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Staphylococcin 1580 Is Identical to the Lantibiotic Epidermin: Implications for the Nature of Bacteriocins from Gram-Positive Bacteria

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Staphylococcin 1580 was purified to homogeneity from culture supernatants of *Staphylococcus epidermidis* 1580 by means of adsorption to XAD 2, cation exchange chromatography, and high-performance liquid chromatography on reversed-phase C_{18}. The purified active substance migrated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis with an apparent M_r of approximately 2,000. Amino acid analysis, mass determination (2,165 Da) and N-terminal sequencing (Ile-Ala-Xaa-Lys-Phe-Ile-Xaa-Xaa-Pro-Gly-Xaa-Ala-Lys-block) demonstrated that staphylococcin 1580 is identical to epidermin, a lanthionine-containing antibiotic peptide (lantibiotic).

Staphylococcin 1580 was first isolated and characterized by Jetten et al. (12) and Jetten and Vogels (9). It was described as a bacteriocin consisting of 20-kDa subunits with a total molecular molecular mass between 150 and 400 kDa composed of 41.8% protein, 34% carbohydrate, and 21.9% lipid. Subsequently, staphylococcin 1580 was investigated in great detail with respect to its mode of action, so that it was considered to be the best-studied bacteriocin of a gram-positive bacterium (16). The mode-of-action studies revealed that staphylococcin 1580 immediately blocked macromolecular biosynthesis, inhibited active transport, induced efflux of ions and accumulated amino acids, and collapsed the proton motive force (10, 11). Moreover, an energy requirement for bacteriocin action was demonstrated (36). In analogy to the channel-forming colicins, it was concluded that its bactericidal activity is based on depolarization of the energy-transducing cytoplasmic membrane.

We have recently described the staphylococcin-like peptide Pep 5 (27). This peptide has a mass of 3,488 Da and is strongly cationic. It contains the unusual amino acids lanthionine, 3-methyllanthionine, and didehydrobutyrine (15), which make it a member of the group of lanthionine-containing peptides recently designated lantibiotics (13, 32). Lantibiotics are synthesized from prepeptides via posttranslational modifications of serine, threonine, and cysteine residues. The modification starts with dehydration of the hydroxy amino acid to yield didehydroalanine (from serine) and didehydrobutyrine (from threonine). Cysteine thiol groups are then added to the α-β double bond of some of the didehydro residues to form lanthionine and 3-methyllanthionine, respectively (37).

Mode-of-action studies conducted with Pep 5 showed strong similarities to staphylococcin 1580. Ions, amino acids, and ATP rapidly leaked from treated cells with a simultaneous drop of the membrane potential (25) and a complete cessation of macromolecular biosynthesis (28); in addition, Pep 5 forms voltage-dependent, short-lived pores of up to 1.2 nm in size in planar membranes (17). Subsequent comparative studies with other lantibiotics such as the food preservative nisin from lactococci (24, 31), subtilin from *Bacillus subtilis* (33), and epidermin (3, 26) demonstrated that this mode of action is characteristic of the elongated, screw-shaped, amphipathic type A lantibiotics (for reviews, see references 3 and 26); like Pep 5, epidermin is produced by *Staphylococcus epidermidis* (1). These results and the published composition data of staphylococcin 1580 led us to speculate that this bacteriocin in fact could be a lantibiotic which was not completely purified, particularly as hydrophobic interaction chromatographies were not employed for its purification (12).

S. epidermidis 1580 was grown in 2 liters of tryptic soy broth, cells were removed by centrifugation, and the culture supernatant was directly applied to a Servachrome XAD 2 column with a 500-ml bed volume (BV). The column was washed with 3 BVs of distilled water and 4 BVs of 50% (vol/vol) methanol. The activity, detected with *Staphylococcus simulans* 22 as an indicator, was eluted with 4 BVs of 90% methanol (vol/vol) complemented with 1 mM acetic acid and adjusted to pH 2 with 1 N HCl. After evaporation of methanol, the pH was adjusted to 5.8 with 200 mM K_2HPO_4, precipitated protein was removed by centrifugation, and the supernatant was applied to a CM Sephadex C 25 column (200-ml BV) equilibrated with 200 mM phosphate, pH 5.8. The resin was washed with 10 BVs of equilibration buffer, and the activity was eluted with a salt gradient (0 to 3 M KCl) in equilibration buffer. Active fractions were concentrated and desalted on a second XAD 2 column (100-ml BV) and subsequently lyophilized. Final purification was achieved by reversed-phase C_{18} high-performance liquid chromatography (HPLC) (Nucleosil; 5-μm particle size, 25 by 0.45 cm) using acetonitrile–0.1% (vol/vol) trifluoroacetic acid as the eluent; the flow rate was 1 ml/min, and the gradient slope was 0.5% in the range of 25 to 40% acetonitrile (Fig. 1). The active fraction was again lyophilized.

The antibacterial substance of *S. epidermidis* 1580 eluted from the reversed-phase column as a single homogeneous peak and migrated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a Coomassie blue-stainable band with an apparent molecular mass of approximately 2 kDa (Fig. 1). The exact mass was determined by ion spray mass spectrometry as described elsewhere (37). The obtained value (mean ± standard deviation) of 2,165 ± 4.1 Da is in excellent agreement with the mass of epidermin, which is 2,164.6 Da (1).

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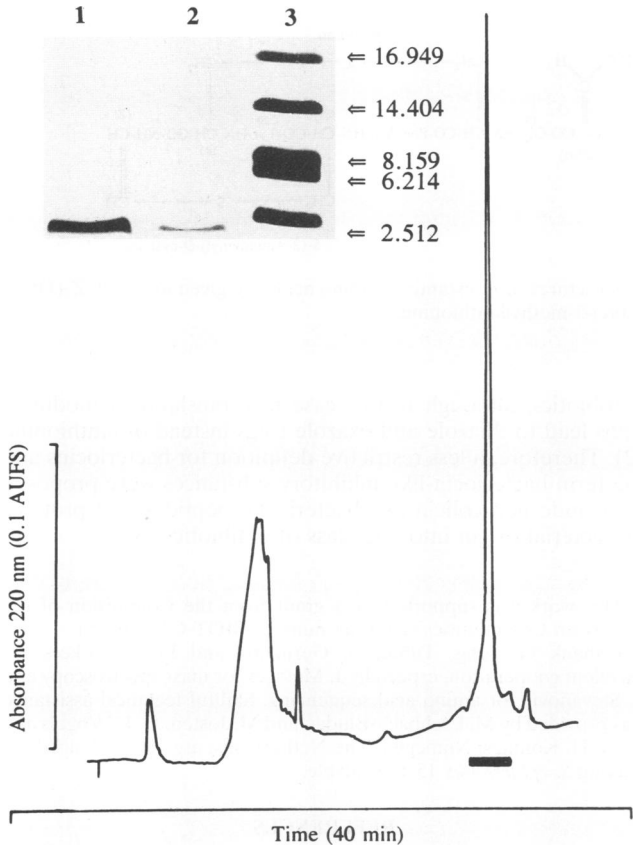

FIG. 1. Chromatography of staphylococcin 1580 on reversed-phase C_{18} HPLC. The active fraction is marked with a bar (AUFS, arbitrary units, full scale). Insert, SDS-PAGE of staphylococcin 1580: 20 μg (lane 1) and 2 μg (lane 2) of lyophilized material from the active HPLC fraction were compared with standard peptides (lane 3); the masses of the standard peptides (Merck; 15124) are given in kilodaltons. Chromatography and SDS-PAGE were performed as described elsewhere (29).

Cochromatography of the active substance from *S. epidermidis* 1580 with epidermin on reversed-phase HPLC resulted in a single peak eluting at the same position as in Fig. 1. We then hydrolyzed the peptide and analyzed its amino acid composition by the *o*-phthaldialdehyde derivatization method as described elsewhere (29). This method is advantageous for detection of lanthionine and 3-methyllanthionine, which could be clearly identified in staphylococcin 1580 (Table 1). Comparison with the amino acid composition of epidermin also suggested that both peptides are identical (Table 1). This was established by amino acid sequencing on an Applied Biosystems pulsed-liquid-phase sequencer (model 477A) and on-line phenylthiohydantoin amino acid analysis; the sequence Ile-Ala-Xaa-Lys-Phe-Ile-Xaa-Xaa-Pro-Gly-Xaa-Ala-Lys-block was found for staphylococcin 1580 and was also obtained with epidermin in control runs. Xaa marks gap positions introduced by lanthionine and 3-methyllanthionine, the bis-phenylthiohydantoin derivatives of which are not identified under routine sequencing conditions. However, these residues do not block Edman degradation, in contrast to didehydroalanine and didehydrobutyrine (for an example, see reference 15). A didehydrobutyrine residue occurs at position 14 of epidermin (Fig. 2) and prevents further sequencing.

TABLE 1. Amino acid composition of purified staphylococcin 1580 and of epidermin

Amino acid(s)	No. of residues found in:	
	Staphylococcin 1580	Epidermin[a]
Alanine	2.1	2
Aspartate or asparagine	0.9	1
Glycine	2.3	2
Isoleucine	2.0	2
Lanthionine and 3-methyllanthionine[b]	2.8	3
Lysine	2.2	2
Phenylalanine	2.0	2
Tyrosine	0.8	1

[a] Taken from the published sequence (1).

[b] Lanthionine and 3-methyllanthionine are not separated under these conditions (29). Didehydrobutyrine and the C-terminal 2-aminovinylcysteine (see Fig. 2) are destroyed during hydrolysis, and proline is not derivatized with *o*-phthaldialdehyde; therefore, these residues are not listed.

To exclude the possibility that *S. epidermidis* 1580 produces epidermin in addition to the previously characterized bacteriocin 1580, we determined activity spectra using the deferred antagonism test as described elsewhere (27) and tryptic soya agar as the medium. We compared the activity of *S. epidermidis* 1580 with those of two epidermin-producing strains, *S. epidermidis* 57 and 175 (6). Besides these strains, we used the following indicators: *S. epidermidis* V1 and V301, both producing Val-1 Leu-6 epidermin (30); *S. epidermidis* 5, producing Pep 5; *B. subtilis* ATCC 6633, producing subtilin; and the routine indicators *B. subtilis* W23, *Micrococcus luteus* ATCC 4698, *Streptococcus zymogenes* 24, and *S. simulans* 22. The activity spectra of *S. epidermidis* 1580, 57, and 175 were identical: none of these three strains inhibited an epidermin- or an epidermin variant-producing strain because of cross immunity; also, *S. zymogenes* was not inhibited by any strain. All other indicators were sensitive to all three producer strains and showed inhibition zones of 1 to 1.5 cm in diameter; *M. luteus* was extremely sensitive, with inhibition zones of more than 5 cm.

The results described above clearly demonstrate that staphylococcin 1580 is identical to the lantibiotic epidermin and that it was not completely purified when studied 20 years ago. At that time, many bacteriocins of gram-positive bacteria with physical properties (size and composition) similar to those of staphylococcin 1580 (35), e.g., the staphylococcins 414 (7), A (18), Bac R1 (23), and C55 (5) were described. In light of the above results, it is tempting to speculate that these, too, were not completely purified and represent small amphiphilic peptides such as lantibiotics. This assumption is based on the following observations. (i) The purification protocols for staphylococcins generally included salt precipitation, ion exchange chromatography, and gel filtration, the latter providing also the basis for the size estimates. These methods do not disrupt hydrophobic interactions, which are common features of amphiphilic peptides. Methods such as XAD adsorption or reversed-phase HPLC were not available or not included because, in analogy to colicins, large proteins which could not withstand drastic conditions such as high organic solvent concentrations or acidic pH were expected. Thus, it seems likely that the active substances were copurified with extracellular material from lysed cells such as cell wall fragments and micellar lipoteichoic acids. This view is supported by published data on the composition of staphylococcins and by the fact that some type A lantibiotics were found to strongly bind to these

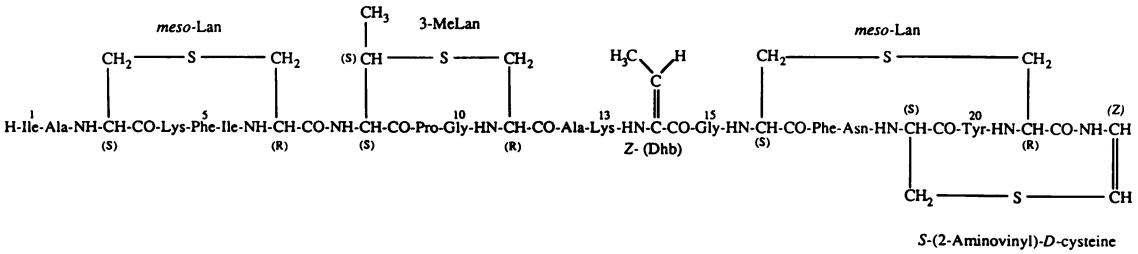

FIG. 2. Structure of the lantibiotic epidermin as taken from reference 13. Structures of nonstandard amino acids are given in detail. *Z*-(Dhb), (*Z*)-2,3-didehydrobutyrine; *meso*-Lan, (2*S*,6*R*)-lanthionine; 3-MeLan, (2*S*,3*S*,6*R*)-3-methyllanthionine.

polymers (4). When, however, denaturing conditions were included during purification, peptides smaller than 10 kDa, such as staphylococcins 462 (8) or IYS2 (20), were found. These were not reported to contain unusual amino acids, but lanthionine and 3-methyllanthionine escape routine amino acid analysis and didehydroamino acids are destroyed unless they are specifically modified before peptide hydrolysis. (ii) Mode-of-action studies with staphylococcin 1580 as well as several type A lantibiotics clearly demonstrated their ability to depolarize the energy-transducing cytoplasmic membrane which results in inhibition of biosynthesis of macromolecules. The latter was also observed for staphylococcins 414 (7), 462 (8), and C55 (5). (iii) Over the last decade, we have studied approximately 750 staphylococcal strains with respect to production of inhibitory substances. Of the strains, 13% were able to inhibit indicator strains but either lost this ability on subculturing or produced too little for characterization (30). Stable producers (a total of eight) all synthesized a lantibiotic; these included two epidermin-producing strains (6), two strains producing Val-1 Leu-6 epidermin (30), two strains producing Pep 5, and two strains producing two peptides which are currently being studied, the Pep 5 variant Pep 280 and K7, which was already described as a peptide micrococcin (21). Furthermore, Leu-6 epidermin from *Staphylococcus gallinarum* has already been described as gallidermin (14). Thus, it is obvious that lantibiotics represent the most important class of inhibitory substances from staphylococci, with epidermin and closely related variants being most commonly produced. The situation appears to be similar to that in lactococci, in which nisin and nisin Z (Asn-27 nisin) are the predominant lantibiotics (19).

Recent years have seen a remarkable revival of interest in bacteriocins of gram-positive bacteria. Numerous studies, initiated in search of novel food preservatives, led to a great number of reports on bacteriocins from, e.g., lactic acid bacteria (22). Application of appropriate purification techniques demonstrated that the majority of these bacteriocins are small polypeptides, either unmodified or modified as lantibiotics. Most authors classify such peptides as bacteriocins in spite of obvious differences from the colicins, the prototype bacteriocins. These are large, domain-structured proteins, which are translocated to their target via a complex uptake machinery including specific receptors. However, the different cell wall architecture of gram-positive bacteria does not require receptors and translocators but rather favors small peptides, reduced to the very minimum size necessary for activity, which can easily penetrate through the peptidoglycan network. Thus, the peptide bacteriocins can be regarded as the functional analogs of colicins. It should also be noted that gram-negative bacteria produce small peptides such as colicin V or microcin B17, the latter being modified similarly to lantibiotics, although in this case posttranslational modifications lead to thiazole and oxazole rings instead of lanthionine (2). Therefore, a less restrictive definition for bacteriocins and the term bacteriocin-like inhibitory substances were proposed to include non-colicin-like bactericidal peptides and proteins of bacterial origin into one class of antibiotics (34).

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