Characterization of an Extracellular Metalloprotease with Elastase Activity from *Staphylococcus epidermidis*

P. TEUFEL and F. GÖTZ*

Mikrobielle Genetik, Universität Tübingen, Waldhäuser Strasse 70/8, 7400 Tübingen, Germany

Received 28 January 1993/Accepted 21 April 1993

The gene *sepA* from *Staphylococcus epidermidis* TÜ3298-P, encoding the extracellular neutral metalloprotease SepP1, was cloned into pT181mcs. DNA sequencing revealed an open reading frame of 1,521 nucleotides encoding a 507-amino-acid protein with an *M* of 55,819. The *sepA*-containing DNA fragment did not hybridize with *Staphylococcus hyicus* or *Staphylococcus carnosus* DNA. Expression of *sepA* in the protease-negative *S. carnosus* (pT181mcsP1) resulted in overproduction of a 33-kDa protease found in the culture medium. The first 15 N-terminal amino acids of the partially purified protease completely matched the deduced DNA sequence starting at GCA (Ala-208). This finding indicated that *SepP1* is synthesized as a preproenzyme with a 28-amino-acid signal peptide, a 179-amino-acid hydrophilic pro region, and a 300-amino-acid extracellular mature form with a calculated *M* of 32,739. In activity staining, the mature protease prepared from *S. carnosus* (pT181mcsP1) corresponded to the extracellular *S. epidermidis* TÜ3298-P protease. The partially purified protease had a pH optimum between 5 and 7, and its activity could be inhibited by zinc- and metal-specific inhibitors such as EDTA and 1,10-phenanthroline, indicating that it is a metalloprotease.

The protease had a low substrate specificity. Glucagon was cleaved preferentially between aromatic (Phe) and hydrophobic (Val) amino acids. The protease hydrolyzed casein and elastin. The amino acid sequence of the mature form of SepP1 revealed pronounced similarities with the thermolabile and thermostable neutral proteases of various bacilli (44 to 58% identity) and a central part of the mature form of the *Pseudomonas aeruginosa* elastase (31% identity). From homology comparison with the *Bacillus thermoproteolyticus* thermolysin, we predict that mature *SepP1* binds one zinc ion at a conserved zinc-binding site.

Most *Staphylococcus aureus* strains secrete proteolytic enzymes; three different types of proteases have been studied in detail. Serine protease from *S. aureus* V8, commonly referred to as V-8 protease or protease I, is an endoprotease which cleaves peptide bonds on the carboxyl-terminal side of glutamic acid and to a lesser extent of aspartic acid (8). The enzyme, having a molecular weight of 12,000, is inhibited by diisopropyl fluorophosphate (DFP) but not by EDTA. Similar serine proteases with the same cleavage preference are also found in other *S. aureus* strains; however, they differ from the above-described enzyme in their size and sensitivity to DFP (2, 24). The amino acid sequence of a 27-kDa serine protease of *S. aureus* revealed a 43-residue C-terminal peptide composed nearly exclusively of aspartic acid, asparagine, and proline (6), proposed to be involved in the transport of the enzyme through the cytoplasmic membrane (7).

The second type of protease from *S. aureus* V8, thiol protease (protease II), is characterized by an isoelectric point of pH 9.4, a molecular weight of 12,500, and a very broad specificity; it also exhibits esterase activity with N-benzoyl-DL-tyrosine ethyl ester as a substrate (2). The enzyme is active only in the presence of reducing agents and is inactivated by heavy metals such as Hg2+, Zn2+, or Ag+, indicating that protease II must possess free SH groups to be active and that it is a cysteine enzyme.

The third protease of *S. aureus* V8, a metalloprotease (2), is completely inactivated by EDTA and is insensitive to sulfhydryl reagents and DFP. Calcium is essential for the stability of the protease. The molecular weight is 28,000, and the pH optimum for hydrolysis of casein is 7.4. Like the thiol protease, the metalloprotease has a rather broad proteolytic specificity, cleaving preferentially at the N-terminal side of hydrophobic residues (1). A metalloprotease with a molecular weight of 38,000 and a pH optimum of 7.0 has been isolated from a mutant of *S. aureus* V8 (5). The protease is fully inactivated by o-phenanthroline but can be reactivated by zinc ions.

Another extracellular protease, lysostaphin, is found in *Staphylococcus simulans* biovar staphylocyticus (27). Lysostaphin is a zinc metalloendopeptidase (34) which hydrolyzes the glycinipeptide bridge of staphylolysin, causing cell lysis. The lysostaphin gene has been recently cloned and sequenced (14, 21). According to its deduced amino acid sequence and processing, lysostaphin appears to be organized as preproenzyme.

In contrast to the well-studied proteases of *S. aureus*, little is known about proteases in *Staphylococcus epidermidis*, the predominant inhabitant of the human skin. Our interest in the role of proteases in skin colonization led to this report on the cloning and sequencing of *sepA* from *S. epidermidis*, the gene encoding the extracellular metalloprotease SepP1, and the biochemical characterization of partially purified SepP1.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** *S. epidermidis* TÜ3298-P, the source of *sepA*, is a mutant of *S. epidermidis* TÜ3298 which has deletions in plasmid TÜ32 and is unable to produce the lantibiotic epidermin (3). *Staphylococcus carnosus* TM300 was used as the cloning host (10). Plasmid pT181mcs, encoding resistance to tetracycline, was used as a cloning vector (15, 23). Staphylococci were cultivated in B broth (10 g of casein hydrolysate 140 [GIBCO], 5 g of yeast extract
[Difco], 5 g of NaCl, 1 g of K₂HPO₄ per liter [pH 7.3]). Unless otherwise stated, the growth temperature was 37°C.

DNA preparation, transformation, and molecular biological techniques. Staphyloccocal DNA was prepared as described previously (12). Cells were lysed by the addition of lyso- 
saphin (8 μg/ml), and DNA was isolated by CsCl centrifugation. S. carnosus was transformed by protoplast transforma-
tion (11). Established protocols were followed for molecular biological techniques (17). DNA hybridization analysis using the ³²P-labeled (9) sepA-containing SacI-PstI (located in the multiple cloning site of pT181mcs) DNA fragment as a probe was carried out according to Southern (29). Hybridization was performed under stringent condi-
tions as follows: the filters were washed twice with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)−0.1% sodium dodecyl sulfate (SDS) at room temperature and twice with 0.1× SSC−0.1% SDS at 65°C. Enzymes for molecular cloning were obtained from Boehringer (Mannheim, Ger-
many), Bethesda Research Laboratories (Eggenstein, Ger-
many), or Pharmacia (Freiburg, Germany). Elastin (bovine 
neck ligament) was obtained from Sigma (Deisenhofen, 
Germany). Polyvinylidene difluoride (PVDF) Immobilon 
membranes were obtained from Amersham (Braunschweig, 
Germany), and DEAE-Sepharose was from Pharmacia.

DNA sequencing. Both strands of the cloned DNA insert of 
pT181mcsPl were sequenced by using the dideoxy pro-
cedure (25), the Pharmacia AutoRead sequencing kit, and the A.L.F. DNA sequencer from Pharmacia LKB. DGTTP was replaced by dITP in some reaction mixtures to overcome compression.

Detection of protease-producing colonies and assay of SepP1 activity. Protease-producing colonies were detected on 
B-agar plates containing 1% skim milk by halo formation 
around the colony. For routine detection of proteolytic 
activity in culture supernatants, column fractions, or poly-
acrylamide gels, 1% agarose containing 1% casein, 50 mM 
Tris-HCl, and 5 mM CaCl₂ was used.

For the photometric determination of proteolytic activity, 
resorufin-labeled casein (35) was used as a substrate accord-
ing to the standard protocol supplied by the manufacturer 
(Boehringer). One hundred microliters of enzyme solution 
was incubated with 50 μl of 0.2 M Tris-HCl (pH 7.8)−20 mM 
CaCl₂ and 50 μl of substrate solution (resorufin-labeled 
casein; 0.4% [wt/vol] in deionized water) for 30 min at 37°C. 
The reaction was stopped by adding 480 μl of stop solution 
containing trichloroacetic acid (5% [wt/vol]). After incuba-
tion at 37°C for 10 min, the reaction mixture was centrifuged 
for 5 min; 400 μl of the supernatant was mixed with 600 μl of 
0.5 M Tris-HCl (pH 8.8). One unit of protease activity is 
defined as the amount of enzyme causing an increase in A₅₇₄ 
of 0.01 during 30 min of incubation.

Elastase activity was detected by using 1% agarose plates 
containing 0.25% elastin, 50 mM Tris-HCl, and 5 mM CaCl₂. 
The plates were first incubated at 37°C for 24 h with a high 
atmospheric humidity and subsequently stored at 4°C for 2 
days.

Partial purification of protease. S. carnosus TM300 
(pT181mcsPl) was grown for 16 h in B broth supplemented 
with tetracycline (25 μg/ml), 0.1% casein, and 2 mM CaCl₂. 
Proteins from the culture supernatant were fractionated by 
ammonium sulfate and precipitated at a final concentration 
of 65% (wt/vol). The pellet was resuspended in 50 mM 
Tris-HCl (pH 7.0)−5 mM CaCl₂ and dialyzed against the 
same buffer overnight. The dialyzed solution was subjected 
to DEAE-Sepharose Fast Flow chromatography (Phar-
macia); equilibration was done with 20 mM Tris-HCl (pH 8.0).

Proteins were eluted with a linear gradient of 0 to 1 M NaCl. 
Each fraction was checked for protease activity. The purity 
of the protease was determined by SDS−12.5% polyacryl-
amide gel electrophoresis (PAGE).

Determination of pH profile and thermostability of partially 
purified SepP1. For pH profile determination, the following 
buffers were used: 100 mM sodium acetate (pH 4 to 5), 25 
mM bis-Tris-HCl (pH 6 to 6.8), 100 mM Tris-HCl (pH 7 to 9), 
and 50 mM sodium bicarbonate (pH 10). Each assay 
was performed with 2.5 mM CaCl₂ and 10 μM ZnCl₂. For 
the determination of thermostability, a buffer containing 100 mM 
Tris-HCl (pH 7) with 2 mM CaCl₂ and 10 μM ZnCl₂ was 
used. The samples were incubated for 25 min at the indicated 
temperatures and cooled rapidly on ice. The residual pro-

tease activity was measured.

Other methods. SDS-PAGE was performed as described 
by Laemmli (16). Electrobolting of proteins to PVDF Im-
mobilon membranes for protein sequence determination 
was performed according to the method of Matsudaira (18). 
The N-terminal sequence of SepP1 was determined by Edman 
degradation, using a model 477A pulsed-liquid protein se-
quence (Applied Biosystems). DNA and protein sequences 
were analyzed by using the computer programs of Microge-
nie (Beckman) and PC/GENE (IntelliGenetics, Inc).

Nucleotide sequence accession number. The novel DNA 
sequence published here has been deposited into the EMBL 
sequence data bank under accession number X69957.

RESULTS

Cloning of sepA from S. epidermidis in S. carnosus. S. 
epidermidis Tu3298-P chromosomal DNA was partially di-
gested with MboI. DNA fragments of 4 to 20 kb were ligated 
to BamHI-digested pT181mcs. The ligated DNA was trans-
formed into S. carnosus TM300, which has very low extra-
cellular protease activity. By screening 8,000 tetracycline-
resistant transformants on B-agar plates containing 1% skim 
milk and tetracycline (25 μg/ml), six positive clones forming 
large halos around the colonies were detected. Restriction 
analysis of the isolated plasmids revealed that all six positive 
clones carried a common restriction fragment within a 4− 
5.7-, or 9.5-kb DNA insert. The plasmid from the S. car-

mosus clone with the smallest insert (4 kb), pT181mcsP1, was 
used for further investigations. S. carnosus(pT181mcsP1) 
produced a much larger halo of proteolytic activity on skim 
milk agar than did S. epidermidis Tu3298-P. The control S. 
carnosus(pT181mcs) had no detectable proteolytic activity in 
this assay, rendering S. carnosus an ideal host for pro-
tease cloning and isolation.

FIG. 1. Restriction map of the 4-kb MboI fragment in 
pT181mcsPl and deletion mapping. The location (deduced from 
the DNA sequence) of the SepP1 gene, sepA, is indicated by an 
open arrow. S. carnosus clones with plasmids having the indi-
cated deletions were protease negative (−). +, protease-positive clone.
The insert of another clone with a very small halo had a different restriction pattern, confirming our observation that *S. epidermidis* Tu3298-P produces various exoproteases.

**Nucleotide sequence of sepA.** The restriction map of the cloned 4-kb DNA fragment is shown in Fig. 1. Deletion analysis of the DNA insert indicated that *HhaI* and *EcoRI* must be located within the protease gene, which was designated *sepA*. The nucleotide sequence of the *HpaI* fragment revealed only one open reading frame (ORF) of the expected size, starting with ATG at nucleotide position 164 and terminating with TAA at position 1687 (Fig. 2). The 1,521-nucleotide ORF could encode a 507-amino-acid protein (*M* = 55,819). The ATG start codon is preceded by a perfect Shine-Dalgarno sequence (AGGAGGT). The first 28 residues of the N terminus resemble a typical signal peptide sequence for secreted proteins of prokaryotic origin. A short sequence containing two positively charged residues is followed by a long hydrophobic sequence and a potential signal peptidase cleavage site (Val–Asp–Ala–28). This cleavage site conforms to the −3, −1 rule (37). Normally a helix-breaking residue (Pro or Gly) or a large polar residue (Glu) occurs four to eight amino acids before the cleavage site (39). In this ORF, we found Glu in position −4. We also found Val in position −7 and Leu in position −8, in keeping with the rules of prokaryotic cleavage sites assigned by using the PC/GENE prediction of prokaryotic secretory signal and by using the method of von Heijne (37). 32P-labeled *sepA*-containing DNA strongly hybridized with DNA from *S. epidermidis* Tu3298-P and its wild type; no hybridization was detected to *S. carnosus* or *S. hyicus* DNA (data not shown).

**Isolation and N-terminal sequence of SepP1 from S.**

![FIG. 2. Nucleotide sequence of sepA and flanking DNA. The sequence contained only one ORF of the expected size. The deduced amino acid sequence can be subdivided into the putative pre (signal), pro, and mature sequences. The amino acids determined by Edman degradation are underlined. The SepP1 coding region is preceded by a perfect Shine-Dalgarno (SD) sequence. The putative −10 and −35 promoter regions are indicated; a typical transcription terminator sequence is missing.](http://jb.asm.org/ Downloaded from http://jb.asm.org/ on October 20, 2017 by guest)
carnosus(pT181mcsP1). The onset of extracellular protease production occurred very early in a batch fermentation of S. carnosus(pT181mcsP1) and correlated strongly with growth. Only in the late stationary phase did protease activity decline. The control, S. carnosus, exhibited no detectable protease activity during batch fermentation (data not shown).

The protease was isolated from 4 liters of culture supernatant of S. carnosus(pT181mcsP1) harvested at the early stationary phase. For partial purification, the protease was precipitated by ammonium sulfate (65%), and after dialysis, the sample was subjected to DEAE-Sepharose Fast Flow chromatography. Each DEAE-Sepharose fraction was checked for protease activity and analyzed by SDS-PAGE (Fig. 3). The protease-positive fractions had a 33-kDa protein band which was absent (in Fig. 3) in the similarly treated culture supernatant of S. carnosus(pT181mcsP1). After SDS-PAGE, no protease activity was detected in the gel, even after removal of SDS and mercaptoethanol by extensive washing. However, after non-denaturing gel electrophoresis, a protein band with proteolytic activity was visible. The bands with proteolytic activity from extracts of S. carnosus(pT181mcsP1) and S. epidermidis Tü3298-P had the same electrophoretic mobility.

The 33-kDa protein on the SDS-polyacrylamide gel was electroblotted onto a PVDF Immobilon-P transfer membrane, and the N-terminal sequence was determined. The first 15 N-terminal residues, Ala-Ala-Thr-Thr-Gly-Thr-Gly-Lys-Gly-Val-Leu-Gly-Asp-Thr-Lys, completely matched that deduced from the nucleotide sequence (Fig. 2), starting at Ala-208. In combination with DNA sequence analysis, this result suggests that the protease is synthesized as a preproenzyme with a 207-amino-acid prepro sequence. If the signal peptide is cleaved at the predicted cleavage site (between Ala-28 and Lys-29), the pro sequence would be 179 amino acids long; the pro region is distinguished by high hydrophilicity. The mature extracellular SepP1 is composed of 300 amino acids with a calculated molecular weight of 32,739.

Enzymatic properties of the partially purified SepP1. The pH optimum of the protease with resorufin-labeled casein as the substrate was between 5 and 7 (Fig. 4); thus, SepP1 can be regarded as a neutral protease. The calculated isoelectric point for the preproprotease is 6.0, and for the mature protease is 5.86. The protease was thermostable up to 50°C; at higher temperatures, stability decreased gradually. However, after incubation at 80°C for 25 min, 20% activity still remained (Fig. 4). The temperature optimum for protease activity was 37°C.

As shown in Table 1, the protease activity can be inhibited by the metal chelator EDTA and the zinc-specific chelator 1,10-phenanthroline. Although SepP1 contains no cysteine residues in its sequence, high concentrations of dithiothreitol exerted an inhibiting effect, very likely by its metal-chelating activity. The proteolytic activity of SepP1 was inhibited with phosphoramidon, an inhibitor of thermolysin and thermolysin-related metalloproteases (40). The enzyme was resistant to phenylmethylsulfonyl fluoride and other inhibitors of serine or cysteine proteases, such as (4-amidinophenyl)methanesulfonyl fluoride, aprotinin, antipain dihydrochloride, and leupeptin. It was also resistant to bestatin, an inhibitor of aminopeptidases, and to pepstatin, an inhibitor of aspartic proteases. The sensitivity of the protease to metal chelators indicates that this neutral SepP1 is a metalloprotease.

SepP1 was also tested for collagenase and elastase activity. We found proteolytic activity only with elastin in the culture supernatants of S. carnosus(pT181mcsP1) and S. epidermidis Tü3298-P.

![FIG. 3. SDS-PAGE (12% polyacrylamide) and Coomassie blue staining of culture supernatants and partially purified SepP1. Lanes: 1, DEAE-Sepharose chromatography fraction with the highest protease activity (4.2 μg of protein); 2, 65% ammonium sulfate precipitation of the culture supernatant of S. carnosus(pT181mcsP1) (25 μg of protein); 3, standard proteins (sizes in kilodaltons are indicated on the right). The arrowhead indicates the 33-kDa protease, from which the N terminus was determined by Edman degradation. Cells were grown for 16 h in B medium with tetracycline (25 μg/ml) and supplemented with 0.1% casein and 2 mM CaCl2.](http://jb.asm.org/)

![FIG. 4. pH profile and thermostability of partially purified SepP1.](http://jb.asm.org/)

### TABLE 1. Inhibition of the S. epidermidis protease P1 activity by various protease inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concn (mM)</th>
<th>Remaining protease activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Phenylmethylsulfonyl fluoride</td>
<td>1</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>74</td>
</tr>
<tr>
<td>EDTA</td>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>EGTA</td>
<td>1</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>1,10-Phenanthrolne</td>
<td>0.1</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>12</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>3,4-Dichloroisocoumarin</td>
<td>0.1</td>
<td>93</td>
</tr>
<tr>
<td>Phosphoramidon</td>
<td>0.2</td>
<td>29</td>
</tr>
</tbody>
</table>

* No inhibition was observed with (4-amidinophenyl)methanesulfonyl fluoride (40 μg/ml), aprotinin (10 μg/ml), leupeptin (0.5 μg/ml), bestatin (40 μg/ml), and pepstatin (0.7 μg/ml).
epidermidis. Elastase activity was weak and detectable only when the supernatants were concentrated 50-fold by lyophilization. S. carnosus (pT181mcs), as a control, showed no detectable elastase activity. Glucagon was cleaved between the amino acids phenylalanine and valine, but there was also a large amount of free amino acids, indicating that SepP1 has a low substrate specificity; with the β chain of insulin, no unambiguous cleavage preference was detectable.

Homology with other proteases. The deduced amino acid sequence of the neutral metalloprotease SepP1 was compared with sequences in the Microgenie database. The amino acid sequences of the mature protease had pronounced similarities with sequences of several other neutral proteases, such as the mature forms of the most stable neutral proteases from Bacillus thermoproteolyticus (53.6% identity), Bacillus stearothermophilus (45.6% identity), and Bacillus cereus (46.6% identity). It also had marked homology with the mature thermolabile neutral proteases from Bacillus amyloquefaciens (55.3% identity) and the neutral proteases A (55.3% identity) and B (44.6% identity) of Bacillus subtilis. A central region of the elastase structural gene (241 amino acids) from Pseudomonas aeruginosa showed 31% identity, while the entire mature elastase showed only 25% identity. The alignment of these sequences is shown in Fig. 5. The catalytic residues in the active site and the zinc-binding site are all conserved in these enzymes.

DISCUSSION

The nucleotide sequence of the neutral metalloprotease gene, sepA, of S. epidermidis revealed an ORF of 1,521 nucleotides which can encode a 507-amino-acid protein of 55.7 kDa. However, the secreted mature form of the protease is only 33 kDa. If the ORF identified corresponds to the primary translation product of sepA, the active protease is synthesized as a preproenzyme, composed of a predicted 28-residue signal peptide, a 179-residue prepropeptide, and the 300-residue mature protease.

Propeptides have various lengths and locations in the precursor protein; their suggested role is to maintain the protease in an inactive state and to mediate the folding of the protease (38). Synthesis as an inactive precursor is a common feature of all extracellular proteases studied so far. The thermolabile proteases from B. thermoproteolyticus (thermolysin) (32) and the thermolysin-related neutral proteases from B. stearothermophilus (31) and B. cereus (28, 42) are synthesized as preproenzymes, as are the thermolabile neutral proteases A (44) and B (31) from B. subtilis and B. amyloquefaciens (36) and all other proteases from B. subtilis, such as subtilisin (30) and bacilliproteinase F (43).

Propeptides are also found in extracellular proteins of various staphylococci, for example, in lysostaphin of S. simulans biovar staphylopteticus (14) and the lipase of S. hyicus (41). The lengths of the propeptides vary, ranging from 10 to over 200 amino acids. In general, the propeptide region is hydrophilic and lacks an extended hydrophobic core. The pro region of SepP1 is also distinguished by a pronounced hydrophilicity.

The mature form of SepP1 was shown here to be a neutral metalloprotease with proteolytic and elastolytic activity. It has similarity to a central region of the P. aeruginosa elastase (LasB), although the amino and carboxy termini of the two molecules are quite different (Fig. 5). LasB (4) is a 23-kDa metalloprotease which cleaves elastin, collagen, immunoglobulin G, serum α1-proteinase inhibitor, and several complement components. The lasB nucleotide sequence revealed that the protein is also organized as a proelastase; the propeptide comprises 150 amino acids (4). However, there is a lasA gene product (23) required for elastase activation. From the studies presented here, it is unlikely that a second gene is involved in elastase activation. From the studies presented here, it is clear that elastase activation varies, ranging from 10 to over 200 amino acids. In general, the propeptide region is hydrophilic and lacks an extended hydrophobic core. The pro region of SepP1 is also distinguished by a pronounced hydrophilicity.

The mature form of SepP1 was shown here to be a neutral metalloprotease with proteolytic and elastolytic activity. It has similarity to a central region of the P. aeruginosa elastase (LasB), although the amino and carboxy termini of the two molecules are quite different (Fig. 5). LasB (4) is a 23-kDa metalloprotease which cleaves elastin, collagen, immunoglobulin G, serum α1-proteinase inhibitor, and several complement components. The lasB nucleotide sequence revealed that the protein is also organized as a proelastase; the propeptide comprises 150 amino acids (4). However, there is a lasA gene product (23) required for elastase activation. From the studies presented here, it is clear that elastase activation varies, ranging from 10 to over 200 amino acids. In general, the propeptide region is hydrophilic and lacks an extended hydrophobic core. The pro region of SepP1 is also distinguished by a pronounced hydrophilicity.

We found no homology of SepP1 with any of the published staphylococcal protease sequences. However, the mature form of SepP1 shows considerable similarities with the primary structures of various Bacillus proteases. Sequence homologies of neutral proteases produced by Bacillus spp. were compared by using the thermolysin sequence as a reference. The thermolysin protein sequence (32), three-dimensional structure (20), and catalytic mechanism (13, 19) have been determined. Thermolysin requires a zinc ion for catalysis and has four calcium-binding sites (22). The zinc-binding site (His-142, His-146, and Glu-166 in the mature thermolysin) is conserved in all Bacillus neutral proteases and in the elastase enzyme of P. aeruginosa (4). These residues are also conserved in SepP1 (His-144, His-148, and Glu-168) (Fig. 5). On the basis of this homology and the inhibition by zinc- and metal-specific inhibitors, we predict that mature SepP1 binds one zinc ion at this conserved zinc-binding site.

In thermolysin, five residues (Asn-112, Ala-113, Glu-143, Tyr-157, and His-231) are essential for the full catalytic activity and the positioning of the substrate backbone in the active site (13). These residues are found at homologous positions in the Bacillus neutral proteases, in LasB (4), and
in SepP1 (Aσn-113, Ala-115, Glu-145, Tyr-159, and His-228) (Fig. 5). The four calcium-binding sites in thermolysin were also compared with those of SepP1. In the latter, the calcium-binding site 3 is missing and there are significant amino acid changes within sites 1, 2, and 4 (Fig. 5). In thermolysin, the removal of calcium ions results in a loss of thermostability (22). Thus, considering the amino acid changes in the calcium-binding sites of SepP1, the relative thermostability of this protease is not surprising.

ACKNOWLEDGMENTS

We thank S. Stevanovic for amino acid sequencing, Arielle Ferrandon and Vera Augsburger for technical assistance, and Karen A. Brune for critically reading the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 253).

REFERENCES


41. Wenzig, E., F. Lottspeich, B. Verheij, G. H. De Haas, and F.