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Elucidation of the primary structure of the lantibiotic epilancin K7 from *Staphylococcus epidermidis* K7

Cloning and characterisation of the epilancin-K7-encoding gene and NMR analysis of mature epilancin K7

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Lantibiotics are bacteriocins that contain unusual amino acids such as lanthionines and α,β-didehydro residues generated by posttranslational modification of a ribosomally synthesized precursor protein. The structural gene encoding the novel lantibiotic epilancin K7 from *Staphylococcus epidermidis* K7 was cloned and its nucleotide sequence was determined. The gene, which was named *elkA*, codes for a 55-residue preprotein, consisting of an N-terminal 24-residue leader peptide, and a C-terminal 31-residue propeptide which is posttranslationally modified and processed to yield mature epilancin K7. In common with the type-A lantibiotics nisin A and nisin Z, subtilin, epidermin, gallidermin and Pep5, pre-epilancin K7 has a so-called class-AI leader peptide. Downstream and upstream of the *elkA* gene, the starts of two open-reading-frames, named *elkP* and *elkT*, were identified. The *elkP* and *elkT* genes presumably encode a leader peptidase and a translocator protein, respectively, which may be involved in the processing and export of epilancin K7. The amino acid sequence of the unmodified pro-epilancin K7, deduced from the *elkA* gene sequence, is in full agreement with the amino acid sequence of mature epilancin K7, determined previously by means of NMR spectroscopy [van de Kamp, M., Horstink, L. M., van den Hooven, M. W., Konings, R. N. M., Hilbers, C. W., Sahl, H.-G., Metzger, J. W. & van de Ven, F. J. M. (1995) *Eur. J. Biochem. 227*, 757–771]. The first residue of mature epilancin K7 appears to be modified in a way that has not been described for any other lantibiotic so far. NMR experiments show that the *elkA*-encoded serine residue at position +1 of pro-epilancin K7 is modified to a 2-hydroxypropionyl residue in the mature protein.

Keywords. Bacteriocin; lanthionine; leader peptide; nucleotide sequence; posttranslational modification; structure determination.

Lantibiotics are gene-encoded polypeptides which contain unusual amino acids such as α,β-didehydroalanine (Dha) and α,β-didehydrobutyrine (Dhb), and thioether-bridge-containing (β-methyl-)lanthionines, that arise from modification of the primary translation product (Fig. 1; Jung, 1991; Bierbaum and Sahl, 1993; Hansen, 1993; de Vos, W. M., Kuipers, O. P., van der Meer, J. R. & Siezen, R. J., unpublished results). These modified residues hamper the determination, by means of standard Edman degradation techniques, of the amino acid sequence of lantibiotics (Jung, 1991; Meyer et al., 1994). α,β-Didehydro residues block the sequence analysis, because removal of the residue that precedes the didehydro residue is followed by hydration of the didehydro residue at the double bond; subsequent deamination then results in the substitution of an oxo group for the amino group of the N-terminal residue. For the N-terminal half of a (β-methyl-)lanthionine, Edman degradation results in a blank cycle because the residue is covalently linked via a thioether bond to an Ala residue or a 2-aminobutyrine residue at a position nearer to the C-terminus. When, prior to sequencing, the thioether bonds of the (β-methyl-)lanthionines are cleaved, the determination of the characteristic (β-methyl-)lanthionine bridging pattern, i.e. the determination of which residues are connected via thioether bonds, is rendered impossible.

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Abbreviations. COSY, correlated spectroscopy; Dha, α,β-didehydroalanine; Dhb, α,β-didehydrobutyrine; $[CD_3]_2SO$, deuterated dimethyl-sulphoxide; DQF-COSY, double-quantum-filtered COSY; epilancin-K7-(3–31)-peptide, peptide fragment of epilancin K7 consisting of the positions 3–31; HMBC, heteronuclear multiple-bond correlation; HSQC, heteronuclear single-quantum coherence; NOESY, nuclear Overhauser enhancement spectroscopy; ORF, open reading frame; ROESY, rotating-frame NOESY; TOCSY, total correlated spectroscopy.

Note. The novel nucleotide sequence data published here has been deposited with the GenBank sequence data bank and is available under accession number U20348.

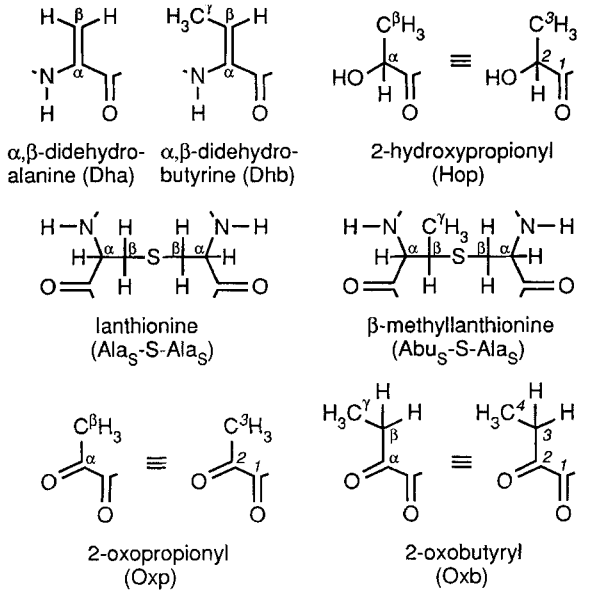

Fig. 1. Modified amino acids as they occur in the peptide chain of epilancin K7 from *S. epidermidis* K7. The modified amino acids 2-oxopropionyl and 2-oxobutyryl represent the first residues of the epilancin-K7-(3−31)-peptide (van de Kamp et al., 1995) and of Pep5 (Kellner et al., 1989, 1991; Freund et al., 1991c), respectively.

NMR spectroscopy, however, does not suffer from these disadvantages. We have recently used this technique to determine the amino acid sequence of the novel (type-A) lantibiotic epilancin K7 from *Staphylococcus epidermidis* K7, including the positions of the lanthionines (van de Kamp et al., 1995). Some uncertainties still remained for the first residue, since the ^{1}H, ^{13}C-NMR experiments that were crucial for the amino acid determination were performed with a degraded form of epilancin K7, called epilancin-K7-(3−31)-peptide, which lacks the first two residues (van de Kamp et al., 1995). In order to resolve the nature of the first residue of mature epilancin K7, successful isolation of sufficient amounts of the polypeptide was undertaken, and new homonuclear and heteronuclear NMR experiments were performed, the results of which are reported here. Analysis of the data shows that the first residue of epilancin K7 represents a novel modified, so-called 2-hydroxypropionyl residue.

Although NMR spectroscopy thus appears to offer the possibility to determine the primary structure of lantibiotics, recently the primary structures of a few lantibiotics, that had been determined by means of ^{1}H-NMR methods, needed correction because they contained mistakes that arose from difficulties in the interpretation of the two-dimensional ^{1}H-NMR spectra (Kessler et al., 1987, 1988; Kettenring et al., 1990; Zimmermann et al., 1993). Therefore, we attempted to confirm the NMR-based amino acid sequence of mature epilancin K7 by employing an independent method. The nucleotide sequence of the epilancin-K7-encoding gene provides a way for confirming the sequence. Knowledge of the gene sequence moreover offers insight into the biosynthesis of the mature protein. For a number of lantibiotics, the structural genes have been cloned and sequenced (Banerjee and Hansen, 1988; Buchmann et al., 1988; Schnell et al., 1988, 1989; Kaletta and Entian, 1989; Kaletta et al., 1989, 1991; Dodd et al., 1990; Mulders et al., 1991; Hynes et al., 1993; Piard et al., 1993; Ross et al., 1993). These sequences reveal that lantibiotics are synthesized as preproteins, consisting of an N-terminal leader peptide, and a C-terminal propeptide, which undergoes maturation to yield the fully modified protein.

Table 1. Two-dimensional ^{1}H-homonuclear NMR experiments and two-dimensional ^{1}H,^{13}C-heteronuclear NMR experiments used to study epilancin K7. ^{1}H-NMR spectra were recorded of a sample containing 2 mM epilancin K7. ^{1}H,^{13}C-NMR spectra were recorded of a sample containing 10 mM epilancin K7, with ^{13}C at natural abundance. In the ^{1}H-TOCSY and ^{1}H,^{13}C-HSQC-TOCSY experiments, spin-locking during the spin-lock time τ_{mix} was achieved using the Clean-MLEV17 sequence (Griesinger et al., 1988), preceded and followed by a trim pulse (1−2 ms). In the ^{1}H-NOESY experiment recorded with presaturation of the water signal, low-power water presaturation was also performed during the NOE build-up time τ_{mix}. In the ^{1}H-ROESY experiment, spin-locking during the ROE buildup or spin-lock time τ_{mix} was achieved with a continuous low-power pulse resulting in a spin-lock field in the range 2−4 kHz. The ^{1}H,^{13}C-HSQC and ^{1}H,^{13}C-HSQC-TOCSY spectra were acquired with the $1/[4\times{}^1J(^1\text{H}-^{13}\text{C})]$ delay set to 1.5 ms (^1J = 167 Hz), and without ^{13}C-decoupling. The ^{1}H,^{13}C-HMBC experiments were optimised for the detection of long-range 2-bond and 3-bond correlations, using a $1/[2\times{}^nJ(^1\text{H}-^{13}\text{C})]$ delay of 67 ms (^nJ = 7.8 Hz; n = 2 or 3). A low-pass J-filter (Kogler et al., 1983) was used to suppress 1-bond correlations, using a $1/[2\times{}^1J(^1\text{H}-^{13}\text{C})]$ delay of 3 ms. Other parameters that are given are, from left to right: the spectrometer frequency for the acquisition (f_2) and the indirect (f_1) dimension, (T) or (S) denoting that either time-proportional phase incrementation (Marion and Wüthrich, 1983) or the method of States et al. (1982) was used for phase cycling in f_1; the number of data points for acquisition (TD f_2) and the number of t_1, increments (TD f_1); the number of accumulated scans per t_1 increment (NS/FID); the spectral width for the f_2 dimension (SW f_2) and for the f_1 dimension (SW f_1); the length of the relaxation delay (RD), during which low-power phase-locked presaturation of the (residual) water signal was performed (p) or not (np); the solvent [$(CH_3)_2SO$, 99.9% $[CD_3]_2SO$; H_2O, 90% H_2O/10% $(CH_3)_2SO$; $(CH_3)_2SO$, 99.9% $(CH_3)_2SO$]; and the sample temperature (T). The quoted references are: (1) Rance et al., 1983; (2) Bax and Davis, 1985; Griesinger et al., 1988; (3) Kumar et al., 1980; Macura et al., 1982; (4) Bax and Davis, 1985; (5) Bax et al., 1990; Norwood et al., 1990; (6) Bax et al., 1989; Kessler et al., 1990; (7) Bax and Summers, 1986; Bax et al., 1988; Bax and Marion, 1988).

Experiment	τ_{mix}	Frequency	TD	NS/FID	SW	RD	Solvent	T	Reference
	ms	MHz			kHz	s		K	
^{1}H-DQF-COSY	–	500/500 (S)	2048/512	96	7.5/ 7.5	1.5 (p)	$(CH_3)_2SO$	308	1
^{1}H-TOCSY	70	400/400 (T)	2048/512	80	6.0/ 6.0	1.5 (p)	$(CH_3)_2SO$	308	2
^{1}H-TOCSY	70	400/400 (T)	2048/512	80	6.0/ 6.0	1.5 (np)	$(CH_3)_2SO$	308	2
^{1}H-TOCSY	70	400/400 (T)	2048/512	48	6.0/ 6.0	1.0 (np)	$(CH_3)_2SO$	298	2
^{1}H-NOESY	450	400/400 (T)	2048/512	72	6.0/ 6.0	1.0 (p)	$(CH_3)_2SO$	308	3
^{1}H-NOESY	450	400/400 (T)	2048/512	72	6.0/ 6.0	1.0 (np)	$(CH_3)_2SO$	308	3
^{1}H-ROESY	50	400/400 (T)	2048/512	48	6.0/ 6.0	1.0 (np)	$(CH_3)_2SO$	298	4
^{1}H, ^{13}C-HSQC	–	400/100 (T)	2048/512	80	4.0/32.3	1.5 (p)	D_2O	298	5
^{1}H, ^{13}C-HSQC-TOCSY	70	400/100 (T)	2048/682	96	4.0/32.3	1.5 (p)	D_2O	298	6
^{1}H, ^{13}C-HMBC	–	500/125 (S)	4096/830	128	5.0/27.5	1.5 (p)	D_2O	298	7
^{1}H, ^{13}C-HMBC	–	600/150 (S)	4096/914	128	7.5/36.0	1.5 (p)	D_2O	298	7

Fig. 2. Deduction of the tentative amino acid sequence of the unmodified epilancin K7 propeptide from the NMR-derived sequence of the mature epilancin K7, and design of the oligonucleotide probes 1 and 2. (A) Amino acid sequence of mature epilancin K7, obtained by NMR spectroscopy (van de Kamp et al., 1995), and (B) deduced amino acid sequence of the unmodified epilancin K7 propeptide [Xaa (Maa), unknown (modified) amino acid]. From the tentative sequence of the unmodified propeptide the nucleotide sequences of two wobbled ofigodeoxyribonucleotides (i.e. mixtures of oligos with degenerate codons) were derived (C) that were used as probes 1 and 2 in the Southern hybridisations. Probe 1 is a 2^7-times degenerated 20-residue nucleotide, and probe 2 is a 2^8-times degenerated 16-residue nucleotide.

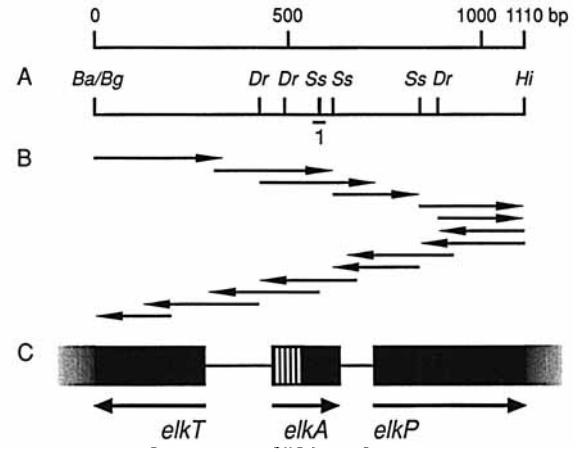

Fig. 3. Restriction map, sequencing strategy, and gene organisation of the *elkA* gene containing a 1.1-kbp *Bgl*II–*Hin*dIII restriction fragment from *S. epidermidis* K7 chromosomal DNA. (A) Restriction map of the 1.1-kbp *Bgl*II–*Hin*dIII restriction fragment as present in plasmid pMK71. Restriction enzymes: Ba, *Bam*HI; Bg, *Bgl*II; Dr, *Dra*I; Hi, *Hin*dIII; Ss, *Ssp*I. Approximately 20 bp from the *Bam*HI/*Bgl*II ligation site, a unique *Eco*RI restriction site is present on the pUC18-vector part of pMK71. The position of the sequence which hybridises with probe 1 is indicated by the horizontal bar with the '1'. (B) Overview of sequenced fragments from which the nucleotide sequence of the full *Bgl*II–*Hin*dIII restriction fragment was obtained. The arrows indicate the sizes and the directions of the fragments. (C) Gene organisation as deduced from the nucleotide sequence. The *elkA* gene is depicted as consisting of two regions, denoting the fact that it encodes a prepeptide which is constituted by an N-terminal leader peptide (hatched box) and a C-terminal propeptide (filled box). The ORFs denoted by *elkT* and *elkP* cross the borders of the fragment; their complete length has not been determined.

Since epilancin K7 is not very similar to any other (type-A) lantibiotic (van de Kamp et al., 1995), a comparison of the nucleotide sequences of the epilancin-K7-encoding gene and of other lantibiotic-encoding genes is considered to be of interest for its own sake. Here, the cloning and sequencing is described of a DNA fragment that harbours the epilancin-K7-encoding gene and the starts of two flanking genes whose products may be involved in the processing and translocation of epilancin K7. The amino acid sequence that is deduced from the nucleotide sequence of the epilancin-K7-encoding gene turns out to correspond completely to the amino acid sequence of mature epilancin K7, determined by NMR spectroscopy. At the position corresponding to the novel 2-hydroxypropionyl residue of mature epilancin K7, the epilancin-K7-encoding gene codes for a Ser residue.

MATERIALS AND METHODS

Bacterial strains, plasmids, media and growth conditions. *S- epidermidis* K7 is a plasmid-free, epilancin-K7-producing strain which was first described by Pulverer and Jeljaszewicz (1976). *S. epidermidis* K7 was grown aerobically at 37 °C in brain-heart-infusion medium (Difco) plus 1% glycine (mass/vol.). *Escherichia coli* strains MC1061 (Casadaban and Cohen, 1980) and JM83 (Vieira and Messing, 1982) were used as hosts in cloning experiments and were handled as described in Sambrook et al. (1989). *E. coli* strains were grown aerobically at 37 °C in tryptone/yeast broth. When appropriate, ampicillin was added to a concentration of 50–100 μg/ml. Plasmid pUC18 (Yanisch-Perron et al., 1985) was used in cloning experiments. Plasmid pMK71 is a pUC18 derivative harbouring a chromosomal 1.1-kbp *Bgl*II–*Hin*dIII fragment encompassing the epilancin-K7-encoding *elkA* gene.

Isolation of *S. epidermidis* K7 chromosomal DNA. Chromosomal DNA of *S. epidermidis* K7 was isolated according to a modification of the procedure of Marmur (1961). *S epidermidis* K7 cells were grown in 100 ml medium to A_{600} 1.5 and harvested by centrifugation. Cells were suspended in 1 ml 25 mM Tris/HCl, pH 8.0, 50 mM glucose and 10 mM EDTA, to which nisin and the lytic endopeptidase lysostaphin had been added to concentrations of 30 μg/ml and 100 μg/ml, respectively, and incubated at 37 °C until lysis had occurred after approximately 30 min. Subsequently, 3 ml 0.15 M NACl, 0.1 M EDTA, pH 8.0, was added to inhibit DNase activity, and the salt concentration was further increased by adding 1 ml 5 M sodium perchlorate. The DNA was purified by two extractions with an equal volume of chloroform/isoamylalcohol (24:1), incubation with RNase (100 μg/ml) at 37 °C for 30 min, and three extractions with an equal volume of phenol/chloroform/isoamylalcohol (25:24:1), followed by precipitation with two volumes of 96% ethanol. After washing with 80% ethanol and drying, the DNA was dissolved in 10 mm Tris/HCl, 1 mM EDTA, pH 8.0.

Recombinant-DNA techniques. Established procedures were used for the isolation of plasmid DNA, agarose-gel electrophoresis, capillary blotting of digested chromosomal and plasmid DNA, colony blotting, end-labelling of oligodeoxyribonucleotide probes with [γ-^{32}P]dATP, and Southern-blot hybridisation with these probes (Sambrook et al., 1989). Restriction endonucleases and other DNA-modifying enzymes, as well as blotting membranes were used as recommended by the manufactur-

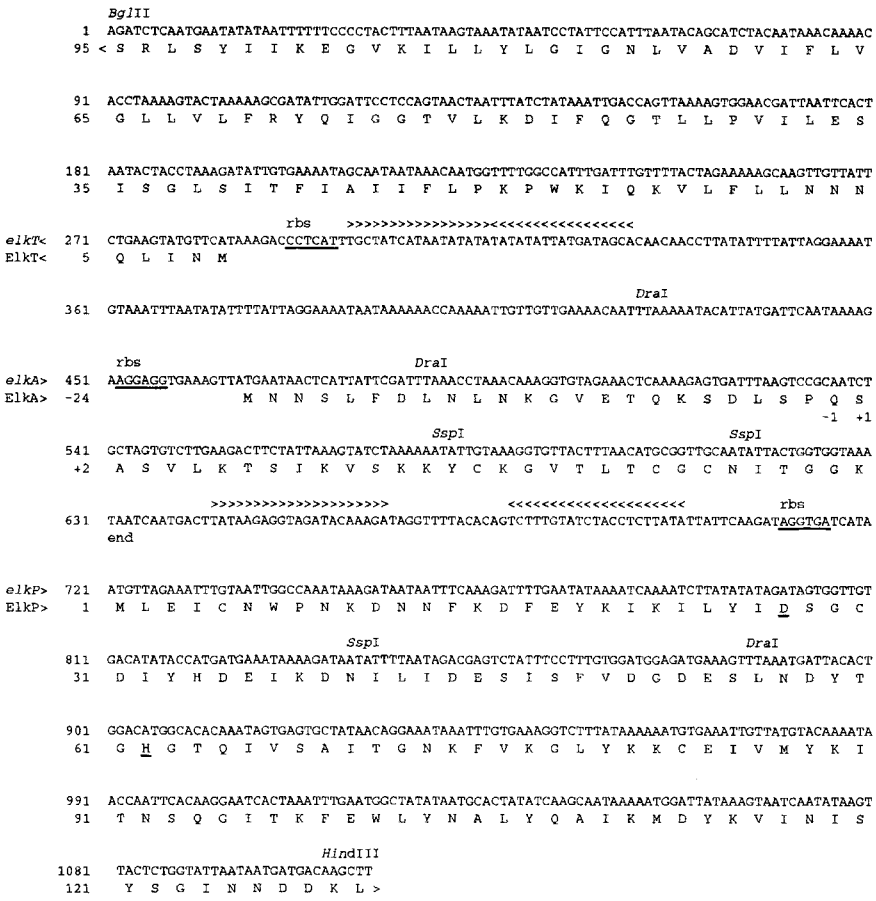

Fig. 4. Nucleotide sequence of the *Bgl*II–*Hin*dIII restriction fragment of *S. epidermidis* K7 chromosomal DNA, harbouring the epilancin-K7-encoding *elkA* gene and part of the *elkT* and *elkP* genes. *Dra*I and *Ssp*I restriction enzyme recognition sites (TTT/AAA and AAT/ATT, respectively) are indicated. Tentative ribosome-binding sites are underlined. Palindromic sequences in the regions between the ORFs are denoted by the ⩾⩽. Underneath the nucleotide sequence, the deduced amino acid sequence of the encoded (unmodified) proteins is given. The position of the cleavage site of pre-epilancin K7 by the tentative ElkP leader peptidase is indicated by '−1 +1'. The active-site residues of the tentative ElkP leader peptidase are underlined.

ers. DNA fragments were purified by the Gene-Clean procedure (US Biochemical).

Sequence analysis and deposition. DNA sequencing was performed using the dideoxynucleotide chain-termination method (Sanger et al., 1977) with double-stranded plasmid DNA that had been purified by ultracentrifugation in a $CsCl_2$/ethidium bromide gradient as a template (Sambrook et al., 1989). Degenerate oligodeoxyribonucleotides used as probes in Southern-blot hybridisations were synthesized on a PCR Mate DNA synthesizer (Applied Biosystems) and purified by reverse-phase HPLC. Oligodeoxyribonucleotides used as primers for sequencing were purchased from Eurogentec S. A. Computer analysis of DNA and amino acid sequences was performed with the PC/GENE program package (Genofit). Sequence alignments were made with the help of the CLUSTAL program of the GCG package (Devereux et al., 1984).

NMR spectroscopy. Epilancin K7 was isolated as described (van de Kamp et al., 1995). For ^1H, ^{13}C-NMR measurements in aqueous solution, epilancin K7 was lyophilised either from a solution in H_2O and dissolved in 90% H_2O/10% D_2O, or from a solution in D_2O and dissolved in 99.9% D_2O. The pH of the sample in 90% H_2O/10% D_2O was adjusted to 4.0 with 0.1 M NAOH and 0.1 M HCl and the pH of the sample in 99.9% D_2O was adjusted to 4.0 (uncorrected meter reading) with 0.1 NAOD and 0.1 M DCl solutions. For ^1H-NMR measurements in deuterated dimethylsulphoxide ($[CD_3]_2SO$), epilancin K7 was lyophilised from a solution in H_2O at pH 4.0, and dissolved in 99.9% $[CD_3]_2SO$. Parameters and conditions for two-dimensional ^1H-homonuclear and ^1H, ^{13}C-heteronuclear NMR experiments, performed with Bruker AM400 and AMX600, and Varian Unity-Plus 500 spectrometers, are summarised (Table 1). Data processing was carried out on a SUN Sparc work station, using the NMRi software package (Tripos).

RESULTS AND DISCUSSION

Cloning of the gene encoding epilancin K7. Based on the amino acid sequence of mature epilancin K7, determined by NMR spectroscopy (van de Kamp et al., 1995), two oligonucleotide probes were designed, termed probes 1 and 2 (Fig. 2). It was assumed that the formation of the α,β-didehydro residues Dha and Dhb and of the (β-methyl-)lanthionines occurs in the same way as in other type-A lantibiotics such as nisin, subtilin, epidermin and Pep5. In these lantibiotics, Dha and Dhb are formed from Ser and Thr residues, respectively; the lanthionines (β-methyllanthionines) are derived from Ser (Thr) and Cys residues, the Cys residues occupying a position nearer to the C-terminus of the unmodified polypeptides than the Ser (Thr) residues (Bierbaum and Sahl, 1993; Hansen, 1993; de Vos, W. M., Kuipers, O. P., van der Meer, J. R. and Siezen, R. J., unpublished results).

Chromosomal *S. epidermidis* K7 DNA was digested with several restriction enzymes and the restriction fragments were

separated on a 0.8% agarose gel, and blotted. Southern hybridisation of the blots with the γ-^{32}P-labelled probes 1 and 2 were performed at 43 C and 38 °C, respectively, and stringent washing at 37 °C and 32 °C, respectively. Both probes hybridised with a *Bgl*II−*Hin*dIII fragment which had a size of approximately 1.1 kbp (data not shown). Since probe 2 was less specific than probe 1, probe 2 was not used in most of the subsequent experiments. A library of *Bgl*II−*Hin*dIII fragments ranging in size over 1.0−1.2 kbp was constructed in pUC18 digested with *Bam*HI and *Hin*dIII. After transformation of *E. coli* MC1061 cells, 200 colonies were selected and analysed on the presence of the epilancin-K7-encoding gene by colony blotting. Nearly all colonies gave a positive signal after Southern-blot hybridisation with probe 1, which was caused by weak hybridisation of probe 1 with the vector DNA. Subsequent Southern-blot hybridisation of plasmid DNA, which was purified from 26 randomly picked colonies and was digested with *Eco*RI and *Hin*dIII, identified six positive clones of which the inserted *Bgl*II−*Hin*dIII fragment strongly hybridised with probe 1. All six inserts had an identical size of 1.1 kbp. One of these clones, pMK71, was used for further analysis.

Nucleotide sequence analysis of the *elkA* gene. The position of the epilancin-K7-encoding gene within the 1.1-kbp *Bgl*II−*Hin*dIII insert of pMK71 was determined by Southern-blot hybridisation of probe 1 with pMK71 digested with both *Eco*RI and *Hin*dIII, in combination with either *Dra*I or *Ssp*I (Fig. 3). Probe 1 hybridised with an internal *Dra*I−*Dra*I fragment. After digestion with *Eco*RI, *Hin*dIII and *Ssp*I, no specific hybridisation with probe 1 was observed, which was later attributed to the presence of a *Ssp*I recognition site in the DNA sequence that hybridises with probe 1 (Figs 3 and 4).

Subsequently, the nucleotide sequence of the *Bgl*II−*Hin*dIII insert was determined (Fig. 3). Comparison of the nucleotide sequenc (Fig. 4) with the amino acid sequence of epilancin K7 revealed the presence of the structural *elkA* gene (elk, epilancin K7) at nucleotide position 466−633, which encodes pre-epilancin K7. Alignment of the deduced unmodified amino acid sequence with the sequences of the prepeptides of the type-A lantibiotics nisin A and nisin Z, subtilin, epidermin and gallidermin, and Pep5, shows that epilancin K7 is a novel member of this class of lantibiotics (Fig. 5). In common with these proteins, lanthionines (β-methyllanthionines) of epilancin K7 are derived from a Ser (Thr) residue with a Cys residue that is only a few (typically 3−5) positions closer to the C-terminus of the propeptide (Fig. 5B). This results in an elongated structure for these type-A lantibiotics (Freund et al., 1991a, b, c; Chan et al., 1992; van de Ven et al., 1991; Lian et al., 1992; van den Hooven, H., unpublished results; van de Kamp, M., unpublished results) in contrast to the more globular structure of type-B lantibiotics that contain head-to-tail linkages (Jung et al., 1991; Kessler et al., 1991, 1992; Zimmermann et al., 1993; Zimmermann, N., Metzger, J. W. & Jung, G., unpublished results). Moreover, similar to what has been found for the type-A lantibiotics mentioned, the primary translation product of the *elkA* gene, pre-epilancin K7 contains a typical class-AI leader peptide (de Vos, W. M., Kuipers, O. P., van der Meer, J. R. and Siezen, R. J., unpublished results) which is characterised by homologous sequences in the −1 to −7 and the −14 to −21 regions and a high content of charged residues (Fig. 5A), and is distinct from the class-AII leader peptides that are found for other lantibiotics such as streptococcin A-FF22 (Hynes et al., 1993), salivaricin A (Ross et al., 1993) and lacticin 481 (Piard et al., 1993). The class-AI leader peptides may serve several purposes, such as directing the transport of the peptides across the cytoplasmic membrane and acting as a template in the maturation reaction(s) of the lantibiotics (see van der Meer et al., 1994). From *in vivo* screening of its bactericidal activity (van de Kamp et al., 1995) and from experiments with liposomes (Driessen et al., 1995), it has become evident that, besides sharing several common structural characteristics with other type-A lantibiotics, epilancin K7 also acts in a way similar to, e.g. nisin and Pep5, i.e. by permeabilising the membranes of susceptible cells (Sahl, 1985, 1991; Benz et al., 1991; Gao et al., 1991; Garcia Garcera et al., 1993).

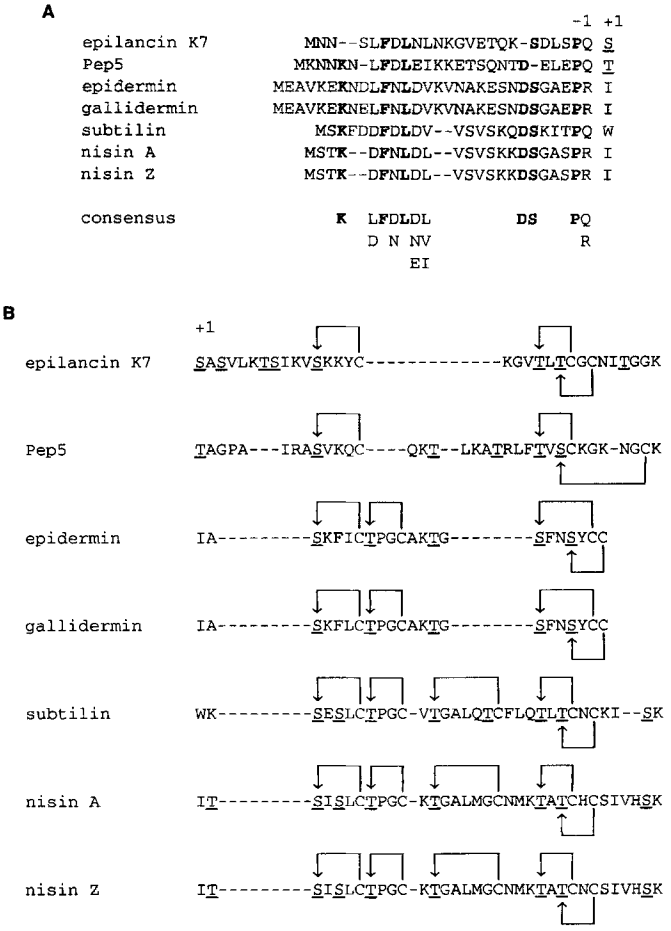

Fig. 5. Alignment of the amino acid sequences of the N-terminal leader peptide parts and the C-terminal propeptide parts of type-A lantibiotics. (A) Alignment of the epilancin-K7 leader peptide sequence with the sequences of the class-AI leader peptides of the type-A lantibiotics nisin A (Buchmann et al., 1988; Kaletta and Entian, 1989; Dodd et al., 1990), nisin Z (Mulders et al., 1991), subtilin (Banerjee and Hansen, 1988), epidermin (Schnell et al., 1988), gallidermin (Schnell et al., 1989) and Pep5 (Kaletta et al., 1989). Gaps are indicated by hyphens. The bottom line gives the consensus sequence for a type-A lantibiotic class-AI leader peptide, with more strictly conserved residues indicated in bold. (B) Alignment of the sequence of the unmodified propeptide of epilancin K7 with the sequences of the unmodified pro-parts of the type-A lantibiotics nisin A, nisin Z, subtilin, epidermin, gallidermin, and Pep5 [for references, see (A)]. Gaps are indicated by hyphens. Ser and Thr residues that undergo posttranslational dehydration, thereby resulting in the formation of Dha and Dhb residues, are underlined. The formation of (β-methyl-)lanthionines by the addition of a Cys-S^4 to the C^3 of a preceding Dha (Dhb) residue is indicated by the arrows. In Pep5 and epilancin K7 the N-terminal residue is further modified to a 2-oxobutyryl residue (Kellner et al., 1989, 1991; Freund et al., 1991c) and a 2-hydroxypropionyl residue (this work), respectively. In epidermin and gallidermin, modification at the C-terminus results in a 2-aminovinyl-cysteine (Allgaier et al., 1986). In line with observations made for Pep5 (Weil et al., 1990) and nisin (Kuipers et al., 1993b; van der Meer et al., 1993, 1994), it is assumed that the Ser and Thr residues in the leader peptides are not prone to dehydration.

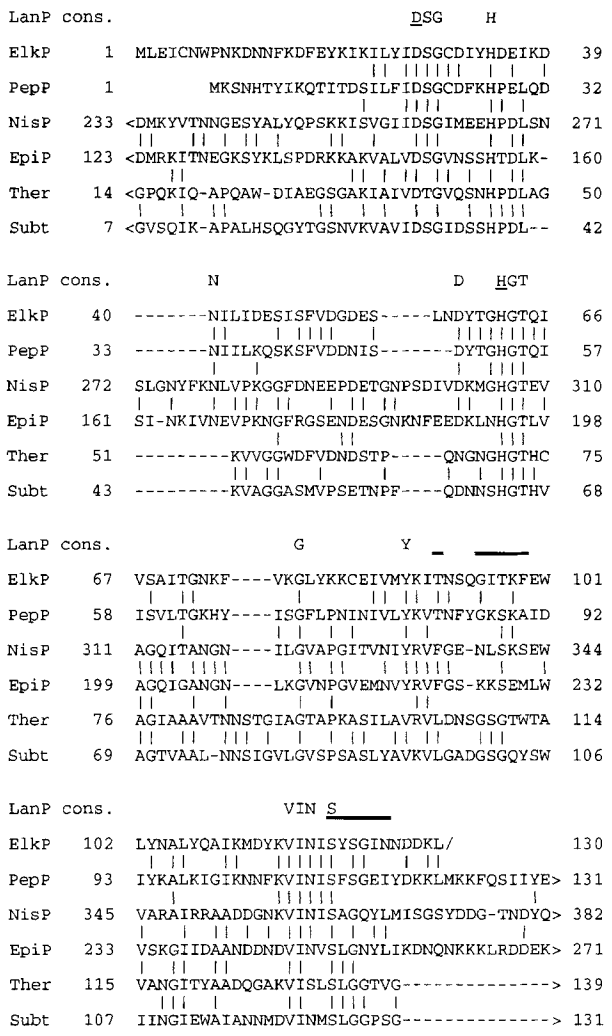

Fig. 6. Alignment of the amino acid sequence of the N-terminal part of the tentative leader peptidase ElkP with the sequences of the lantibiotic leader peptidases PepP (Meyer et al., 1995), NisP (van der Meer et al., 1993; Engelke et al., 1994) and EpiP (Schnell et al., 1992), and the sequences of the well-characterised serine proteases subtilisin BPN′ (Subt) from *Bacillus amyloliquefaciens* (Wells et al., 1983) and thermitase (Ther) from *Thermoactinomyces vulgaris* (Meloun et al., 1985). Pairwise identities between ElkP and PepP, PepP and NisP, NisP and EpiP, EpiP and Ther, and Ther and Subt are indicated by the vertical bars (|). Gaps are indicated by hyphens. The numbering of Ther and Subt starts at the N-terminus of the maturated proteins. The top lines show the residues which are identical in all four lantibiotic leader peptidases. Catalytic residues are underlined, and the region of the substrate-binding site is indicated by the doubled lines (Siezen et al., 1991). Whereas the overall amount of identity between the presented parts of the lantibiotic proteases is low, the pairs ElkP and PepP, and NisP and EpiP are significantly more similar (44% and 49% identity, respectively) than the other pairwise combinations ElkP and NisP, ElkP and EpiP, PepP and NisP, and PepP and EpiP (22%, 22%, 24%, and 19% identity, respectively).

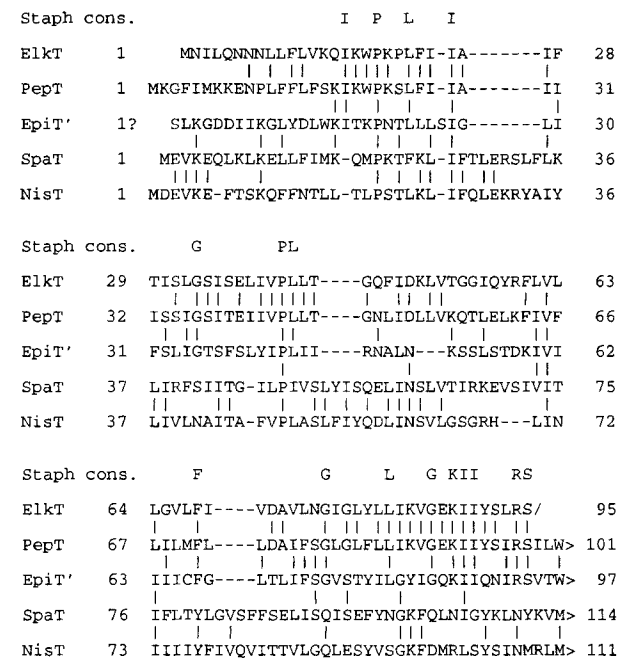

Fig. 7. Alignment of the amino acid sequence of the N-terminal part of the tentative translocator protein ElkT with the sequences of the lantibiotic translocators PepT (Meyer, C., Bierbaum, G., Heidrich, C., Süling, J., Iglesias-Wind, M. I., Gnau, V., Kempter, C. and Sahl, H.-G., unpublished results), SpaT (Klein et al., 1992), NisT (Engelke et al., 1992; Kuipers et al., 1993a), and the truncated EpiT′ protein (Schnell et al., 1992). Pairwise identities between ElkT and PepT, PepT and EpiT′, EpiT′ and SpaT, and SpaT and NisT are indicated by the vertical bars (|). Gaps are indicated by hyphens. The top lines show the residues which are identical in the three staphylococcal lantibiotic translocators ElkT, PepT and EpiT′. Whereas the overall amount of identity between the shown parts of the lantibiotic translocators is very low, the pair ElkT and PepT is significantly more similar (55% identity) than any of the other pairwise combinations (ElkT and EpiT′, 20%; ElkT and SpaT, 15%; ElkT and NisT, 18%; PepT and EpiT′, 32%; PepT and SpaT, 17%; PepT and NisT, 18%; EpiT′ and SpaT, 16%; EpiT′ and NisT, 17%; SpaT and NisT, 33%).

Nucleotide sequence analysis of other *elk* genes. Downstream of the *elkA* gene and with the same polarity, an open-reading-frame (ORF; nucleotide positions 721−1110) is present that crosses the border of the fragment at the *Hin*dIII site (Fig. 4). Analysis of the deduced amino acid sequence shows that the N-terminal part of the tentatively encoded protein is homologous to the N-terminal part of the PepP leader peptidase and also (although to a lesser extent) to the NisP and EpiP leader peptidases. These leader peptidases are involved in the processing of prePep5, prenisin and of pre-epidermin, respectively (Augustin et al., 1992; Schnell et al., 1992; van der Meer et al., 1993; Engelke et al., 1994; Meyer, C., Bierbaum, G., Heidrich, C., Süling, J., Iglesias-Wind, M. I., Gnau, V., Kempter, C. and Sahl, H.-G., unpublished results). Therefore, the ORF downstream of the *elkA* gene is named *elkP*. In Fig. 6, an alignment is given of the currently known part of the amino acid sequence of ElkP with the corresponding parts of the sequences of the NisP and EpiP peptidases and with homologous sequences of the well-characterised serine proteases subtilisin BPN′ from *Bacillus amyloliquefaciens* (Wells et al., 1983) and thermitase from *Thermoactinomyces vulgaris* (Meloun et al., 1985). The alignment shows that the ElkP and PepP proteins lack an N-terminal prepro-sequence similar to the prepro-sequences in NisP and in EpiP peptidases. This suggests that the ElkP and PepP proteins have a cytoplasmic location, whereas the NisP and EpiP proteins have an extracellular location (Augustin et al., 1992; Schnell et al., 1992; van der Meer et al., 1993; Engelke et al., 1994). The overall similarity between the aligned parts of the lantibiotic leader peptidases is highest near the active-site residues.

Upstream of the *elkA* gene and with an opposite polarity, an ORF (nucleotide positions 1−285) is present that crosses the border of the fragment at the *Bgl*II site (Fig. 4). Analysis of the deduced amino acid sequence shows a high degree of similarity

Table 2. ^{13}C-NMR chemical shifts for *S. epidermidis* epilancin K7 in aqueous solution (pH 4.0, T = 298 K). ^{13}C-NMR chemical shift values (±0.1 ppm) are expressed relative to $(^{13}CH_3)_3Si(CD_2)_2CO_2H$ (Bax and Subramanian, 1986; van de Ven et al., 1993). Hop, 2-hydroxypropionyl; X_s, X residue of lanthionine.

Residue	Chemical shift of						
	$^{13}C'$ ($^{13}C{=}O$)	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	$^{13}C^{\gamma}$	$^{13}C^{\delta}$	$^{13}C^{\varepsilon}$	$^{13}C^{\zeta}$
	ppm						
Hop1	180.3 ($^{13}C^{1}$)	70.3 ($^{13}C^{2}$)	22.4 ($^{13}C^{3}$)				
Ala2	177.0	52.4	19.3				
Dha3	170.1	138.2	115.6				
Val4	176.3	63.1	32.4	20.7			
Leu5	177.6	55.1	42.2	27.1	25.0, 32.4		
Lys6	176.5	56.8	32.8	25.1	29.2	42.1	
Dhb7	168.6	130.8	137.2	15.5			
Dha8	169.9	138.5	116.7				
Ile9	176.3	62.2	38.7	27.9, 17.8	13.3		
Lys10	177.2	56.8	32.8	25.1	29.0	42.0	
Val11	176.3	63.1	32.6	21.2			
Ala_S12	174.7	56.3	36.2				
Lys13	176.8	57.7	31.9	25.2	29.1	42.1	
Lys14 S	176.1	57.7	33.4	24.6	29.2	42.0	
Tyr15	176.0	58.4	37.4	131.4	133.1	118.2	157.2
Ala_S16	174.5	56.3	36.9				
Lys17	176.8	56.7	32.9	24.9	29.0	42.1	
Gly18	174.2	45.4					
Val19	177.8	63.4	31.9	21.2			
Abu_S20	175.8	61.6	50.6	23.4			
Leu21 S	178.4	55.6	40.5	27.3	24.0		
Abu_S22	176.1	62.3	48.1	22.7			
Ala_S23	175.3	56.9	39.3				
Gly24 S	174.9	45.9					
Ala_S25	174.9	56.2	39.8				
Asn26	175.5	53.2	38.5	177.1			
Ile27	176.4	61.8	38.5	27.5, 17.7	13.0		
Dhb28	170.0	130.7	137.5	15.6			
Gly29	175.0	45.8					
Gly30	173.4	45.4					
Lys31	180.8	57.3	33.6	24.9	29.0	42.1	

to the N-terminal part of the tentative Pep5-translocator protein PepT (Meyer, C., Bierbaum, G., Heidrich, C., Süling, J., Iglesias-Wind, M. I., Gnau, V., Kempter, C. and Sahl, H.-G., unpublished results). Correspondingly, the ORF upstream of the *elkA* gene is named *elkT*. Alignment of the currently known part of the ElkT sequence with the sequences of the N-terminal parts of the PepT, NisT, SpaT and EpiT′ translocators (Engelke et al., 1992; Klein et al., 1992; Schnell et al., 1992; Kuipers et al., 1993a; Meyer, C., Bierbaum, G., Heidrich, C., Süling, J., Iglesias-Wind, M. I., Gnau, V., Kempter, C. and Sahl, H.-G., unpublished results; Fig. 7) shows that the overall similarity between these hydrophobic parts of the tentative lantibiotic translocators is rather low. The translocator proteins are implicated to function in the export of the lantibiotics.

Besides the fact that the currently known parts of the tentative ElkP and ElkT proteins particularly resemble the PepP and PepT equivalents (Figs 5 and 6), the organisation of the *elkA*, *elkP* and *elkT* genes also resembles the unique organisation of the corresponding genes in the *S. epidermidis pep* gene cluster involved in the biosynthesis of Pep5 (Meyer, C., Bierbaum, G., Heidrich, C., Süling, J., Iglesias-Wind, M. I., Gnau, V., Kempter, C. and Sahl, H.-G., unpublished results). However, in the *pep* gene cluster, a small gene, *pepI*, coding for a 69-residue immunity protein, is located between the *pepA* and *pepT* genes, having the same polarity as the *pepA* gene (Reis and Sahl, 1991; Meyer, C., Bierbaum, G., Heidrich, C., Süling, J., Iglesias-Wind, M. I., Gnau, V., Kempter, C. and Sahl, H.-G., unpublished results). In contrast, no ORF of significant size, which would resemble the *pepI* gene, is present between the *elkA* and *elkT* ORFs. The organisation of the presently described *elk* genes differs from the gene organisation in the *S. epidermidis epi* cluster where the *epiP* gene has a different position relative to the structural *epiA* gene (Schnell et al., 1992), and differs even more from the gene organisation of the *Lactococcus lactis nis* and the *Bacillus subtilis spa* clusters, where both the translocator-encoding and protease-encoding genes have other locations relative to the structural *nisA* and *spaS* genes (Steen et al., 1991; Chung and Hansen, 1992; Chung et al., 1992; Engelke et al., 1992, 1994; Klein et al., 1992, 1993; Kuipers et al., 1993a; van der Meer et al., 1993; Gutowski-Eckel et al., 1994).

The nucleotide sequence was finally analysed for the occurrence of other possible functional elements. Each of the ORFs *elkA*, *elkP* and *elkT* is preceded by a tentative ribosome-binding site at an appropriate distance (5–10 bp) upstream of the ATG start codon (Fig. 4). Between the *elkA* and the *elkP* ORFs and directly upstream of the *elkT* ORF, large palindromic sequences are found, which could be involved in transcription regulation and termination (Fig. 4). Identification of tentative promoter sequences based on similarity with canonical −35 and −10 sequences was considered to be unreliable because of the high A+T content of the staphylococcal DNA.

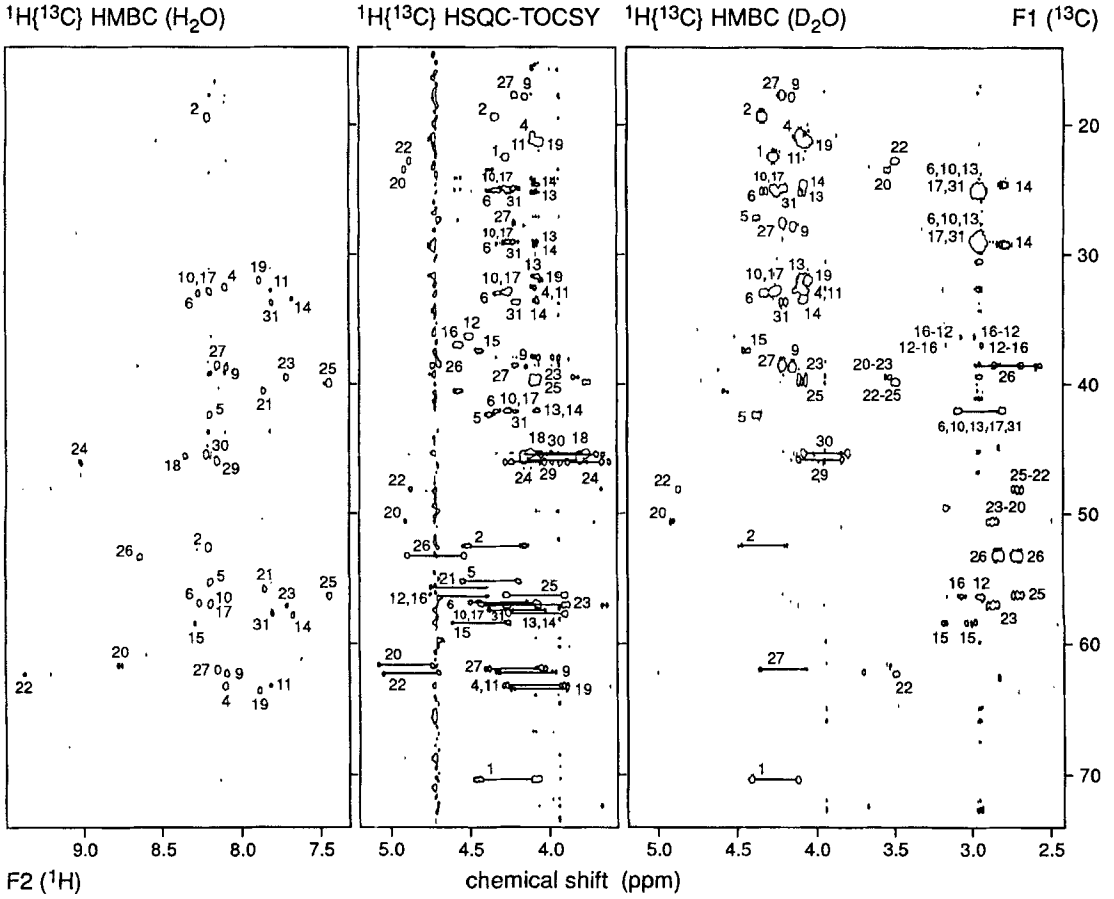

Fig. 8. Alignment of regions of ^{1}H,^{13}C-NMR spectra of epilancin K7. Numbers refer to the position of the residues in the amino acid sequence of epilancin K7. Left panel, $^1H^N$-^{13}C region of the ^{1}H,^{13}C-HMBC spectrum recorded for epilancin K7 in H_2O (90% H_2O/10% D_2O), showing intraresidue 2-bond $^1H^N$-$^{13}C^2$ and 3-bond $^1H^N$-$^{13}C^3$ cross-peaks. Middle panel, $^1H^2$-^{13}C region of the ^{1}H,^{13}C-HSQC-TOCSY spectrum recorded for epilancin K7 in D_2O (99.9% D_2O), showing intraresidue connectivities between the $^1H^2$ resonances and main-chain $^{13}C^2$ and side-chain ^{13}C resonances. The $^1H^2$-$^{13}C^2$ cross-peaks show up as doublets in the f_2 dimension ($^1J \approx 165$ Hz) since the spectrum was acquired without ^{13}C decoupling. The $^1H^2$-$^{13}C^2$ (C^2-^1H-$^{13}C^2$) connectivity for residue 1 (2-hydroxypropionyl) occupies a unique position in the spectrum (f_2 = 4.26 ppm, f_1 =70.3 ppm). Right panel, $^1H^2$-^{13}C region and part of the $^1H^3$-^{13}C region of the ^{1}H,^{13}C-HMBC spectrum recorded for epilancin K7 in D_2O (99.9% D_2O), showing intraresidue 2-bond $^1H^2$-$^{13}C^3$ and 3-bond $^1H^2$-$^{13}C^3$ cross-peaks, as well as 3-bond cross-thioether-bridge $^1H^3_i$-$^{13}C^3_j$ cross-peaks that prove the presence of the lanthionine connecting residue 12 with 16 and of the two β-methyllanthionines connecting residue 20 with 23 and 22 with 25 (van de Kamp et al., 1995). In addition, $^1H^6$-$^{13}C^5$ and $^1H^6$-$^{13}C^4$ cross-peaks for the Lys residues show up in this region. The application of a ^1J filter (Kogler et al., 1983) in the ^{1}H,^{13}C-HMBC pulse sequence efficiently suppressed 1-bond correlations, but a few are visible as doublets in f_2 ($^1J \approx 165$ Hz).

NMR spectroscopy and the nature of the first residue. Previously we have reported the primary structure of mature epilancin K7 which was determined by (^{1}H) homonuclear NMR spectroscopy of the native protein, and by ^{1}H-homonuclear and ^{1}H,^{13}C-heteronuclear NMR spectroscopy of a degraded form, the epilancin-K7-(3−31)-peptide (van de Kamp et al., 1995). The results presented above show that the amino acid sequence established by NMR spectroscopy corresponds completely with the amino acid sequence deduced from the nucleotide sequence of the *elkA* gene. Apparently, at positions where Thr and Ser residues occur in the deduced amino acid sequence of pre-epilancin K7, dehydration results in Dhb and Dha residues, followed by the formation of one lanthionine and two β-methyllanthionines. Also, the reported length of 31 residues for mature epilancin K7 (van de Kamp et al., 1995) is in agreement with the position of the typical cleavage site just at the N-terminal side of residue +1 in pre-epilancin K7. The nucleotide sequence data prompted us to investigate what the exact nature of the first residue of epilancin K7 is; it had previously remained enigmatic (van de Kamp et al., 1995).

The first residue of mature epilancin K7 constitutes a block in the sequence determination by Edman degradation (van de Kamp et al., 1995). The amino acid sequence which is deduced from the *elkA* gene sequence shows that, in the unmodified primary translation product, pre-epilancin K7, the corresponding residue is a Ser (this work; Fig. 4). The previously published analysis of the ^{1}H-NMR spectrum of mature epilancin K7 in aqueous solution indicated that the first residue of mature epilancin K7 has 'Ala'-like properties (i.e. it has C^2H-like and C^3H_3-like proton resonances at 4.26 ppm and 1.33 ppm (van de Kamp et al., 1995). The data strongly suggest that the Ser at position +1 of the epilancin K7 propeptide is subjected to a modification reaction. In Pep5, the Thr at position +1 of the Pep5 propeptide (Kaletta et al., 1989) is modified, probably via dehydration followed by spontaneous rehydration and deamination to a 2-oxobutyryl residue (Fig. 1) as the first residue of mature Pep5; this residue blocks Edman degradation of the protein (Kellner et al., 1989, 1991; Freund et al., 1991c). Similarly, for epilancin K7, modification of the Ser at position +1 would be expected to yield a 2-oxopropionyl residue (Fig. 1) as the first residue of the mature protein. This is not the case, however, since a 2-oxopropionyl residue lacks the C^2H-like proton that is detected by NMR spectroscopy (van de Kamp et al., 1995). What then is the nature of the first residue of epilancin K7, why does it pre-

Table 3. ^{1}H-NMR chemical shifts for *S. epidermidis* epilancin K7 in aqueous solution (pH 4.0, $T = 298$ K). ^{1}H-NMR chemical shift values (± 0.02 ppm) are expressed relative to $(C^1H_3)_3Si(CD_2)_2CO_2H$. The δ value of the Asn26 $^1H^2$ resonance is indicated by (H_2O) because the Asn26 $^1H^2$ resonance coincides with the water line (δ is approximately 4.7 ppm; van de Kamp et al., 1995) and is not detected due to presaturation of the water magnetisation. Hop, 2-hydroxypropionyl; X_s, X residue of lanthionine.

Residue	Chemical shift of			
	$^1H^N$	$^1H^2$	$^1H^3$	other
	ppm			
Hop1	–	4.26 (C^2-^1H)	1.33 (C^3-$^1H_3$)	
Ala2	8.21	4.36	1.42	
Dha3	9.83	–	5.58, 5.50	
Val4	8.10	4.09	2.11	$^1H_3^4$ 0.93
Leu5	8.20	4.36	1.67, 1.52	$^1H^4$ 1.57; $^1H_3^5$ 0.86, 0.80
Lys6	8.27	4.31	1.85, 1.78	$^1H_2^4$ 1.44, 1.37; $^1H_2^5$ 1.64; $^1H_2^6$ 2.93
Dhb7	9.47	–	6.50	$^1H_3^4$ 1.72
Dha8	9.39	–	5.57, 5.42	
Ile9	8.09	4.14	1.91	$^1H_2^4$ 1.45, 1.24; $^1H_3^4$ 0.90; $^1H_3^5$ 0.83
Lys10	8.21	4.25	1.78	$^1H_2^4$ 1.40; $^1H_2^5$ 1.63; $^1H_2^6$ 2.93
Val11	7.81	4.07	2.05	$^1H_3^4$ 0.92
Ala_s12	8.21	4.49	3.16, 2.95	
Lys13	8.48	4.08	1.86	$^1H_2^4$ 1.47, 1.34; $^1H_2^5$ 1.65; $^1H_2^6$ 2.97
Lys14 S	7.67	4.08	1.51	$^1H_2^4$ 0.93; $^1H_2^5$ 1.47; $^1H_2^6$ 2.78
Tyr15	8.30	4.43	3.17, 3.00	$^1H^5$ 7.13; $^1H^6$ 6.80
Ala_s16	7.98	4.56	3.05, 2.98	
Lys17	8.20	4.26	1.84, 1.78	$^1H_2^4$ 1.40; $^1H_2^5$ 1.63; $^1H_2^6$ 2.94
Gly18	8.35	3.95, 3.85		
Val19	7.90	4.04	2.04	$^1H_3^4$ 0.94
Abu_s20	8.78	4.90	3.53	$^1H_3^4$ 1.32
Leu21 S	7.85	4.55	1.74, 1.49	$^1H^4$ 1.45; $^1H_3^5$ 0.88
Abu_s22	9.39	4.87	3.48	$^1H_3^4$ 1.35
Ala_s23	7.71	4.08	3.64, 2.85	
Gly24 S	9.03	4.07, 3.82		
Ala_s25	7.44	4.08	3.57, 2.69	
Asn26	8.64	(H_2O)	2.82, 2.71	$^1H_2^5$ 7.55, 6.86
Ile27	8.15	4.20	1.93	$^1H_2^4$ 1.49, 1.22; $^1H_3^4$ 0.97; $^1H_3^5$ 0.88
Dhb28	9.53	–	6.72	$^1H_3^4$ 1.74
Gly29	8.15	3.98		
Gly30	8.21	3.93		
Lys31	7.79	4.18	1.82, 1.68	$^1H_2^4$ 1.35; $^1H_2^5$ 1.62; $^1H_2^6$ 2.96

vent Edman degradation of epilancin K7, and in which way may it originate from a Ser residue?

^{13}C-NMR assignments. To reveal the nature of the first residue of mature epilancin K7, we investigated the ^{13}C-NMR spectrum of the protein. This had not been accomplished for mature epilancin K7 in the previous study (van de Kamp et al., 1995), since the available amount of the protein was insufficient for a study of its ^{13}C-NMR spectrum at natural abundance. Instead, in the previous investigation, the ^{13}C-NMR spectrum of a degraded form, the epilancin-K7-(3–31)-peptide, was analyzed; this lacks the two first residues of the native protein. A new large-scale isolation of epilancin K7 facilitated the present analysis of its ^{13}C-NMR spectrum. Complete ^{13}C-NMR assignments (Table 2) were obtained by combining the information present in ^{1}H,^{13}C-heteronuclear single-quantum coherence (HSQC), ^{1}H,^{13}C-HSQC-total correlated spectroscopy (TOCSY) and ^{1}H,^{13}C-heteronuclear multiple-band correlation (HMBC) spectra recorded for the mature protein in aqueous solution (either 90% H_2O/10% D_2O or 99.9% D_2O) with ^{13}C at natural abundance (Fig. 8), using the ^{1}H resonance assignments for the protein in aqueous solution (Table 3; van de Kamp et al., 1995). The ^{13}C chemical shift of 70.3 ppm for the C^2 atom of residue 1 differs by nearly 20 ppm from the $^{13}C^2$ chemical shift of an Ala residue (52.4 ppm for Ala2 in Fig. 8; Richarz and Wüthrich, 1978; Spera and Bax, 1991; Wishart et al., 1991). Instead, it resembles the (unique) ^{13}C chemical shift of a Thr C^3 atom (69.2 ppm) (Richarz and Wüthrich, 1978; Spera and Bax, 1991). This indicates that the first residue has a hydroxy substituent at its C^2 atom, and represents a 2-hydroxypropionyl residue (Fig. 1). The presence of an electronegative substituent at the C^2 atom of residue 1 is in line with the position of the main-chain carbonyl $^{13}C^1$ resonance of residue 1 (180.3 ppm), which is shifted downfieid by approximately 4 ppm relative to the $^{13}C^1$ resonances of normal amino acids (176–177 ppm; Table 2; Richarz and Wüthrich, 1978; Wishart et al., 1991), resembling the $^{13}C^1$ resonance position of the C-terminal Lys31 (180.8 ppm; Table 2).

^{1}H-NMR assignments in $[CD_3]_2SO$. Further evidence for a 2-hydroxypropionyl residue as the first residue of epilancin K7 came from the analysis of the ^{1}H-NMR spectrum of epilancin K7. In line with the expectation that the postulated C^2OH proton of residue 1 will be in rapid exchange with water, no C^2O^1H resonance was detected in the ^{1}H-NMR spectrum recorded for epilancin K7 in aqueous solution (van de Kamp et al., 1995). In order to observe the hydroxy proton, the ^{1}H-NMR spectrum of epilancin K7 in 99.9% $[CD_3]_2SO$ was investigated. Complete ^{1}H assignments (Table 4) were obtained from the conventional analysis of two-dimensional ^{1}H-homonuclear DQF-COSY

Table 4. ^{1}H-NMR chemical shifts for *S. epictermidis* epilancin K7 in 99.9% $[CD_3]_2SO$ (T = 308 K). $^1H^N$-NMR chemical shifts are also given for T = 298 K. ^{1}H-NMR chemical shift values (±0.02 ppm) are expressed relative to $(C^1H_3)_3Si(CD_2)_2CO_2H$. For 2-hydroxypropionyl at position 1, two sets of resonances were detected (see text and Fig. 9). n.d., not detected. Hop, 2-hydroxypropionyl; X_s, X residue of lanthionine.

Residue	Chemical shift of				
	$^1H^N$	$^1H^2$	$^1H^3$	other	$^1H^N$ (298 K)
	ppm				
Hop1	–	4.18 (C^2-^1H)	1.20 (C^3-$^1H_3$)	O^3-^1H 7.57 (C^2O^1H)	– (O^3-^1H 7.59)
	–	3.98 (C^2-^1H)	1.20 (C^3-$^1H_3$	O^3-^1H 7.78 (C^2O^1H)	– (O^3-^1H 7.81)
Ala2	7.88	4.37	1.27		7.92
Dha3	n.d.	–	5.99, 5.56		9.24
Val4	8.04	4.14	2.04	$^1H^4_3$ 0.84	8.09
Leu5	8.01	4.30	1.61, 1.48	$^1H^4$ 1.57; $^1H^5_3$ 0.84, 0.80	8.03
Lys6	7.99	4.29	1.79, 1.62	$^1H^4_2$ 1.37; $^1H^5_2$ 1.57; $^1H^6_2$ 2.75	8.05
Dhb7	n.d.	–	6.42	$^1H^4_3$ 1.62	9.38
Dha8	n.d.	–	6.01, 5.64		8.92
Ile9	8.15	4.22	1.88	$^1H^4_2$ 1.41, 1.11; $^1H^4_3$ 0.84; $^1H^5_3$ 0.79	8.19
Lys10[a]	8.20	4.31	1.68, 1.60	$^1H^4_2$ 1.39, 1.33; $^1H^5_2$ 1.58; $^1H^6_2$ 2.73	8.22
Val11	7.98	4.20	2.06	$^1H^4_3$ 0.84	8.08
Ala_s12	8.61	4.36	2.95		8.57
Lys13	8.46	3.97	1.74, 1.68	$^1H^4_2$ 1.32, 1.28; $^1H^5_2$ 1.55; $^1H^6_2$ 2.75	8.44
Lys14 S	8.23	4.04	1.45	$^1H^4_2$ 1.05, 0.82; $^1H^5_2$ 1.43; $^1H^6_2$ 2.64	8.18
Tyr15	8.46	4.35	3.02, 2.85	$^1H^5$ 7.02; $^1H^6$ 6.64	8.45
Ala_s16	7.80	4.45	2.91		7.77
Lys17	7.85	4.12	1.66, 1.54	$^1H^4_2$ 1.31; $^1H^5_2$ 1.52; $^1H^6_2$ 2.71	7.87
Gly18	8.18	3.69			8.23
Val19	7.67	4.05	1.89	$^1H^4_3$ 0.87	7.67
Abu_s20	8.47	4.74	3.48	$^1H^4_3$ 1.21	8.54
Leu21 S	7.69	4.57	1.62, 1.47	$^1H^4$ 1.41; $^1H^5_3$ 0.88, 0.80	7.71
Abu_s22	8.95	4.73	3.35	$^1H^4_3$ 1.20	9.02
Ala_s23 S	7.51	3.91	3.37, 2.91		7.52
Gly24	8.75	3.69, 3.56			8.82
Ala_s25	7.22	3.91	3.34, 2.53		7.22
Asn26	8.33	4.51	2.57, 2.45	$^1H^5_2$ 7.42, 6.91	8.35
Ile27	7.92	4.14	1.81	$^1H^4_2$ 1.46, 1.12; $^1H^4_3$ 0.87; $^1H^6_3$ 0.83	7.94
Dhb28	9.21	–	6.35	$^1H^4_3$ 1.61	9.26
Gly29	7.87	3.72			7.90
Gly30	7.93	3.71			7.96
Lys31	7.78	4.11	1.69, 1.58	$^1H^4_2$ 1.31; $^1H^5_2$ 1.51; $^1H^6_2$ 2.73	7.87

(Fig. 9), TOCSY, NOESY and rotating-frame NOESY (ROESY) spectra (Wüthrich, 1986). A new resonance, i.e. without a counterpart in the ^{1}H-NMR spectrum of epilancin K7 in water, was detected at 7.57 ppm (T = 308 K; δ = 7.59 at 298 K). The corresponding proton has scalar, through-bond interactions with the C^2-^1H (δ = 4.18 ppm) and C^3-$^1H_3$ (δ = 1.20 ppm) protons of the first residue, which were detected by DQF-COSY (Fig. 9) and TOCSY (data not shown). The chemical shift of 7.57 ppm is in agreement with the expected position for the O^1H resonance of a 2-hydroxypropionyl residue, i.e. downfield from the random-coil Thr and Ser O^4H resonance positions in $[CD_3]_2SO$ (5.92 ppm and 4.79 ppm, respectively; Bundi et al., 1975) and upfield from the random-coil Asp O^5H, O^6H, and Tyr C^7OH resonance positions in $[CD_3]_2SO$ (12.23, 12.08 and 9.13 ppm, respectively; Bundi et al., 1975). From the combined ^{13}C-NMR and ^{1}H-NMR data, we conclude that the first residue of mature epilancin K7 is a 2-hydroxypropionyl residue.

In addition to the C^2-^1H resonance at δ = 4.18 ppm for 2-hydroxypropionyl at position 1, a second C^2-^1H resonance for this residue was detected at δ = 3.98 ppm by the observation of a scalar connectivity with the C^3-$^1H_3$ resonance of 2-hydroxypropionyl at position 7, at δ = 1.20 ppm in the DQF-COSY spectrum (Fig. 9). In the TOCSY spectrum at T = 298 K, very weak connectivity was observed between this im C^2-^1H resonance and the signal (δ = 7.81 ppm) of a proton which appears to be in relatively fast exchange with the residual water in the $[CD_3]_2SO$ sample (data not shown). Presumably, in $[CD_3]_2SO$, the 2-hydroxypropionyl residue may exist in two slowly interchanging conformations, one with the C^2OH in a position where its proton can exchange freely [$\delta(C^2O^1H)$ = 7.81 ppm; $\delta(C^2$-$^1H)$ = 3.98 ppm; T = 298 K], the other in which exchange of the C^2OH proton is much slower, possibly because it is involved in an intraresidue hydrogen bond with the main-chain carbonyl [$\delta(C^2O^1H)$ = 7.59 ppm; $\delta(C^2$-$^1H)$ = 4.18 ppm; T = 298 K].

Formation of the 2-hydroxypropionyl residue. The finding that position 1 is a 2-hydroxypropionyl residue is in agreement with mass spectrometric measurements on complete epilancin K7 (van de Kamp et al., 1995) and on N-terminal epilancin K7

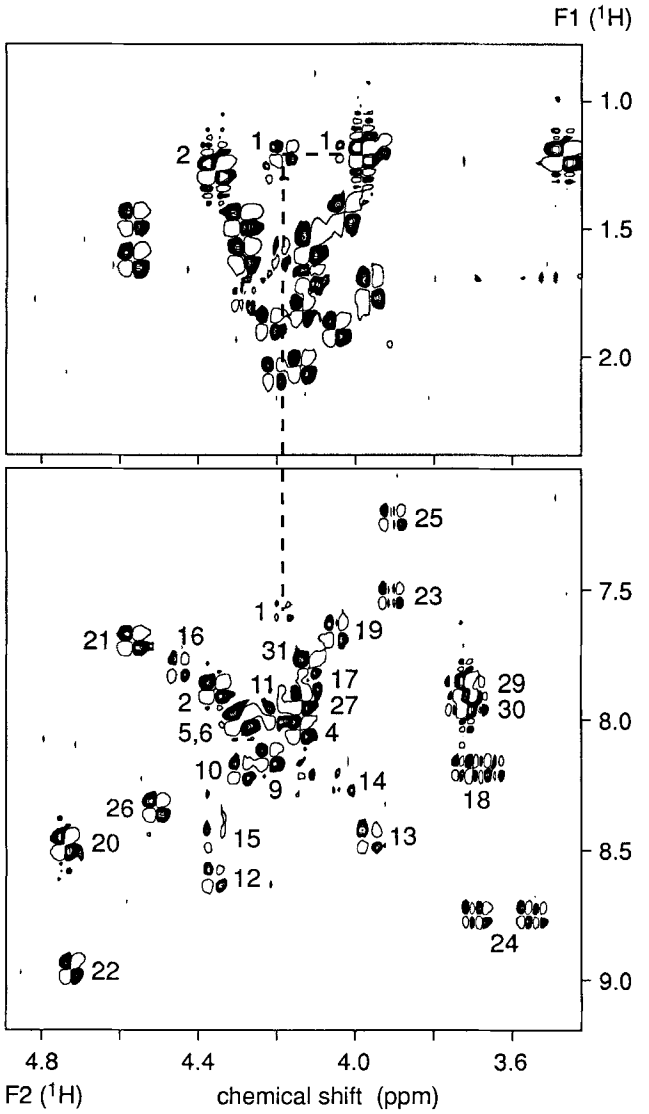

Fig. 9. Regions of the ^{1}H DQF-COSY spectrum of mature epilancin K7 in 99.9% [CD_3]$_2$SO (T = 308 K). Numbers refer to the position of the residues in the amino acid sequence of epilancin K7. Lower panel, so-called fingerprint region containing the 3-bond $^1H^N$-$^1H^2$ cross peaks for residues 2 to 31 (except for the α,β-didehydro residues 3, 7, 8 and 28) and the 3-bond cross peak between the 2-hydroxy ^{1}H (C^2O^1H) resonance at f_1 = 7.57 ppm and the C^2-^{1}H resonance at f_2 = 4.18 ppm of the 2-hydroxypropionyl residue at position 1 (Hop1). Upper panel, region containing 3-bond $^1H^2$-$^1H^3$ cross-peaks. The following cross-peaks are labelled: two C^2-^{1}H−C^3-$^1H_3$ cross-peaks for Hop1 at (f_2, f_1) = 4.18, 1.20 and 3.98, 1.20 (see text and Table 4) and one $^1H^2$-$^1H^3$, cross peak for Ala2 at (f_2, f_1) = 4.37, 1.27. The cross peaks for Hop1 are connected by dashed lines (- - -).

fragments (Metzger, J. W. and Jung, G., unpublished results). Moreover, the absence of a free amino group explains why mature epilancin K7 is blocked for Edman degradation (van de Kamp et al., 1995). The data reported here give us some clues to the mechanism of formation of 2-hydroxypropionyl group at position 1 from a Ser residue. The possibility that the 2-hydroxypropionyl residue is formed spontaneously after dehydration of residue 1 (resulting in a Dha residue) and subsequent cleavage of the leader sequence is disfavoured by the finding that in the epilancin-K7-(3−31)-peptide the third residue (2-oxopropionyl) (which is the first residue in this peptide; van de Kamp et al., 1995) is not converted spontaneously into a 2-hydroxypropionyl residue. Moreover, in Pep5 the first, 2-oxopropionyl residue (Kellner et al., 1989, 1991; Freund et al., 1991c) is also not converted spontaneously into a 2-hydroxybutyryl residue. We postulate that the 2-hydroxypropionyl residue is the result of four subsequent steps as follows: (a) dehydration of the Ser at position +1 in pre-epilancin K7, resulting in a Dha residue; (b) cleavage of the leader sequence by the tentative ElkP leader peptidase; (c) spontaneous conversion of Dha1 to a 2-oxopropionyl residue; (d) enzymic reductive conversion of a 2-oxopropionyl residue at position 1 to 2-hydroxypropionate on the cytoplasmic side of the cell membrane. This would be followed by export of the mature polypeptide. In line with this scheme, the ElkP peptidase lacks an N-terminal signal sequence, and thus is probably localised in the cytosol. Generally, an intracellular position of the leader peptidase may correlate with the discovery of novel types of modifications at the N-terminus of lantibiotics. In the case of epilancin K7, cleavage of the leader by ElkP (step 2) might endow the N-terminus of epilancin K7 accessible to cytoplasmic enzymes. The spontaneously formed 2-oxopropionyl (or puryvyl) residue (step 3) might be the accidental substrate for an enzyme such as lactate dehydrogenase, converting 2-oxopropionate at position 2 to a 2-hydroxypropionate residue (step 4). For reasons of simplicity, we assume that there is no requirement for a dedicated special biosynthetic enzyme, encoded by a separate ORF in the *elk* gene cluster, that completes the maturation of epilancin K7 by converting the first residue into 2-hydroxypropionate; instead, another enzyme involved in a different, unrelated metabolic route may very well be responsible for the N-terminal modification. Consistently, the activity of epilancin K7 is barely dependent on the presence of 2-hydroxypropionyl residue at position 1; the epilancin-K7-(3−31)-peptide which misses a 2-hydroxypropionyl residue at position 1 and Ala at position 2, still has approximately 75% of the activity of mature epilancin K7 (van de Kamp et al., 1995). The finding that the first 2-oxopropionyl residue at position 3 of the epilancin-K7-(3−31)-peptide is not converted into a 2-hydroxypropionyl residue, is in agreement with an intracellular location of the modification reaction, since the epilancin-K7-(3−31)-peptide is very likely the product of a degradation reaction at the Dha at position 3 that occurs during isolation and purification of epilancin K7. The latter degradation probably reflects an intrinsic chemical instability of Dha residues, since a similar vulnerability to degradation at Dha residues has been observed for nisin (Chan et al., 1989; Rollema et al., 1991).

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