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The molecular organization of the lysostaphin gene and its sequences repeated in tandem

Peter Heinrich [1], Ralf Rosenstein [3], Maria Böhmer [3], Peter Sonner [2], and Friedrich Götz [3]

[1] Institut für Biochemie der Universität München
[2] Zoologisches Institut der Universität München
[3] Lehrstuhl für Mikrobiologie, Technische Universität München, Arcisstrasse 21, D-8000 München 2, Federal Republic of Germany

Summary. The gene encoding lysostaphin of *Staphylococcus staphylolyticus* was cloned in *Escherichia coli* and its DNA sequence was determined. The complete coding region comprises 1440 base pairs corresponding to a precursor of 480 amino acids (molecular weight 51669). It was shown by NH_2-terminal amino acid sequence analysis of the purified extracellular lysostaphin from *S. staphylolyticus* that the mature lysostaphin consists of 246 amino acid residues (molecular weight 26926). Polyacrylamide gel electrophoresis revealed a similar molecular weight for the most active form. By computer analysis the secondary protein structure was predicted. It revealed three distinct regions in the precursor protein: a typical signal peptide (ca. 38 aa), a hydrophilic and highly ordered protein domain with 14 repetitive sequences (296 aa) and the hydrophobic mature lysostaphin. The lysostaphin precursor protein appears to be organized as a preprolysostaphin.

Key words: Lysostaphin gene – Preprolysostaphin – Repetitive sequences – Secondary structure – *Staphylococcus staphylolyticus*

Introduction

Lysostaphin has been reported as an extracellular enzyme of *Staphylococcus staphylolyticus* that lyses *S. aureus* cells (Schindler and Schuhardt 1964). Purification and characterization of lysostaphin revealed that the cell wall lytic activity is due to a glycylglycine endopeptidase which is capable of specifically lysing staphylococcal cells (Schindler and Schuhardt 1965). The endopeptidase is a zinc-metalloenzyme with a molecular weight of 25000, an isoelectric point of 9.5 and a pH optimum of 7.5 (Browder et al. 1965; Trayer and Buckley 1970). The main target of the endopeptidase is the staphylococcal interpeptide bridge which in *S. aureus* and some other staphylococcal species is composed of five glycine residues (Schleifer and Kandler 1972). If two or more glycine residues of the interpeptide bridge are replaced by serine, as in *S. epidermidis*, the cell become less susceptible to lysostaphin (Schleifer and Kloos 1975; Robinson et al. 1979).

Lysostaphin is indispensable to staphylococcal genetics and is used for DNA isolation and protoplast formation. In this study we report on the cloning and sequencing of the lysostaphin gene and on the secondary structure of the protein.

Abbreviations: aa, amino acid(s)

Offprint requests to: F. Götz

Materials and methods

Bacterial strains and plasmids. Staphylococcus staphylolyticus ATCC1362 (NRRL-B2628) was the DNA donor strain. DNA from *S. staphylolyticus* was cloned in *Escherichia coli* RR28, $r^-m^-recA^-$ (Hennecke et al. 1982). Subcloning of the lysostaphin-containing DNA fragment was carried out with pUC19 as vector and *E. coli* JM83 (*ara, Δlac-proAB, rpsL, φ80, lacZΔ*M15) as host organism (Yanisch-Perron et al. 1985).

DNA preparation. Chromosomal DNA of *S. staphylolyticus* was prepared essentially by the cleared lysate method as described by Novick and Bouanchaud (1971). Cells were lysed with a high concentration of lysostaphin (50 μg/ml) and the DNA was isolated after CsCl-centrifugation. The supercoiled plasmid DNAs of *E. coli* were prepared by the procedure of Birnboim and Doly (1979) with a few modifications. Briefly, cells from 5 ml of bacterial cultures grown overnight were spun and resuspended in 2.5 ml TSE buffer (10 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 10 mM EDTA). Following centrifugation, 0.1 ml lysozyme solution (10 mg/ml freshly dissolved in 50 mM glucose, 50 mM Tris-HCl, pH 8.0, and 10 mM EDTA) were added, the tubes were incubated at room temperature for 5 min and gently shaken after addition of 0.2 ml of freshly prepared SDS solution (1% sodiumdodecylsulfate in 0.2 M NaOH) Upon adding 0.15 ml of 3 M sodiumacetate solution, pH 4.8, tubes were gently shaken and incubated on ice for 15 min. Following centrifugation, the supernatants were extracted with phenol/chloroform and DNA was precipitated by adding 2.5 volumes of ethanol at room temperature and centrifuging. The dried pellets were dissolved in 0.1 ml of RNase buffer (0.1 mg RNase A per millilitre 10 mM Tris-HCl, pH 7.5, 15 mM NaCl) and incubated for 15 min at room temperature. Water was added to a final volume of 400 μl and the samples were extracted with phenol/chloroform and chloroform. Upon addition of a one-tenth volume of 3 M sodium acetate, pH 5.2, the DNAs were recovered by ethanol precipitation. The pellets were rinsed with 70% ethanol, dried in vacuo and dissolved in 20 μl TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA).

Targeting of deletions. The unidirectional digestion of DNA, first cut with two different restriction enzymes generating 5′ and 3′ protrusions and then treated with exonuclease III and S1 nuclease allows a very defined removal of DNA for sequencing (Henikoff 1984) or mapping of biological functions. The DNA was cloned into the polylinker of the *Eco*RI/*Sma*I site of pUC19 (Yanisch-Perron et al. 1985); 5 µg of the recombinant plasmid were double digested with *Pst*I/*Bam*HI, phenol extracted, ethanol precipitated and taken up in 25 µl of 10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0, 10 µl 5× exo-buffer (330 mM Tris-HCl, pH 8.0, 3.3 mM $MgCl_2$) and water to a final volume of 50 µl, mixed and incubated for 5 min at 37° C. Then 150 Units (U) exonuclease III/pmol susceptible 3′ ends were added at time zero. Aliquots of 2.5 µl were removed at 35 sec intervals, mixed with 5 µl water and incubated for 10 min at 70° C to stop and inactivate exonuclease III. On ice, 12.5 µl of S1 buffer (2 mM sodium acetate, pH 4.6, 500 mM NaCl, 2 mM $ZnSO_4$, 10% glycerol) and 5 µl S1 nuclease (4 U/µl) were added and incubated for 20 min to digest single-stranded DNA and stopped by the addition of 5 µl 0.8 M Tris-HCl, pH 8.0, 20 mM EDTA. Seven microlitre aliquots were examined by electrophoresis on agarose gel to decide the extent of deletion. The remaining 23 µl were incubated for 2 min at 37° C with 1 µl Klenow fragment (5 U); 2 µl of a mixture of the four dNTPs (each at 0.125 mM) were added for 10 min at 37° C to create blunt ends. Samples were phenol extracted and ethanol precipitated. Each pellet was dissolved in 4 µl 10× ligase buffer (0.5 M Tris-HCl, pH 7.6, 0.1 M $MgCl_2$, 10 mM DTE), 1 µl T4 DNA ligase (2 U) and water to a final volume of 40 µl and the ligation was run for 6 h at 25° C. Calcium-treated competent JM83 cells were transformed with 10 µl of the ligation mix and spread on LB plates containing ampicillin (100 µg/ml).

DNA sequence analysis. Dideoxy-DNA sequence analysis (Sanger et al. 1977) was carried out using the convenient technique of sequencing supercoiled plasmid DNA (Chen and Seeburg 1985) according to the method as described by Heinrich (1986). In brief, 0.5 pmol of supercoiled plasmid DNA was denatured with 40 µl of 0.2 M NaOH, 0.2 mM EDTA. After 5 min at room temperature, the solution was neutralized by addition of 4 µl of 2 M ammonium acetate-acetic acid at pH 4.5. DNA was precipitated with 2.5 volumes of ethanol, mixed with 70% ethanol, dried in vacuo and used for sequencing. The denatured DNA was mixed with 5 pmol oligonucleotide primer, ^{35}S-dATP (Amersham, 10 µCi/µl, 650 Ci/mmol) and annealing buffer. The mixture was incubated at 37° C for 20 min. Following primer annealing, 2 U *E. coli* polymerase I (large fragment) were added. The sample was dividied into four tubes containing the appropriate dideoxy- and deoxynucleotide triphosphates. Following the "chase", the samples were dried in vacuo, dissolved in 4 µl formamide-dye mix and 1 µl aliquots were applied to the gel. Gel electrophoresis was carried out using "wedge-shaped" polyacrylamide sequencing gels.

DNA samples and enzymes. The synthetic primers 5′–GTAAAACGACGGCCAGT–3′ (universal) and 5′–CAGGAAACAGCTATGAC–3′ (reversal) for sequencing pUC plasmids (Vieira and Messing 1982) were synthesized by the solid phase phosphoamidite method (Matteuci and Caruthers 1980). Each oligonucleotide was purified by polyacrylamide (16%) gel electrophoresis. The UV quenching band of the product was eluted with H_2O and purified over a DEAE-cellulose column (bed volume 200 µl). Enzymes, such as restriction enzymes, DNA polymerase, alkaline phosphatase, S1 nuclease or exonuclease III were purchased from Boehringer Mannheim or New England Bio Labs GmbH, Schwalbach and were used according to the manufacturer's specifications.

Computer analysis of the sequence. Computer analysis was carried out in the Max von Pettenkofer Institut (Munich) with Dr. S. Modrow and Prof. H. Wolf, as described by Modrow et al. (1987). Briefly, the secondary structure of lysostaphin was predicted by a computer program written for a VAX750, based on suggestions by Cohen et al. (1984) by using the algorithms of Chou and Fasman (1974) or Garnier et al. (1978). These predictions were superimposed on local hydrophilicity values (Hopp and Woods 1981).

Other techniques. The N-terminal amino acid of lysostaphin was determined by the DABITC/PITC double coupling method (Chang et al. 1978).

Extracellular proteins from the culture supernatant of *S. staphylolyticus* were concentrated by lyophilization and dialysed against 10 mM Tris buffer, pH 7.5, with 0.01% SDS. Periplasmatic proteins from *E. coli* were isolated by the "cold osmotic shock" procedure as described by Neu and Heppel (1965). The efficiency of the cell fractionation procedure was confirmed by assaying the catalytic activities of β-lactamase (O'Callaghan et al. 1972) and lactate dehydrogenase using NADH and pyruvate as substrates.

The exoproteins were solubilized in sample buffer (2.25% glycerol, 0.25% β-mercaptoethanol, 2.3% SDS, 0.001% bromphenol blue, and 62 mM Tris-HCl, pH 6.8) and heated for 2 min at 100° C prior to sodium dodecyl sulfate polyacrylamide (15%) gel electrophoresis (SDS-PAGE) (Laemmli 1970). Prestained molecular weight markers for SDS-PAGE were obtained from Sigma (Deisenhofen). The approximate molecular weights of the standard proteins are: 26500 (triosephosphate isomerase); 36500 (lactic dehydrogenase); 48500 (fumarase); 58000 (pyruvate kinase); 84000 (fructose-6-phosphate kinase); 116000 (β-galactosidase); 180000 (a_2-macroglobulin).

Lysostaphin was still active after separation by SDS-PAGE if SDS was removed from the gel (Thudt et al. 1985). To detect the cell wall lytic activity of lysostaphin on the washed polyacrylamide gel, the gel was laid onto a thin (1.5 mm) agarose (1.2%) gel. The agarose contained 50 mM Tris-HCl, pH 7.5, 0.15 M NaCl and heat-inactivated *S. carnosus* cells (pretreated at 60° C for 20 min), giving an optical density at 578 nm of ca. 2.0. Lysostaphin activity was indicated by clearing of the turbid agarose due to cell lysis. *S. carnosus* was used as an indicator strain because of its high sensitivity to lysostaphin (Götz et al. 1983).

Results and discussion

Cloning of the lysostaphin gene

Chromosomal DNA of *S. staphylolyticus* was partially digested with *Mbo*I and enriched for 4–20 kb fragments. These fragments were ligated with *Bam*HI-cut and phosphatase-treated vector, pBR322. Cells of *E. coli* RR28 were

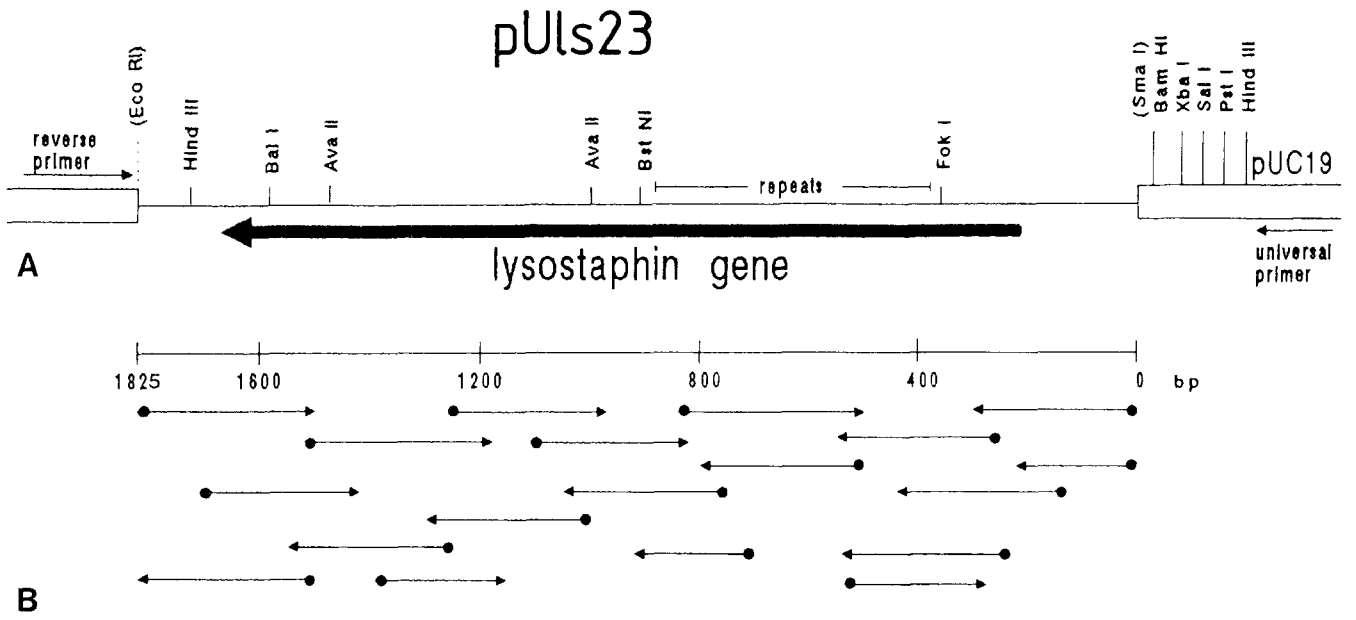

Fig. 1. **A** Genetic map of the lysostaphin gene encoding the DNA fragment cloned in pUC19. The *Eco*RI and the *Sma*I sites are destroyed due to blunt end ligation. **B** Sequencing strategy. *Arrows* indicate the direction of individual sequencing for one or other strand, respectively

transformed with the ligated mixture and plated on LB medium with an agar overlay containing heat-killed *S. aureus* cells. After 2–3 days of cultivation, five colonies among ca. 2300 Ap^R transformants were found to form a clearing at the colony/agar contact site. The clearing was only visible after removing the colony. The recombinant plasmids of the clones contained DNA inserts of 25, 15, 13 and 4.7 kb, respectively. By cutting the smallest recombinant plasmid with *Eco*RI and *Pst*I, a 3.2 kb fragment was isolated and recloned in *E. coli* JM83 using pUC19 as vector; transformants which contained the fragment were still lysostaphin positive. With BAL31, the 3.2 kb fragment was successively shortened from both ends until a ca. 1.8 kb fragment was generated. The recombinant plasmid was called pUls23 (Fig. 1 A).

The lysostaphin gene

The DNA fragment in pUls23 was shortened by *Exo*III treatment as described in Materials and methods. A set of 18 subclones was isolated, each deletion increased between 100 and 180 bp. The deletions were unidirectional, starting from *Bam*HI in the insert direction. Most of the 1825 bp fragment was sequenced from both ends using either the universal or the reverse sequencing primer as outlined in Fig. 1 B.

The nucleotide sequence of the 1825 bp DNA fragment revealed one major open reading frame (ORF) (Fig. 2). The coding region from position 219 with the start codon TTG to position 1659 comprises 1440 nucleotides. Seven nucleotides upstream of the start codon is a Shine-Dalgarno (1974) sequence –AGGAGGT–, which exhibits complementarity to the 3'end of the 16 *S* ribosomal RNA of *Bacillus subtilis* (Green et al. 1985). Preceding the ORF there is no consensus promotor sequence although the sequences TTGAGT (position 92–97) and TCAAAG (position 114–119) might in *E. coli* function as a −35 and a −10 promotor region, respectively. Immediately adjacent to the stop codon TGA is a GC-rich region ordered as a short inverted repeat that is followed by a T– stretch. This sequence may represent a transcriptional termination sequence (Platt 1986). There are two additionaly inverted repeats further downstream which may also function as transcription terminators.

Size of the extracellular and mature lysostaphin

Extracellular proteins form *S. staphylolyticus*, the periplasmatic proteins from *E. coli* (pUls23) and the purified lysostaphin preparation from Sigma were analyzed by SDS-PAGE (Fig. 3). The lysostaphin preparation from Sigma revealed one prominent protein band (molecular weight 25000) that exhibits high staphylolytic activity (Fig. 3 A and B, lane 2). The extracellular proteins from *S. staphylolyticus* exhibit a protein band at the same position (Fig. 3 A, lane 3) with staphylolytic activity (not shown). Periplasmatic proteins of *E. coli* (pUls23) are shown in Fig. 3 A, lane 1. The activity blot revealed two active bands corresponding to proteins with a molecular weight of 25000 and 68000, respectively (Fig. 3 B, lanes 1 a and 1 b). While the calculated molecular weight of 25000 correlates quite well with the predicted molecular weight (26926) of the mature lysostaphin (Table 1), it is more difficult to explain the large size of the active 68 kDa protein; however, it is likely that the protein represents the immature form of lysostaphin, which exhibits for unknown reasons a reduced migration rate in SDS-PAGE.

The commercially available enzyme from Sigma showed only one prominent protein band in SDS-PAGE (Fig. 3 A). Its NH_2-terminal aa sequence revealed Ala-Ala-Thr-His-Glu as the first five aa. This sequence completely matched that deduced from the nucleotide sequence (Figs. 2, 4). It is remarkable that the mature lysostaphin starts only 5 aa after the last repeat at aa position Ala^{235}. The mature enzyme consists of 246 aa (Table 1). Some aa are highly un-

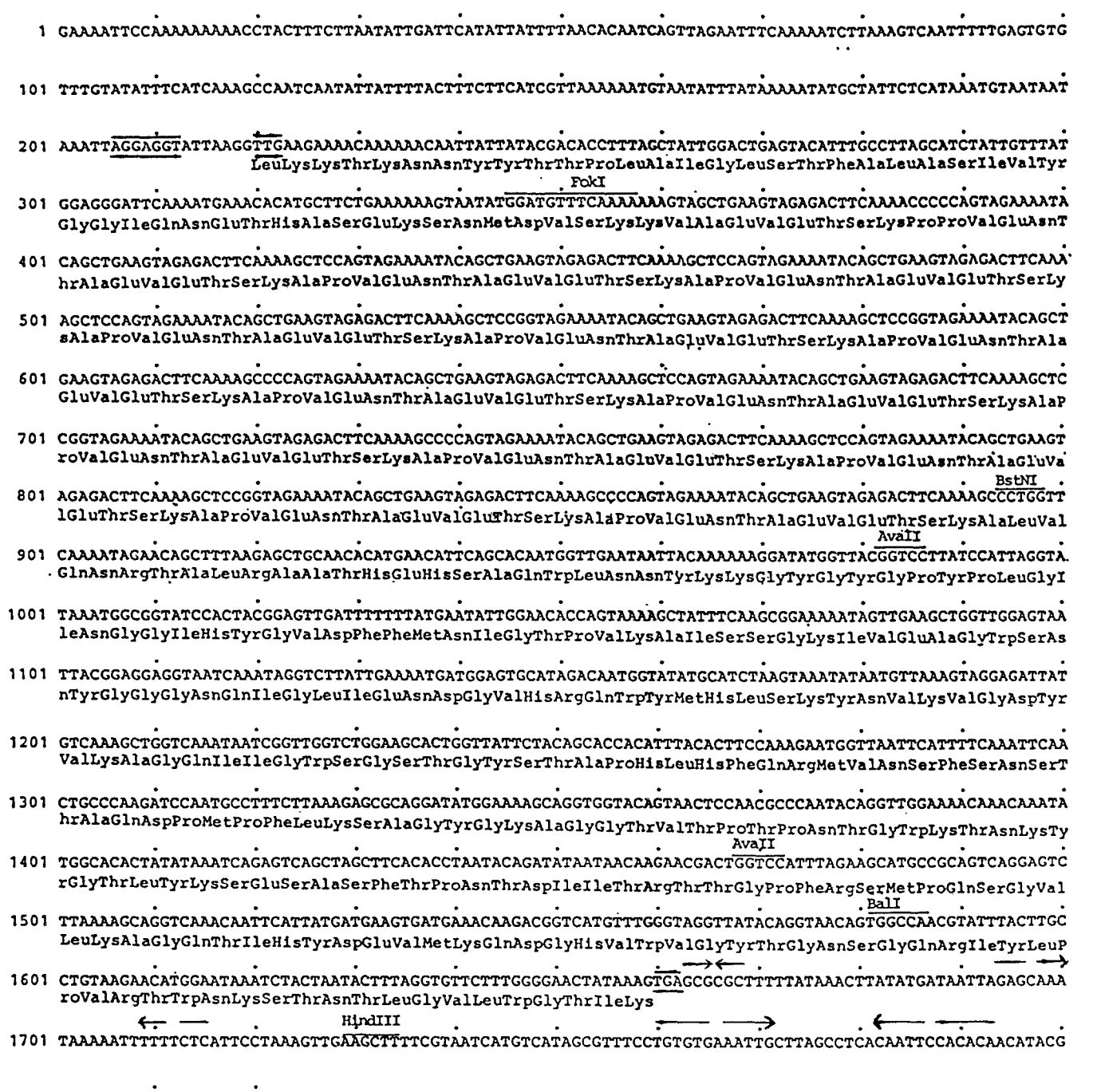

Fig. 2. Nucleotide and amino acid sequence of the lysostaphin gene. The nucleotide sequence comprises the entire (1825 bp) DNA insert of pUls23. The Shine-Dalgarno sequence, the start codon (TTG) and the stop codon (TGA) are indicated by *double lines*. The palindromic sequences at the 3′end are indicated by arrows above the nucleotide sequence

evenly distributed in the preprolysostaphin and the mature lysostaphin. Nearly 90% of Glu is in the prepropeptide, underlining its hydrophilic character, while the majority of Asp, Gly, Met, Gln, and Trp are localized in the mature lysostaphin.

Repetitive sequences

The DNA sequence of the lysostaphin gene revealed an unusual feature. There are 14 repetitive sequences beginning at nucleotide position 364 and ending at position 909 (Fig. 4). Each repeat consists of 39 nucleotides and codes for 13 amino acids (aa). The conserved aa sequence is interrupted in the first repeat where Ala^8 is exchanged by Pro, and in the second half of the last repeat where 3 aa are substituted, signalling the end of the repeats. Within the nucleotide sequence of certain repeats there are, in the third position of the codons for Ala^8 and Pro^9, some changes from T to C and from A to G, respectively. However, these changes do not alter the aa sequence (see Fig. 4).

The significance of these tandem repeats is not clear. Nevertheless, there must be an enormous evolutionary pressure to maintain the repeats nearly unchanged; the DNA sequence revealed only few silent mutations. Repetitive se-

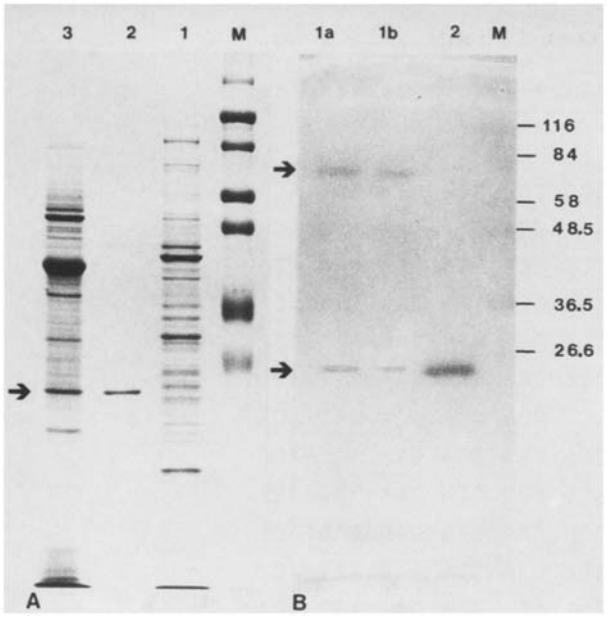

Fig. 3A–B. SDS-polyacrylamide gel electrophoresis (PAGE) of extracellular and periplasmatic proteins from *Staphylococcus staphylolyticus* an *Escherichia coli* (pUls23), respectively. **A** Protein staining with Coomassie brilliant blue. **B** Corresponding staining of lysostaphin activity (lysostaphin is still active after removal of SDS from the gel). Protein source and (protein concentration): Lanes 1 (20 μg), 1a (40 μg) and 1b (20 μg), periplasmatic proteins from *E. coli* (pUls23); lane 2 (1 μg), purified lysostaphin from Sigma; lane 3 (40 μg), culture supernatant of *S. staphylolyticus*; lane M, migration rate of the prestained protein standards (kDa are shown at the right hand margin). *Arrows* indicate the protein and staphylolytic activity of lysostaphin

Table 1. Amino acid composition of the preproenzyme and the mature lysostaphin predicted from the DNA sequence

Amino acids	Number of amino acids	
	Preproenzyme	Mature lysostaphin
Ala	44	12
Cys	0	0
Asp	8	7
Glu	48	5
Phe	8	7
Gly	38	35
His	10	9
Ile	17	14
Lys	36	16
Leu	17	11
Met	7	6
Asn	34	16
Pro	27	12
Gln	12	10
Arg	8	6
Ser	38	19
Thr	55	22
Val	46	15
Trp	8	8
Tyr	19	16
Total	480	246
Molecular weight	51669	26926
Average residue weight	107	109
Average residue charge	−12	+10

quences have also been found in other organisms. Thus the murein lipoprotein of the outer membrane of *E. coli* that is covalently attached via a C-terminal lysine to the peptidoglycan contains internal repeats (Handtke and Braun 1973). Group A streptococci possess an antiphagocytic surface antigen known as M protein. The M protein contains regions which exhibit a repeating seven residue periodicity (Beachey et al. 1978). Interestingly, the repeats are composed on non-polar and charged residues and reveal a high α-helical potential – like the repeats of the lysostaphin propeptide. The M protein also shows a high similarity with mammalian tropomyosin which exhibits a heptate periodicity (Manjula and Fischetti 1980). The cell wall-bound protein A of *S. aureus* reveals two regions of internal repeats, namely, a 58 aa unit, responsible for IgG binding, repeated 5 times and an 8 aa unit, possibly responsible for binding to the cell wall of *S. aureus*, repeated 12 times (Sjödahl 1977; Uhlén et al. 1984). The so-called proline-rich proteins constitute the largest group of proteins in mammalian salivary secretions. The aa sequence revealed that the proteins contain 13 tandemly repeated regions of a 14-residue proline-rich peptide (Clements et al. 1985).

Secondary structure of lysostaphin

The primary sequence of the lysostaphin precursor protein was further analysed by a computer program that predicts the secondary protein structure and superimposes values for hydrophilicity (Fig. 5). The secondary structure revealed three characteristic regions: the signal peptide, the propeptide and the mature lysostaphin.

The signal peptide comprises a 6 aa long hydrophilic region at the extreme NH_2-terminus and a 21 aa long hydrophobic region ranging from aa position 9 to 30. The hydrophobic region is further characterized by a long β-pleated sheet. The signal peptidase cleavage site is not yet known, although, the sequence Ala-Ser-Glu at position 36–38 is a potential candidate (Perlman and Halvorson 1983).

Adjacent to the signal peptide is a nearly 200 aa long putative propeptide which ends at aa position 234. The propeptide contains the 14 repeats (Fig. 4). Twelve repeats have an identical structure which is characterized by a hydrophilic stretch of aa and a strong tendency to form an α-helix. The latter characteristic is missing in repeats 1 and 14 due to the altered aa sequence. The function of the propeptide is not clear. However, the propeptide might be involved in protein transport. We demonstrated recently by gene fusions between the *S. hyicus* lipase gene and the *E. coli* β-lactamase gene that most of the lipase propeptide is necessary for efficient secretion of the hybrid proteins in *S. carnosus* (Götz et al. 1985; Liebl and Götz 1986).

The mature lysostaphin starts at aa position 235, indicated by the arrow in Fig. 5. Its secondary structure, which is totally different from that of the propeptide, is characterized by several hydrophobic domains and several β-turns.

REPETITIVE SEQUENCES OF THE PROLYSOSTAPHIN

Amino acid sequence		1 Ala	2 Glu	3 Val	4 Glu	5 Thr	6 Ser	7 Lys	8 Ala	9 Pro	10 Val	11 Glu	12 Asn	13 Thr
Sequence position	No. of repeats													
									Pro					
364-402	(1)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	CCC	CCA	GTA	GAA	AAT	ACA
403-441	(2)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCA	GTA	GAA	AAT	ACA
442-480	(3)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCA	GTA	GAA	AAT	ACA
481-519	(4)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCA	GTA	GAA	AAT	ACA
520-558	(5)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCG	GTA	GAA	AAT	ACA
559-597	(6)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCG	GTA	GAA	AAT	ACA
598-636	(7)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCC	CCA	GTA	GAA	AAT	ACA
637-675	(8)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCA	GTA	GAA	AAT	ACA
676-714	(9)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCG	GTA	GAA	AAT	ACA
715-753	(10)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCC	CCA	GTA	GAA	AAT	ACA
754-792	(11)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCA	GTA	GAA	AAT	ACA
793-831	(12)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCT	CCG	GTA	GAA	AAT	ACA
832-870	(13)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCC	CCA	GTA	GAA	AAT	ACA
										Leu		Gln		Arg
871-909	(14)	GCT	GAA	GTA	GAG	ACT	TCA	AAA	GCC	CTG	GTT	CAA	AAT	AGA
910-		ACA	GCT	TTA	AGA	GCT	GCA	ACA	CAT	GAA	CAT	...	...	...
		Thr	Ala	Leu	Arg	/Ala	Ala	Thr	His	Glu	His	...	...	...
						/235 →		MATURE LYSOSTAPHIN						

Fig. 4. Thirteen residue periodicity within the propeptide of lysostaphin. The sequences of the respective regions have been aligned to indicate periodicity and homology. The repeating aa are numbered from 1–13; deviations from the aa sequence occur only in repeats 1 and 14. Altered nucleotides are *underlined*. The mature lysostaphin starts at aa position 235

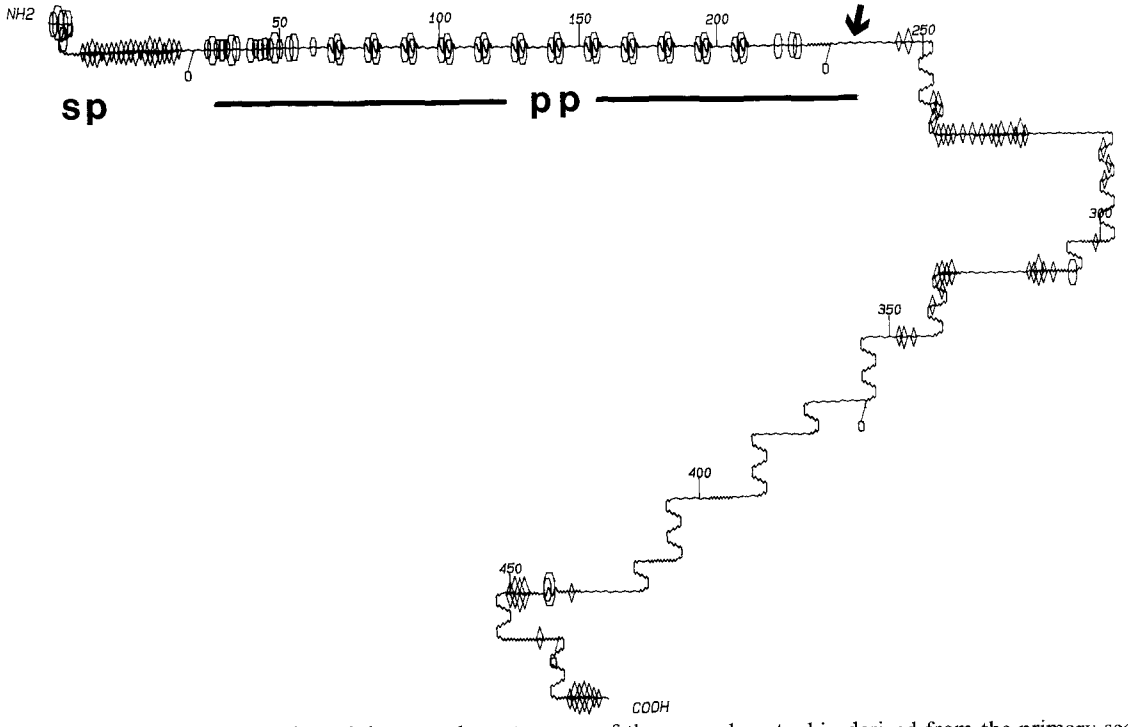

Fig. 5. Chou-Fasman prediction of the secondary structure of the preprolysostaphin derived from the primary sequence. The possibility of the occurrence of α-helices, β-pleated sheets, random coils and β-turn regions were evaluated using stringent conditions. The parameters for hydrophilicity were averaged over seven aa residues, with a limit of 0.7 for hydrophilicity. The values for hydrophilicity are superimposed on the secondary structure. The *arrow* indicates the processing site of the mature lysostaphin.
sp, putative signal peptide; pp, putative propeptide region
Symbols: α-helices (〰), β-pleated sheets (〰), random coil (〰), β-turn regions (ʃ), hydrophilic regions (0), hydrophobic regions (◊)

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Very recently the DNA sequence of the lysostaphin gene from *S. simulans* was published (Recsei et al. 1987).