Structure of the pheromone peptide of the *Staphylococcus epidermidis agr* system

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Abstract The *agr* quorum-sensing system is responsible for the regulation of several virulence factors in staphylococci, with an extracellular pheromone peptide as signalling molecule. By monitoring the biological activity of synthetic peptides, it could be demonstrated that the pheromone of the *agr* system in *Staphylococcus epidermidis* is an octapeptide containing a thiolester linkage between the central cysteine and the C-terminal carboxyl group. The peptide was active at nanomolar concentrations. The N-terminus of the peptide pheromone, which is encoded as part of a protein precursor, proved to be crucial for biological activity.

Key words: Agr; Quorum sensing; Posttranslational modification; Pheromone; *Staphylococcus epidermidis*

1. Introduction

The accessory gene regulator (*agr*) of *Staphylococcus aureus* controls the expression of several virulence factors, including α-toxin, β-toxin, δ-toxin, serine protease, DNase, fibrinolysin, enterotoxin B and toxic shock syndrome toxin-1. Through the activities of the *agr* system, the production of these exoproteins is increased in post-exponential growth phase, whereas the expression of cell wall-associated proteins such as protein A, coagulase, and fibronectin-binding protein is decreased [1–4].

Among the coagulase-negative staphylococci, *S. epidermidis* is considered the prime nosocomial pathogen. The nature of the regulation of virulence factors in *S. epidermidis*, including proteases, δ-toxin, lipase and exopolysaccharide ('slime'), is unknown. However, an *agr*-like system would be a likely candidate for such regulation, and has been proposed, for example, in the case of slime production [5].

The *agr* locus of *S. aureus*, about 3.5 kbp in size, comprises the *agrA*, *agrC*, *agrD* and *agrB* genes, which are cotranscribed (RNAII), and the gene for a regulatory RNA molecule, RNAIII, which also encodes the gene for the δ-toxin (*hld*). RNAIII controls the expression of target genes in an unknown fashion. Transcription of RNAII is controlled by the P2 promoter, and transcription of RNAIII by the P3 promoter [6–8].

The gene products of the *agrA* and *agrC* genes form a classical two-component regulatory system. AgrC is a membrane-associated signal-dependent histidine kinase, which, upon binding of an extracellular signal, is autophosphorylated at a histidine residue [7]. As a next step, the response regulator protein, AgrA, is phosphorylated and activates the transcription of RNAII and RNAIII in an unknown manner. It has been shown that this activation is dependent on the presence of an additional regulatory protein, SarA [9,10]. The roles of AgrB and AgrD have recently become more clear. A small peptide is excised from the AgrD protein, modified, and secreted into the surrounding medium. This peptide represents the autoinductive signal of the *agr* system [11,12] and has been regarded as a pheromone because it signals the state of cell density to all members of the bacterial population and, accordingly, represents a means of cell-cell communication [13]. AgrB seems to be involved in the maturation and secretion process of the modified peptide. The sequences of the pheromone peptides vary among different groups of *S. aureus* strains, and the pheromone peptide of one group can inhibit the expression of the *agr* system of another group, while stimulating expression in cells of its own group [12].

Here we report the sequence of the *agr* system in *S. epidermidis*, which appears to be very similar to the analogous sequences in *S. aureus* and *S. lugdunensis*. The region within AgrD that encodes the peptide pheromone was sequenced in 15 *S. epidermidis* strains; only a single type of peptide pheromone sequence was found. The biological activity of synthetic peptides was determined by assaying the expression of a reporter gene that was under the control of the *S. epidermidis agr* P3 promoter. Our results provide evidence that the synthetic octapeptide DSVCASYF, which was modified by the introduction of a thiolester bond between the cysteine thiol and the C-terminal carboxyl group, is identical to the naturally occurring inducing agent.

2. Materials and methods

2.1. Bacterial strains

S. epidermidis ATCC 14990, *S. epidermidis* Tü3298 (DSM 3095 [14]), *S. epidermidis* O-47 [15], *S. epidermidis* RP62A [16], and 12 *S. epidermidis* strains of hospital origin (kindly provided by Prof. G. Pulverer, Universität Köln) were used for sequence determination of the entire, or parts of, the *agr* coding region.

S. epidermidis Tü3298 (DSM 3095) was the host for the promoter test plasmid pRB594P3.

2.2. Plasmids

The promoter test plasmid pRB594 is a derivative of pRB373 [17]. It contains the promoterless pUB112 *cat* gene [18] adjacent to a multiple cloning site and carries the erythromycin resistance gene *ermB* from transposon Tn551. The plasmid pRB594P3 was constructed by insertion of a *Bam*HI-digested PCR product with the *agr* P3 region (and the P2 region in the opposite orientation) of *S. epidermidis* ATCC 14990 into the *Bam*HI site of the MCS. *Bam*HI sites were introduced via the PCR primers at both ends of the PCR product. The fidelity and orientation of the inserted PCR product were determined by DNA sequencing.

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2.3. General methods

Escherichia coli and staphylococcal plasmid DNA was prepared using Nucleobond AX-100 columns (Macherey-Nagel, Düren, Germany). For staphylococci, cells were first treated with 25 μg/ml lysostaphin (Sigma, St. Louis, MO). Transformation of *S. epidermidis* was performed by electroporation as described [19]. *E. coli*-specific recombinant DNA techniques were performed according to standard procedures [20]. Computer analyses of DNA and polypeptide sequences were performed using MacDNASIS Pro (Hitachi Software Engineering, San Bruno, CA). Enzymes for molecular cloning were obtained from Boehringer-Mannheim or New England Biolabs. *S. epidermidis* cells were grown in BM (1% tryptone (Difco), 0.5% yeast extract (Gibco BRL), 0.5% NaCl, 0.1% K_2HPO_4, 0.1% glucose). Staphylococcal cells were disrupted in 20 mM Tris-HCl (pH 7.8) by glass beads as described [21]. Cell debris was removed by centrifugation (10 min, 5000×*g*). PCR was performed using Vent DNA polymerase (New England Biolabs). PCR primers were purchased from Interactiva (Ulm, Germany). Chromosomal staphylococcal DNA was prepared according to the method of Mamur [22].

2.4. DNA sequence analysis

DNA was sequenced by cycle sequencing on a DNA sequencer 4000 L (LI-COR Inc., Lincoln, NE) using the Thermo Sequenase fluorescent-labelled prime cycle sequencing kit (Amersham, Little Chalfont, UK). All fluorescent-labelled primers were purchased from MWG-Biotech (Ebersberg, Germany). The first oligonucleotide that was used for sequence determination by primer walking was 5′-GGCAGCAGATATCATTTCAACAATCGG-3′.

2.5. Electrospray mass spectrometry

ESI-MS was performed on an API III TAGA Triple Quadrupole (Perkin Elmer Sciex, Thornhill, Ont., Canada). Samples were dissolved in acetonitrile/water (1:1) and introduced into the ion source at a constant flow rate of 70 μl/min. The orifice voltage was set at 80 V.

2.6. Preparative and analytical HPLC

Purification of crude peptides was performed on a Waters 600 Multi Solvent Delivery System equipped with a Lambda Max Model 481 as detector. A semipreparative column (Nucleosil C18, 4×250 mm; 5 μm; Grom, Herrenberg, Germany) was used at a flow rate of 3.5 ml/min and a linear gradient (10–100% B in 45 min; solvent A: 0.1% TFA (trifluoroacetic acid) in water; solvent B: 0.1% TFA in acetonitrile) with λ = 214 nm as detection wavelength.

The concentration of purified peptides, which were redissolved in DMSO (dimethylsulphoxide), was determined using analytical HPLC on a Kontron HPLC system with Kroma System 2000 software. An analytical column (Spherisorb ODS2 2×100 mm; 5 μm; Grom, Herrenberg, Germany) was used at a flow rate of 250 μl/min and a linear gradient (0–100% B in 30 min; solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in acetonitrile) with λ = 214 nm as detection wavelength. A weighed sample of the (unmodified) peptide DSVCASYF was used as a reference.

2.7. Synthesis of peptides

The linear and cyclic peptides (SVCASYF/DSVCASYF/GDSVCASYF) were synthesised manually using the fluorenylmethoxycarbonyl (Fmoc) protocol for solid phase synthesis. All amino acids were from Novabiochem (Läufelfingen, Switzerland). A Trityl-resin (PepChem; Clausen and Goldammer, Tübingen, Germany) was loaded with Fmoc-phenylalanine residue used as solid support. Resin substitution was 0.7 mmol/g and 0.1 mmol amino acid was used for each coupling. The *tert*-butyl group was used to protect serine and tyrosine residues. For linear peptides, a trityl group and for cyclised peptides a methoxytrityl group (Mmt) was used to protect cysteine residues. The amino acids were coupled using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and hydroxybenzotriazole (TBTU/HOBt). Deprotection of the Fmoc-amino acid attached to the resin was accomplished by using piperidine. For the N-terminal amino acids, Boc (*tert*-butyloxycarbonyl) protection was used. Linear peptides were directly cleaved with TFA:TIS (triisopropylsilane) (95:5). The synthesis route for cyclic peptides comprised cleavage of the peptides from the resin and removal of the Mmt group performed in a one-step procedure using DCM (dichloromethane):TFA:TIS (94:1:5) for 15 min. The filtrates were evaporated, dissolved in DCM and cyclised with DCC (dicyclohexylcarbodiimide)/DMAP (4-dimethylaminopyridine) (3 eq.) for 12 h [23]. The solvents were then evaporated and the other protecting groups were finally cleaved with TFA:water (90:10) for 2 h. The TFA was removed and the peptides were dissolved in acetonitrile:water (1:1). The solutions were filtered and purified by preparative HPLC. The HPLC fractions were collected and lyophilised. Purity of peptides was confirmed by HPLC and ESI-MS.

2.8. Chloramphenicol acetyltransferase (CAT) assay

CAT activity was determined according to the method of Shaw [24]. The assay mixture contained 100 mM Tris-HCl (pH 7.8), 0.1 mM acetyl-Coenzyme A, and 0.4 mg/ml 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). Assays were performed in 96-well microtitre plates using a SpectraMax 340 microtitre plate reader (Molecular Devices, Sunnyvale, CA) with SpectraMaxPro software. 5 μl cell extract and 5 μl 5 mM chloramphenicol in 100% ethanol (or 5 μl 100% ethanol in controls) were added to 90 μl of the assay mixture. Cell extracts were diluted 1:10 or 1:100 with 20 mM Tris-HCl (pH 7.8) when necessary. Absorption at 412 nm was measured every 15 s for 20 min. The linear part of the resulting curve was used to determine the CAT activity (absorption coefficient ε = 13 600 M^{-1} for DTNB). For calculation of the specific activity, protein contents of the cell extracts were determined using the Bio-Rad DC protein assay for detergent-containing samples (Bio-Rad Laboratories GmbH, Munich, Germany).

2.9. Sequence data

Sequence data have been submitted to the GenBank database under accession number AF012132.

3. Results

3.1. The peptide pheromone of the S. epidermidis agr system

The presence of the *agr* system in the nosocomial pathogen *S. epidermidis* was shown by sequencing the entire *agr* coding region in strains ATCC 14990 and Tü3298 (see below). The

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                      10        20        30        40        50
S. lugdunensis      1 MNLLSGLFTK GISAIFEFIG NFSAQDICNA YFDEPEVFQE LIDLQRKQLIESV
S. aureus 1         1 MNTLFNLFFD FITGILKNIG NIAAYSTCDF IMDEVEVFKE LTQLHE
S. epidermidis      1 MEIIFNLFIK FFTTILEFIG TVAGDSVCAS YFDEPEVFEE LTKLYE
S. aureus 3         1 MKKLLNKVIE LLVDFFNSIG YRAAYINCDF LLDEAEVFKE LTQLHE
S. aureus 2         1 MNTLVNMFFD FIIKLAKAIG IVGGVNACSS LFDEPKVFAE LTNLYDK
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Fig. 1. Comparison of the AgrD proteins of *S. aureus*, *S. lugdunensis*, and *S. epidermidis*. The AgrD protein comprises the sequence of the peptide pheromone, which is cleaved from AgrD and posttranslationally modified by AgrB [11,12]. The sequences of the peptides purified from *S. aureus* subgroups and from *S. lugdunensis* [11,12] are underlined. Identical amino acid boxes within the AgrD sequence in the N-terminal and C-terminal vicinity of the peptide pheromone sequence are shown. The important Cys residue is in bold letters. The sequence directly C-terminal to the pheromone sequence is more conserved than the N-terminal sequence. The lengths of the pheromone peptide sequences of *S. aureus* and *S. lugdunensis* are within 7–9 amino acids [12]. In this study we demonstrate that the modified octapeptide DSVCASYF (the sequence is identical in 15 different *S. epidermidis* strains) is the biologically active component, whereas the modified nonapeptide GDSVCASYF and the modified heptapeptide SVCASYF show almost no activity.

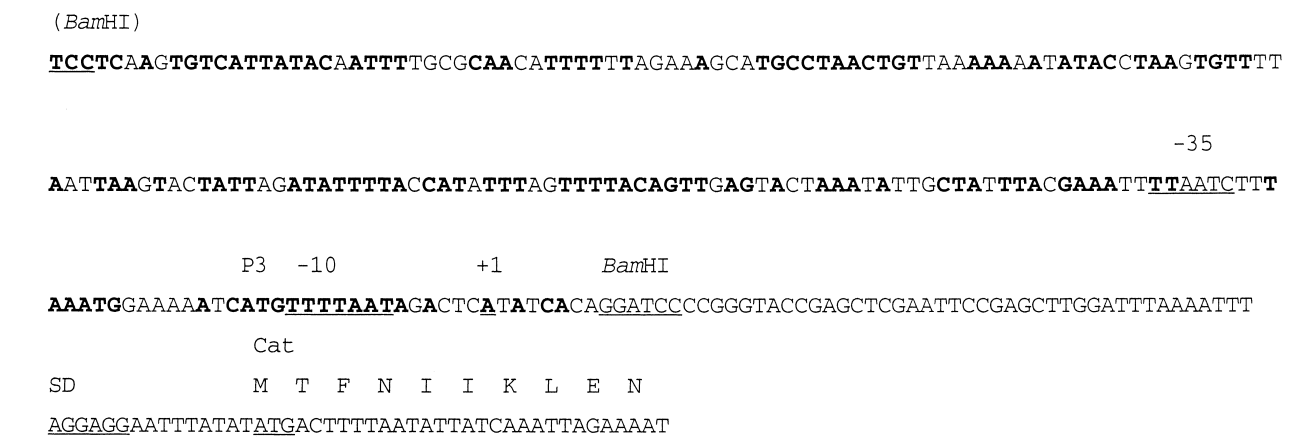

Fig. 2. P2/P3 promoter region of *S. epidermidis* ATCC 14990 cloned into pRB594. The figure shows the *Bam*HI fragment obtained by PCR amplification comprising the P2/P3 promoter region of *S. epidermidis* ATCC 14990 which was cloned into the promoter test plasmid pRB594. Nucleotides shown in bold letters are identical to the *S. aureus* sequence [3]. The −35 and −10 regions of the P3 promoter, and the transcription start site of RNAIII were deduced from the homology to the *S. aureus* sequence. The Shine-Dalgarno (SD) sequence and the translation start with the N-terminal sequence of the CAT reporter enzyme are marked.

coding region of only the peptide pheromone was sequenced in 15 *S. epidermidis* strains (strains ATCC 14990, Tü3298, O-47 and 12 strains of hospital origin). The corresponding amino acid sequence was the same in all strains sequenced.

The pheromone peptide of the *agr* system has been described in *S. aureus*. The mass difference of −18 between the synthetic, unmodified and the purified pheromone peptide of *S. aureus* implied that the modification necessary for biological activity could be a dehydration reaction. Cleavage analyses suggested the presence of a thiolester bond. It has been proposed that this thiolester bond is formed between the central cysteine thiol and the C-terminal carboxyl group [11,12]. In order to prove that the active peptide in vivo contains the hypothetical thiolactone structure, the stimulatory biological activity of a pure synthesised thiolactone-containing peptide needed to be demonstrated.

The pheromones found in *S. aureus* and *S. lugdunensis* are hepta-, octa- or nonapeptides, which differ in the length of the N-terminus, whereas the distance from the central cysteine to the C-terminus is conserved (Fig. 1). In order to elucidate the type of modification in the pheromone as well as to analyse the influence of the N-terminal structure of the *S. epidermidis* peptide pheromone on biological activity, we synthesised three peptides with lengths of 7, 8 or 9 amino acids, each differing in the length of the N-terminus (SVCASYF, DSVCASYF, GDSVCASYF). The primary sequence was based on the

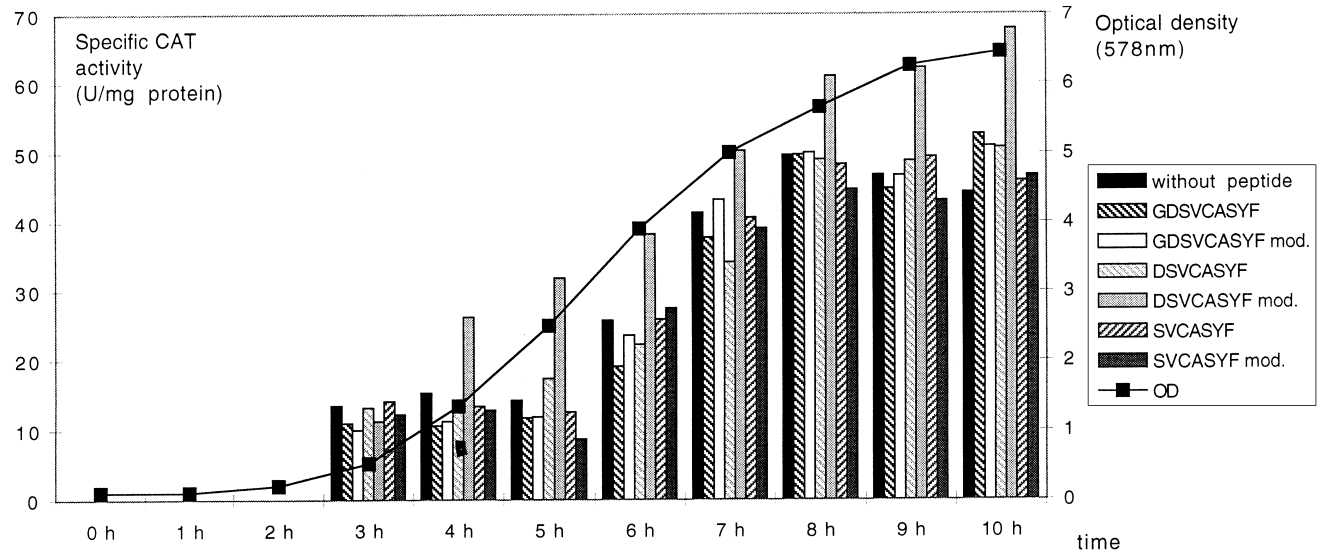

Fig. 3. CAT expression in *S. epidermidis* Tü3298 (pRB594P3) upon addition of various synthesised peptides. Flasks containing 100 ml BM were inoculated with 1/100 vol. of an *S. epidermidis* Tü3298 (pRB594P3) preculture and grown with aeration for 10 h. The plasmid pRB594P3 harbours the *cat* reporter gene under the control of the *agr* P3 promoter. Optical densities of the cultures were determined hourly. Growth curves of all cultures were comparable. After 3 h of growth, the peptides SVCASYF, DSVCASYF, GDSVCASYF, as well as their thiolactone-containing modified counterparts ('mod.'), were added to a final concentration of 20 nM. From this time on, cell extracts were prepared from samples taken every hour. Specific CAT activities were calculated from CAT activities and protein content determined in triplicate in every cell extract. Black bars show the CAT activities in the control to which no peptide was added, where CAT activity is affected only by the host-encoded *agr* system.

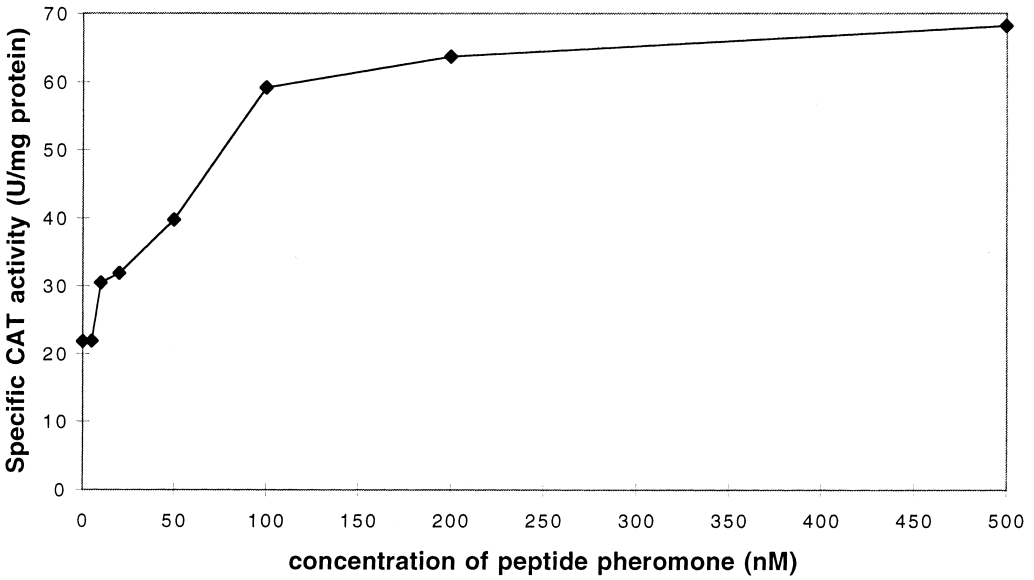

Fig. 4. CAT activity in cultures of *S. epidermidis* (pRB594P3) upon addition of different concentrations of the thiolactone-containing peptide DSVCASYF. Flasks containing 50 ml BM were inoculated with precultures of *S. epidermidis* (pRB594P3), which contained the *cat* reporter gene under control of the *agr* P3 promoter, and were grown with aeration for 3 h. The thiolactone-containing peptide DSVCASYF was then added to different final concentrations of 5, 10, 20, 50, 100, 200, and 500 nM. No peptide was added to the control. After an additional 2 h of growth, cells were harvested, disrupted and the CAT activities as well as the protein content of the cell extracts were determined in triplicate. Specific CAT activities were calculated from the values obtained.

DNA sequence encoding the peptide pheromone of *S. epidermidis*. In addition to the linear unmodified peptides, cyclic peptides with a thiolester bond between the cysteine thiol and the C-terminal carboxyl group were synthesised. The peptides were shown by ESI-MS and analytical HPLC to be 100% pure. The peptides were diluted in DMSO and the concentrations were determined by analytical HPLC (absorption at 214 nm) relative to a weighed standard of the unmodified peptide DSVCASYF.

In order to assay the biological activity of the synthetic peptides, we developed an assay that uses CAT as a reporter enzyme. The *cat* gene was cloned under the control of the *S. epidermidis agr* P3 promoter region (Fig. 2). The resulting promoter test plasmid pRB594P3 harbours an erythromycin resistance gene. It was transformed in the erythromycin-sensitive *S. epidermidis* Tü3298.

By measurement of CAT activity at different times during the growth of *S. epidermidis* Tü3298 (pRB594P3), we could demonstrate that the activity of the *agr* system in *S. epidermidis* Tü3298 is growth phase-dependent. At the end of exponential growth phase the CAT activity increased rapidly to a level that was maintained during stationary phase (Fig. 3, black bars).

When added to the test strain cultures of *S. epidermidis* Tü3298 (pRB594P3), the correct synthetic pheromone should increase CAT expression by its action on the host-encoded AgrA/AgrC two-component regulatory system, which regulates transcription from the P3 promoter. Because the host-encoded *agr* peptide pheromone was assumed to superimpose the exogenous activity during stationary phase, the synthetic peptides were added 3 h after inoculation when the background CAT activity was still low. All synthesised modified and unmodified peptides were applied at a concentration of 20 nM in this experiment (Fig. 3). None of the unmodified peptides, the modified hepta- or the modified nonapeptide increased CAT activity when compared to the control. A significant increase of CAT activity (twofold after 1 h of growth) was only found when the thiolactone-containing octapeptide DSVCASYF was added. This strongly suggests that this peptide is identical to the naturally occurring inducing agent, i.e. the natural *agr* peptide pheromone in *S. epidermidis*.

The relative inducing effect of the modified DSVCASYF peptide was highest at 1 h and 2 h after addition, and decreased afterwards, probably due to the onset of host-encoded pheromone expression. In the sample containing the modified DSVCASYF peptide, CAT activity was still somewhat higher in stationary phase compared to the controls (approximately 20%), which implies that the activity of the peptide added early in growth phase is still present after several hours.

The concentration-dependent effect of the modified octapeptide DSVCASYF on CAT expression was determined using concentrations between 5 and 500 nM (Fig. 4). CAT activities were determined 2 h after addition of the peptide. A concentration of 10 nM was sufficient to result in a detectable increase in CAT activity, whereas concentrations higher than 100 nM could only slightly further increase the effect.

At a concentration of 200 nM, which is 10 times the concentration used in the experiment illustrated in Fig. 3, the

Table 1
Inducing activities of the modified (thiolactone-containing) and unmodified peptides at a concentration of 200 nM

Peptide (200 nM)	Specific CAT activity (U/mg protein)
Without peptide	12.5
GDSVCASYF	11.2
GDSVCASYF modified	15.6
DSVCASYF	9.8
DSVCASYF modified	53.3
SVCASYF	8.9
SVCASYF modified	16.1

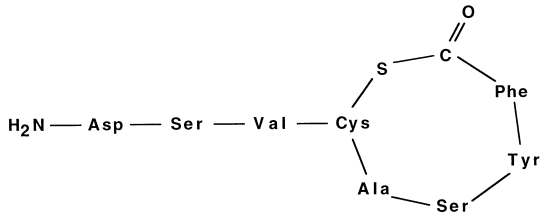

Fig. 5. Structure of the *agr* peptide pheromone of *Staphylococcus epidermidis*. The structure of the pheromone molecule was determined in this study by demonstration of the biological activity of a chemically synthesised compound. It consists of the peptide DSVCASYF containing a thiolester linkage between the central cysteine and the C-terminal carboxyl group. Only the central cysteine, but not the rest of the amino acid sequence, is conserved in the *agr* type of pheromones [12].

unmodified peptides still did not show any effect, whereas the two modified peptides (thiolactone-containing GDSVCASYF and SVCASYF) seemed to cause a small stimulatory effect (Table 1). However, compared to the activity of the thiolactone-containing DSVCASYF, this effect was almost negligible.

3.2. Sequence analysis of the S. epidermidis agr system

The sequence of the *S. epidermidis agr* system was determined by primer walking. The first oligonucleotide that was used for sequencing was based on the DNA sequence of the 5′ end of the coding region for the δ-toxin in *S. aureus* and the N-terminal amino acid sequence of the δ-toxin of *S. epidermidis* Tü3298. The entire coding region of the *agr* system was sequenced in *S. epidermidis* strains ATCC 14990 and Tü3298. The DNA sequences of the two strains were almost identical.

The *hld* region was sequenced in two additional *S. epidermidis* strains (O-47, RP62A). The δ-toxin amino acid sequence of RP62A was the same as that of *S. epidermidis* ATCC 14990 and Tü3298. The *hld* sequence of *S. epidermidis* O-47 revealed an additional methionine codon directly upstream of the coding region, which was the consequence of a transition point mutation at position −1 from A to G.

4. Discussion

4.1. Biological activity of the pheromone peptide

Our results show that the *agr* system is present and functional in *S. epidermidis*. The *agr* system is known to play a pivotal role in the regulation of virulence factors in *S. aureus* [1–4] and we assume that it has the same role in *S. epidermidis*.

The biological activity of a chemically synthesised octapeptide containing a thiolester bond between the central cysteine thiol and the C-terminal carboxyl group was demonstrated, which strongly suggests that this compound is the naturally occurring pheromone of the *S. epidermidis agr* system (Fig. 5). This also implies that the pheromones of the highly similar systems of *S. lugdunensis* and *S. aureus*, where the *agr* pheromone was first described [11], most likely harbour the same kind of posttranslational modification.

In our assay, a detectable activity of the pheromone could be found at a minimal concentration of 10 nM. At concentrations higher than 100 nM, the effect could be further increased only slightly. We therefore assume that the pheromone peptide concentration threshold which leads to the activation of the *agr* system is within this concentration range.

Because the synthetic hepta- and nonapeptides containing thiolester bonds that were derived from the *agrD* DNA sequence did not reveal significant biological activity, the structure at the N-terminus of the pheromone seems to be very important for activity. This also means that the enzyme(s) excising the pheromone from the AgrD precursor must work with high precision.

After addition of the pheromone in the exponential growth phase, an increased reporter enzyme activity was still detectable in stationary growth phase when compared to controls suggesting that the pheromone is stable at least for several hours in the extracellular medium.

Posttranslationally modified peptides are often found as mediators of bacterial cell-cell communication in Gram-positive bacteria [13,25]. The nature of many of these modifications is still unknown. The thiolactone structure has not previously been found in signalling molecules of bacteria. There is some structural similarity to other quorum sensing systems, which use non-peptide pheromones with oxygen- (instead of sulphur-) containing lactone structures. The quorum sensing signal molecules of many Gram-negative bacteria are *N*-acylhomoserine lactones [26]. In the Gram-positive bacterium *Streptomyces griseus*, the control of antibiotic synthesis is mediated by a γ-butyrolactone [27]. Thus, the lactone motif appears to be widespread in cell-cell communication systems, and staphylococci, by posttranslational modification of peptides, seem to have developed a different approach for its synthesis. An interesting question is what makes this chemical structure so suitable for signalling purposes.

4.2. Sequence analysis of the S. epidermidis agr system

DNA sequence data from *S. epidermidis* ATCC 14990 showed that the entire *agr* system of *S. epidermidis* is very similar to the system of *S. aureus*. The response regulator AgrA shows the highest identity to that of *S. aureus* (87.3%). The histidine kinase AgrC (overall identity to *S. aureus* AgrC 50.5%) has a pronounced similarity to *S. aureus* in the C-terminal part of the protein, whereas the N-terminal part shows only low similarity. Comparison of the *S. aureus* and *S. lugdunensis* AgrC sequences had already led to speculation that the N-terminal portion might represent the region that binds the structurally divergent pheromone peptides, whereas the C-terminal region interacts with the highly conserved response regulator AgrA [12], a hypothesis which is supported by our sequence data.

The AgrD sequence reveals a pronounced similarity in the region directly C-terminal to the pheromone peptide (Fig. 3). This region might represent a structural element important for the modifying reaction presumably carried out by AgrB. With the exception of the central cysteine residue, the sequence of the *S. epidermidis* peptide pheromone is not related to any known *S. aureus* subgroup, nor to *S. lugdunensis*. In the *agr* type of pheromones, only the central cysteine and its distance to the C-terminus is conserved. These conserved structural features are probably important for thiolactone formation. Sequence data obtained from 15 *S. epidermidis* strains showed that *S. epidermidis* does not reveal a heterogeneity of the peptide pheromones as has been found in *S. aureus* [12], at least among the strains investigated in this study.

The protein believed to be involved in the maturation of the

peptide pheromone, AgrB, shows a relatively high identity to those of *S. aureus* (51.3%) and *S. lugdunensis* (53.7%). The peptide sequence of the *S. epidermidis* δ-toxin has already been known for some time [28]. As in *S. aureus*, it appears to be encoded within the RNAIII region. In contrast, the RNAIII region of *S. lugdunensis* encodes no δ-toxin-like peptides [29].

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