the location of the thioether bridges

Ala_5 -9- ${}_5Ala$ -14	Ala_5 -11- ${}_5Ala$ -25	Ala_5 -18- ${}_5Ala$ -26
αH - βH^a	NH- βH	αH - αH
αH - βH	αH -NH	αH - βH
βH - αH	αH - $\beta' H$	$\beta' H$ - αH
βH - βH	$\beta H/\beta' H^b$ -NH	$\beta' H$ - βH
βH - $\beta' H$	$\beta H/\beta' H^b$ - αH	
γCH_3 - αH	$\beta H/\beta' H^b$ - βH	
γCH_3 - βH		
γCH_3 - $\beta' H$		

^aThe low-field βH resonance of Ala_5 and ${}_5Ala$ (residues 11, 14, 18, 25 and 26) is arbitrarily named βH and the high-field one $\beta' H$ because these resonances are not assigned stereospecifically.

^bThe βH resonances of Ala_5 -11 overlap.

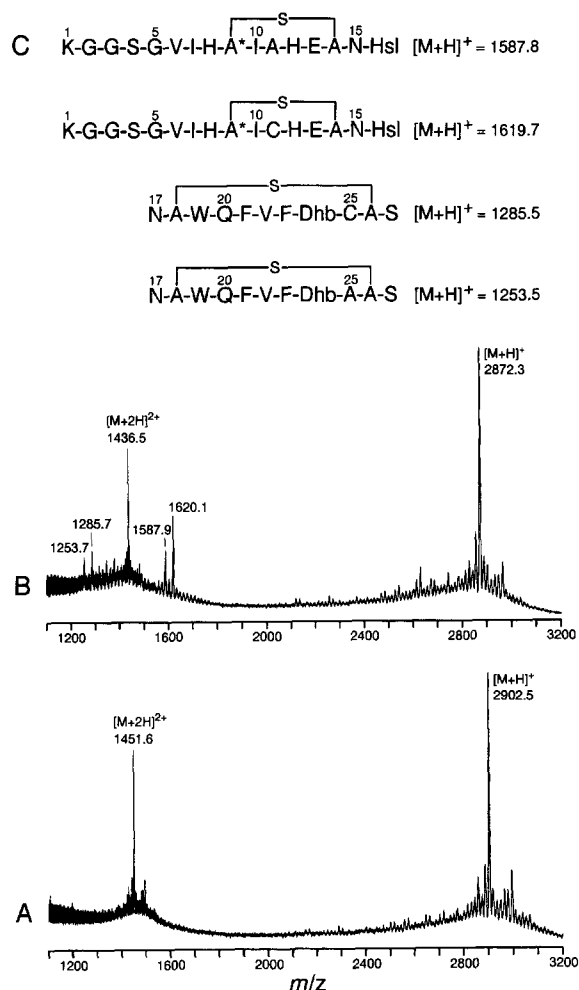


Fig. 3. Part of FAB-MS spectra of (A) lacticin 481 and (B) its CNBr peptide. In the latter spectrum additional peaks were observed, as indicated. (C) Peptides corresponding to the additional peaks in the mass spectrum of the CNBr-treated lacticin 481, together with the calculated masses of the protonated molecules (Hsl, homoserine lactone; Dhb, dehydrobutyrine). The C-terminal peptides are given with the correct thioether bridging, as determined in this study.

most unstructured. However, a number of NOEs were observed for the side chain of Phe-21, the aromatic ring of which points to the lanthionine of the cyclic structure in which Phe-21 is located. Thus, the amino-acid sequence together with the CNBr-induced modifications could be confirmed and the resonances could be completely assigned (Table 1). The majority of the observed NOEs were intra-residue or sequential. Almost all of the remaining NOEs were observed between the (3-methyl)lanthionine-forming residues 9 and 14, 11 and 25, and 18 and 26 (Table 2 and Fig. 4). The distances between β H protons in different halves of a (3-methyl)lanthionine (CH-S-CH) are always between 2.0 and 4.5 Å; thus, these contacts should be observable in NOESY spectra. Every residue involved in (3-methyl)lanthionine bridging shows only NOE contacts to a single other (3-methyl)lanthionine-forming residue. These NOEs necessarily reflect the bridging pattern of lacticin 481. The previously established location of the 3-methylanthionine between residues 9 and 14 is now confirmed. In addition, the NOEs between the two residues forming a (3-methyl)lanthionine in lacticin 481 are comparable to

those observed between the cross-linked (3-methyl)lanthionine-forming residues of nisin. These observations lead to the complete structure of lacticin 481 as [3-methylanthionine^{9,14},lanthionine^{11,25},lanthionine^{18,26}]lacticin 481 (Fig. 5).

Now that the bridging pattern is known, the line broadening in the NMR spectrum of intact lacticin 481 can be reconsidered. By modelling two low-energy structures can be built with the rings formed by the residues 9-to-14 and 18-to-26 folded with the one above the other and vice versa (H.S. Rollema, unpublished results). An exchange between these two proposed conformations on the ms time scale would explain the observed line broadening in the NMR spectrum.

Currently about 25 lantibiotics are known, which are subdivided into two types, A and B [30]. The type-A lantibiotics, like nisin, subtilin, epidermin, Pep5 and epilancin K7, are elongated cationic polypeptides, which exert their bactericidal function primarily via membrane perturbation. Type-B lantibiotics, like the duramycins, actagardine and mersacidin, are globular, have a low net charge, and are enzyme inhibitors. Lacticin 481 has been classified as type A [5]. Both the lantibiotic and the leader sequence of lacticin 481 show significant homology to the corresponding sequences of the lantibiotics streptococcin A-FF22 (SA-FF22) [31,32], salivaricin A [33] and variacin [34] (see Fig. 6), but not to those of the other

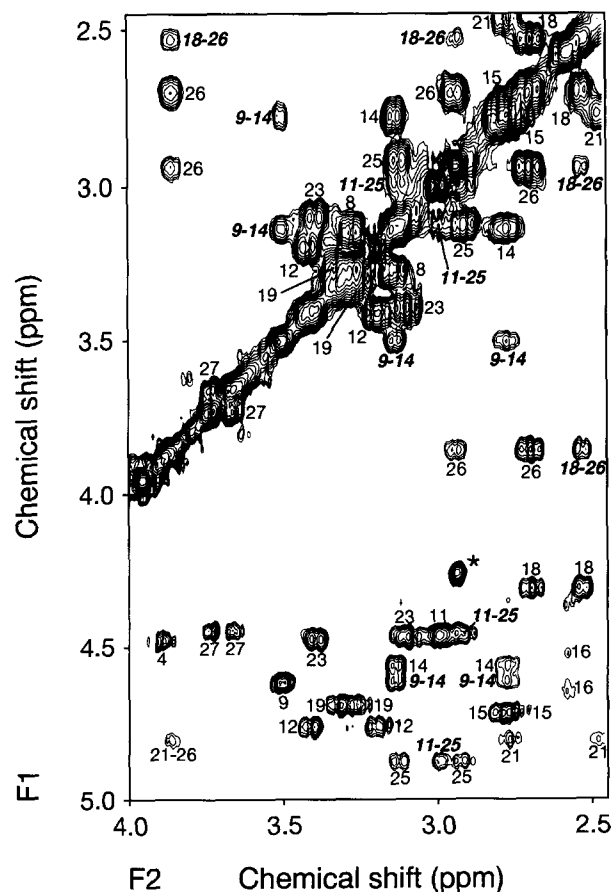


Fig. 4. Inter-residue NOEs establishing the thioether bridges between residues 9 and 14, 11 and 25, and 18 and 26 in a part of a NOESY spectrum of the CNBr peptide of lacticin 481 in aqueous solution (²H₂O) at 5°C and pH 3.5. The numbers in the NOESY spectrum refer to residue positions in the sequence of the polypeptide. The NOEs over the thioether bridges are indicated in bold italics. A cross-peak of an impurity is indicated by an asterisk.

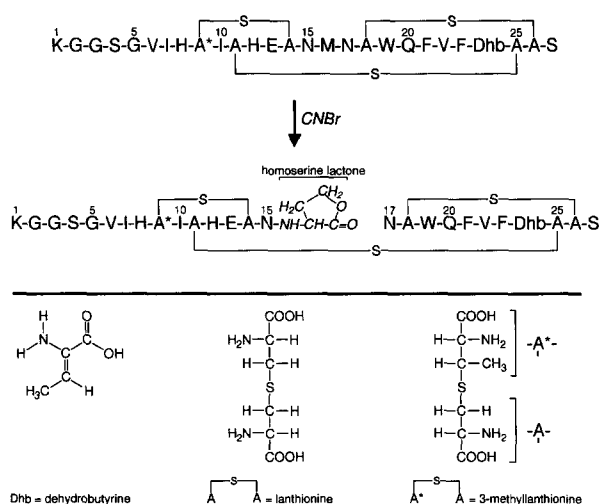


Fig. 5. Representation of the covalent structure of lacticin 481, its CNBr peptide, and structures of the unusual residues.

type-A lantibiotics [5,30]. Thus, it appears that these four polypeptides form a new group of type-A lantibiotics. For this group of lantibiotics mode of action studies have only been performed for SA-FF22: this polypeptide acts by the voltage-dependent formation of pores in bacterial membranes, similar to that displayed by several other type-A lantibiotics [35]. The overlapping ring systems make lacticin 481 rather compact, in contrast to the type-A lantibiotics not belonging to the lacticin-481 group, which are elongated and have a membrane-spanning length.

The lantibiotics lacticin 481, SA-FF22, salivaricin A and variacin are produced by microorganisms from different genera, i.e. *L. lactis*, *Streptococcus pyogenes*, *S. salivarius* and *Micrococcus varians*, respectively. However, in addition to sequence homology these four lantibiotics also show an identical position of potential (3-methyl)lanthionine-forming residues. Of these four lantibiotics, the thioether-bridging pattern has only been elucidated completely for lacticin 481. It is tempting to speculate that the bridging patterns of SA-FF22, salivaricin A and variacin are similar to that determined for lacticin 481.

This work indicates that the combination of NMR and MS is highly suitable for detailed structure elucidation of lantibiotics, including the determination of the location of (3-methyl)lanthionine bridges. For a large number of lantibiotics (e.g. nisin, actagardine and epilancin K7) NMR can be applied

directly, while for the lacticin-481 type lantibiotics chemical modification is necessary prior to NMR analysis. A similar CNBr reaction as used for lacticin 481 can be applied for variacin, while SA-FF22 can be cleaved within the overlapping ring systems as shown before [32].

Lacticin 481 shows a new type of bridging pattern not observed for the other lantibiotics (e.g. [30]), demonstrating the large structural variety of lantibiotics. The elucidation of the complete covalent structure of lacticin 481 is of importance for the clarification of its structure/function relationship and for a rational design of mutants.

Acknowledgements: The authors wish to thank Charles Slangen and Dr. Ser Visser, NIZO (Netherlands Institute for Dairy Research), and Prof. G.I. Tesser, University of Nijmegen, for a variety of helpful suggestions and discussions.

References

- [1] Klaenhammer, T.R. (1993) FEMS Microbiol. Rev. 12, 39-86.
- [2] Rogers, L.A. and Whittier, E.O. (1928) J. Bacteriol. 16, 211-229.
- [3] Piard, J.-C., Delorme, F., Giraffa, G., Commissaire, J. and Desmazeaud, M. (1990) Neth. Milk Dairy J. 44, 143-158.
- [4] Thuault, D., Beliard, E., Le Guern, J. and Bourgeois, C.M. (1991) J. Dairy Sci. 74, 1145-1150.
- [5] Piard, J.-C., Kuipers, O.P., Rollema, H.S., Desmazeaud, M.J. and De Vos, W.M. (1993) J. Biol. Chem. 268, 16361-16368.
- [6] Rince, A., Dufour, A., Le Pogam, S., Thuault, D., Bourgeois, C.M. and Le Pennec, J.P. (1994) Appl. Environ. Microbiol. 60, 1652-1657.
- [7] Siezen, R.J., Kuipers, O.P. and De Vos, W.M. (1996) Antonie van Leeuwenhoek 69, 171-184.
- [8] Demel, R.A., Peelen, T., Siezen, R.J., De Kruijff, B. and Kuipers, O.P. (1996) Eur. J. Biochem. 235, 267-274.
- [9] Ingram, L. (1970) Biochim. Biophys. Acta 224, 263-265.
- [10] Gross, E. and Morell, J.L. (1971) J. Am. Chem. Soc. 93, 4634-4635.
- [11] Van de Kamp, M., Horstink, L.M., Van den Hooven, H.W., Konings, R.N.H., Hilbers, C.W., Frey, A., Sahl, H.-G., Metzger, J.W. and Van de Ven, F.J.M. (1995) Eur. J. Biochem. 227, 757-771.
- [12] Van de Kamp, M., Van den Hooven, H.W., Konings, R.N.H., Bierbaum, G., Sahl, H.-G., Kuipers, O.P., Siezen, R.J., De Vos, W.M., Hilbers, C.W. and Van de Ven, F.J.M. (1995) Eur. J. Biochem. 230, 587-600.
- [13] Meyer, H.E., Heber, M., Eisermann, B., Korte, H., Metzger, J.W. and Jung, G. (1994) Anal. Biochem. 223, 185-190.
- [14] Zimmermann, N., Metzger, J.W. and Jung, G. (1995) Eur. J. Biochem. 228, 786-797.
- [15] Piard, J.-C., Muriana, P.M., Desmazeaud, M.J. and Klaenhammer, T.R. (1992) Appl. Environ. Microbiol. 58, 279-284.
- [16] Bax, A. and Davis, D.G. (1985) J. Magn. Reson. 65, 355-360.
- [17] Griesinger, C., Otting, G., Wüthrich, K. and Ernst, R.R. (1988) J. Am. Chem. Soc. 110, 7870-7872.

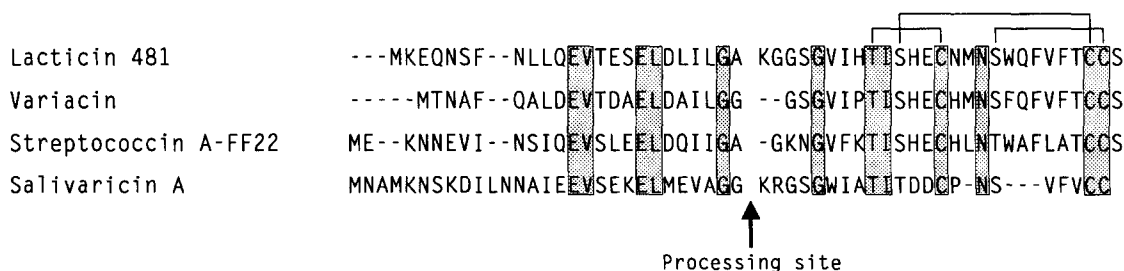


Fig. 6. Alignment of the lacticin-481 type lantibiotic prepeptides [5,6,31,33,34]. Identical residues are indicated in shaded boxes. The prepeptide cleavage sites are shown by an arrow. The thioether bridges as determined for lacticin 481 are given. At the corresponding positions in the other lacticin-481 type lantibiotics potential (3-methyl)lanthionine-forming residues are found; a number of them have already been proven to be involved in bridging.

- [18] Jeener, J., Meier, B.H., Bachmann, P. and Ernst, R.R. (1979) *J. Chem. Phys.* 71, 4546–4553.
- [19] Bodenhausen, G., Kogler, H. and Ernst, R.R. (1984) *J. Magn. Reson.* 58, 370–388.
- [20] Van den Hooven, H.W., Doeland, C.C.M., Van de Kamp, M., Konings, R.N.H., Hilbers, C.W. and Van de Ven, F.J.M. (1996) *Eur. J. Biochem.* 235, 382–393.
- [21] Van den Hooven, H.W., Spronk, C.A.E.M., Van de Kamp, M., Konings, R.N.H., Hilbers, C.W. and Van de Ven, F.J.M. (1996) *Eur. J. Biochem.* 235, 394–403.
- [22] Gross, E. and Witkop, B. (1962) *J. Biol. Chem.* 237, 1856–1860.
- [23] Arlandini, E., Gioia, B., Perseo, G., Danieli, B. and Rubino, F.M. (1987) *Biomed. Environ. Mass Spectrom.* 14, 487–493.
- [24] Biemann, K. (1989). in: *Protein Sequencing: A Practical Approach* (Findlay, J.B.C. and Geisow, M.J. eds.) pp. 99–118, IRL Press, Oxford.
- [25] Barber, M., Elliot, G.J., Bordoli, R.S., Green, B.N. and Bycroft, B.W. (1988) *Experientia* 44, 266–270.
- [26] Lipták, M., Vékey, K., Van Dongen, W.D. and Heerma, W. (1994) *Biol. Mass Spectrom.* 23, 701–706.
- [27] Roepstorff, P. and Fohlman, J. (1984) *Biomed. Mass Spectrom.* 11, 601.
- [28] Eckart, K. (1994) *Mass Spectrom. Rev.* 13, 23–55.
- [29] Slijper, M., Hilbers, C.W., Konings, R.N.H. and Van de Ven, F.J.M. (1989) *FEBS Lett.* 252, 22–28.
- [30] Sahl, H.-G., Jack, R.W. and Bierbaum, G. (1995) *Eur. J. Biochem.* 230, 827–853.
- [31] Hynes, W.L., Ferretti, J.J. and Tagg, J.R. (1993) *Appl. Environ. Microbiol.* 59, 1969–1971.
- [32] Jack, R.W., Carne, A., Metzger, J., Stevanovic, S., Sahl, H.-G., Jung, G. and Tagg, J. (1994) *Eur. J. Biochem.* 220, 455–462.
- [33] Ross, K.F., Ronson, C.W. and Tagg, J.R. (1993) *Appl. Environ. Microbiol.* 59, 2014–2021.
- [34] Pridmore, D., Rekhif, N., Pittet, A.-C., Suri, B. and Mollet, B. (1996) *Appl. Environ. Microbiol.* 62, 1799–1802.
- [35] Jack, R., Benz, R., Tagg, J. and Sahl, H.-G. (1994) *Eur. J. Biochem.* 219, 699–705.