Structural gene isolation and prepeptide sequence of gallidermin, a new lanthionine containing antibiotic

Norbert Schnell, Karl-Dieter Entian, Friedrich Götz, Thomas Hörner, Roland Kellner, and Günther Jung


Received 2 December 1988
Accepted 4 December 1988

Key words: Lantibiotic; Gallidermin; Epidermin; Nucleotide sequence; Post translational modification; Staphylococcus gallinarum

1. SUMMARY

Peptide antibiotics containing lanthionine and 3-methylanthionine bridges, named lantibiotics [1], are of increasing interest. A new lantibiotic, gallidermin, has been isolated from Staphylococcus gallinarum. Here we report the isolation of its structural gene which we name gdmA. In all lantibiotics so far studied genetically, three peptides can be formally distinguished: (i) the primary translation product, which we call the prepeptide; (ii) the propeptide lacking the leader sequence and (iii) the mature lantibiotic. Unlike the plasmid-coded epidermin, gdmA is located on the chromosome. The gdmA locus codes for a 52 amino acid residue prepeptide, consisting of an α-helical leader sequence of hydrophilic character, which is separated from the C-terminus (propeptide) by a characteristic proteolytic processing site (Pro^2 Arg^1 Ile^1). Although pro-gallidermin differs from pro-epidermin (a recently isolated lantibiotic) only by a single amino acid residue exchange, Leu instead of Ile, the N-terminus of the prepeptide differs by an additional two exchanges.

2. INTRODUCTION

A group of staphylococci with common markers was discovered during studies on the characteristics of isolates from the skin of poultry. These strains belonged to a new staphylococcal species, Staphylococcus gallinarum. This species is distinguishable from the other staphylococcal species by DNA-homology, the peptidoglycan type, novobiocin resistance and the wide range of carbohydrates utilized [2].

We have found that several S. gallinarum strains produced a potent antibiotic which inhibited the growth of Gram-positive bacteria especially. One of the strains, S. gallinarum DSM4616, was investigated further. After isolation, purification and elucidation of its structure, the antibiotic, gallidermin was found to be a lanthionine-containing...
polypeptide [3]. Gallidermin is very similar to epidermin, the structure of which has already been determined [4,5]. Lantibiotics are important for studying the biosynthesis of peptides containing unusual amino acids. Nine lantibiotics have already been described. In addition to epidermin and gallidermin, they include for example the food preservative nisin [6], subtilin [7,8], ancovenin [9], Ro 09-0198 [10] and Pep5 [11]. Epidermin and gallidermin are also of potential interest for treating skin disorders associated with acne, caused by certain Gram-positive bacteria. Recently, we isolated the epidermin structural gene from \textit{S. epidermidis} and thus obtained evidence that the prepeptide of epidermin is synthesized ribosomally.

In this study we report the cloning and sequencing of the gallidermin structural gene and its homology with the epidermin gene.

3. MATERIALS AND METHODS

3.1. Strains, plasmids and media

\textit{Staphylococcus gallinarum} DSM4616 was used as a source for chromosomal and plasmid DNA. Recombinant plasmids were amplified in \textit{Escherichia coli} strain RR1 (F$^-$ hsdS20 supE44 ara-14 proA2 lacY galK2 rpsL20 xyl-5 mtl-1). \textit{S. gallinarum} and \textit{E. coli} strains were both grown on LB-media (Gibco, Neu-Isenburg, F.R.G.).

3.2. Plasmid isolation

Plasmids and chromosomal DNA were isolated from \textit{S. gallinarum} [12]. Plasmids of \textit{E. coli} were isolated according to a slightly modified Birnboim-Doly procedure [13] and afterwards purified in an ultra microcentrifuge (Beckmann TL100, rotor TLA 100.2) at 80 000 rpm for 12 h.

3.3. Molecular biological techniques

Established protocols were followed for molecular biological techniques [14]. Restriction enzyme cut DNA was eluted from the 0.7% agarose gels by the freeze-squeeze method [15]. The hybridization mix, HCl titrated to pH 7, contained sodium citrate (22 g/l), sodium chloride (44 g/l), sonicated salmon sperm DNA (1 g/l) and sodium lauroyl sarcosinate (final concentration 1%). After hybridization [16] at 31°C with 50 ng $^{32}$P-labelled synthetic oligonucleotide, Southern blots and colony filters were washed thrice, also at 31°C, with the buffer described above, to which sodium dodecyl sulfate was added to a final concentration of 0.1%.

3.4. Oligonucleotides

Oligonucleotides were synthesized on a 380B DNA synthesizer (Applied Biosystems, Weiterstadt, F.R.G.) and used without further purification.

3.5. DNA sequencing

DNA was sequenced bidirectionally by the di-deoxy chain termination method [17], using the plasmid sequencing technique [18]. Synthetic oligonucleotides were used as primers.

![Fig. 1. Southern hybridization of digested S. gallinarum chromosomal and plasmid DNA with the labelled 15 b oligonucleotide (specific activity 4000 GBq/g DNA). Lanes H, HindIII Bg, BglII E/H, EcoRI-HindIII E/Bg, EcoRI-BglII E, EcoRI digests of chromosomal DNA; lane Plas, undigested plasmids.](image-url)
4. RESULTS

4.1. Cloning of the gallidermin structural gene, gdmA

As we had recently cloned the epidermin structural gene, epiA, from a 54 kbp plasmid [1], we used one of the epidermin sequencing primers (5' CAC ATC CAG GAG TAC 3'), because of sequence similarities among the antibiotics probably also homologous to the gallidermin coding region, to screen chromosomal and plasmid DNA from S. gallinarum DSM4616. Although this S. gallinarum strain harboured several plasmids, all of them failed to hybridize with the synthetic oligonucleotide used. Instead, chromosomal DNA gave a distinct signal with the probe (Fig. 1). Thus, a 1.7 kbp HindIII/EcoRI fragment was cloned into pUC19 and was found to correspond to the gallidermin structural gene, gdmA, shown by DNA-sequencing.

4.2. Characterization of the gallidermin structural gene, gdmA

Sequencing of the gdmA gene, shown in (Fig. 2), was consistent with the previously published sequence of the gallidermin propeptide [3]. A single methionine codon is at an appropriate distance to the Shine-Dalgarno sequence. The gdmA open reading frame terminates at a TAA stop codon. Hence, the gallidermin prepeptide consists of 52

```
Fig. 2. Nucleotide sequences of the epidermin- (epiA) or gallidermin (gdmA) structural genes, respectively. Deduced amino acid sequences of pre-epidermin and -gallidermin are shown. Differing nucleotides or amino acids are indicated for epidermin in the upper, for gallidermin in the lower line, respectively. The Shine-Dalgarno sequence in front of the ATG codon is boxed and the proteolytic cleavage site at which the propeptide is processed is indicated by an arrow. The potential stop codons are given (am, amber; oc, ochre; op, opal).
```
amino acid residues including the starting methionine. The propeptide sequence of gallidermin corresponds to the C-terminus of the prepeptide. At a characteristic proteolytic cleavage site (Pro-2 Arg-1 Ile1), the propeptide is matured from the prepeptide. The N-terminus extends the propeptide by 30 amino acid residues (Fig. 2).

4.3. Homologies between pre-gallidermin and pre-epidermin

As recently reported, the propeptides of gallidermin and epidermin differ in one amino acid residue. Ile6 from epidermin is replaced by Leu6 in the gallidermin propeptide (Fig. 2). Comparison of the DNA sequences, however, revealed stronger differences, but 30 bp upstream of the start codon are highly conserved. This region includes the ribosomal binding site. Within the leader sequences of the peptides, two differences occur within their primary prepeptide structures. Glu-22 (gallidermin) replaces Asp-22 (epidermin) and Asp-19 (gallidermin) replaces Asn-19 (epidermin) (Fig. 2). Homology is 85% for the coding nucleotide sequences, whereas 94% of the amino acids are conserved. This finding might justify the following conclusions. (i) The antibiotic function of epidermin and gallidermin depends on a strongly conserved propeptide sequence and (ii) the N-terminal part of the prepeptide is also important during lantibiotic biosynthesis and only minor changes are possible.

5. DISCUSSION

Both the structural elucidation of gdmA and its similarity to epiA clearly indicate that both genes originated from a common ancestor, but the DNA structure of S. gallinarum and S. epidermidis have diverged. This is in agreement with earlier experiments, where the DNA-DNA-hybridization between the organisms was determined to be 21% [2], and is exemplified at the molecular level for the respective lantibiotic structural genes in the present study. Strong evolutionary pressure must have conserved the structures necessary for antibiotic function and biosynthesis. Schnell et al. [1] have proposed an important role for the N-terminal amino acids in lantibiotic biosynthesis. Perhaps post-translational modification reactions are dependent on the α-helical conformation of the N-terminus. These maturation reactions are serine- and threonine-dehydratization, nucleophilic sulfur addition to the resulting double bonds and decarboxylation of the C-terminal amino acid residue, which probably yields the C-terminal unsaturated structure. As within epidermin, gallidermin’s 30 amino terminal residues are mostly hydrophilic, assuming partly at least, an α-helical conformation in an appropriate environment. The two alterations in comparison to epidermin leave the structure unchanged, thus strengthening the hypothesis of its importance, either in lantibiotic maturation or secretion, or possibly both.

The above results give evidence of a completely identical biosynthetic pathway for the production of both gallidermin and epidermin. The isolation of their respective structural genes will provide a solid basis for future studies on the biosynthesis of lantibiotics.

ACKNOWLEDGEMENTS

This work was supported by Deutsche Forschungsgemeinschaft (SFB 323) and by the Fonds der Chemischen Industrie. We thank L. Hofmann for oligonucleotide synthesis and C. Kaletta and Prof. D. Mecke for discussions. Dr. J.A. Barnett is thanked for critical reading of the manuscript. N.S. is supported by a Dechema grant and K.-D.E. was a recipient of a Heisenberg grant of the Deutsche Forschungsgemeinschaft.

REFERENCES