Inhibitors and Specificity of \textit{Pseudomonas aeruginosa} LasA$^*$

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LasA is an extracellular protease of \textit{Pseudomonas aeruginosa} that enhances the elastolytic activity of \textit{Pseudomonas} elastase and other proteases by cleaving elastin at unknown sites. LasA is also a staphyloytic protease, an enzyme that lyses \textit{Staphylococcus aureus} cells by cleaving the peptidoglycan pentaglycine interpeptides. Here we showed that the staphyloytic activity of LasA is inhibited by tetrathylepentamine and 1,10-phenanthroline (zinc chelators) as well as excess Zn$^{2+}$ and dithiothreitol. However, LasA was not inhibited by several serine or cysteine proteinase inhibitors including diisopropyl fluorophosphate, phenylmethylsulfonyl fluoride, leupeptin, and N-ethylmaleimide. LasA staphyloytic activity was also insensitive to N-$\text{N}^\prime$-$\text{p}$-tosyl-$\text{l}$-lysine chloromethyl ketone or phosphoramidon. EDTA and EGTA were inhibitory only at concentrations greater than 20 mM. Without added inhibitors, LasA obtained by DEAE-cellulose fractionation was active toward $\beta$-casein, but the same cleavage patterns were observed with column fractions containing little or no LasA. The $\beta$-casein cleaving activity was fully blocked in the presence of inhibitors that did not affect staphyloytic activity. In the presence of such inhibitors, purified LasA was inactive toward acetyl-Ala$_4$ and benzyloxycarbonyl-Gly-Pro-Gly-Gly-Pro-Ala, but it degraded soluble recombinant human elastin as well as insoluble elastin. N-terminal amino acid sequencing of two fragments derived from soluble elastin indicated that both resulted from cleavages of Gly-Ala peptide bonds located within similar sequences, Pro-Gly-Val-Gly-Gly-Ala-Xaa (where Xaa is Phe or Gly). In addition, Ala was identified as the predominant N-terminal residue in fragments released by LasA from insoluble elastin. A dose-dependence study of elastase stimulation by LasA indicated that a high molar ratio of LasA to elastase was required for significant enhancement of elastolysis. The present results suggest that LasA is a zinc metalloendopeptidase selective for Gly-Ala peptide bonds within Gly-Gly-Ala sequences in elastin. Substrates that contain no Gly-Gly peptide bonds such as $\beta$-casein appear to be resistant to LasA.

\textit{Pseudomonas aeruginosa}, a Gram-negative opportunistic pathogen, produces several extracellular proteolytic enzymes that are thought to play a role in the pathogenesis of this organism. Most studies of \textit{P. aeruginosa} proteases (for reviews, see Refs. 1–4) have emphasized three extracellular endopeptidases, elastase (also termed pseudolysin), alkaline proteinase, and LasA. A lysine-specific endopeptidase (5) and an endopeptidase that lyses \textit{Staphylococcus aureus} cells by cleaving the pentaglycine pentaglycine interpeptides. Here we showed that the staphyloytic activity of LasA is inhibited by tetrathylepentamine and 1,10-phenanthroline (zinc chelators) as well as excess Zn$^{2+}$ and dithiothreitol. However, LasA was not inhibited by several serine or cysteine proteinase inhibitors including diisopropyl fluorophosphate, phenylmethylsulfonyl fluoride, leupeptin, and N-ethylmaleimide. LasA staphyloytic activity was also insensitive to N-$\text{N}^\prime$-$\text{p}$-tosyl-$\text{l}$-lysine chloromethyl ketone or phosphoramidon. EDTA and EGTA were inhibitory only at concentrations greater than 20 mM. Without added inhibitors, LasA obtained by DEAE-cellulose fractionation was active toward $\beta$-casein, but the same cleavage patterns were observed with column fractions containing little or no LasA. The $\beta$-casein cleaving activity was fully blocked in the presence of inhibitors that did not affect staphyloytic activity. In the presence of such inhibitors, purified LasA was inactive toward acetyl-Ala$_4$ and benzyloxycarbonyl-Gly-Pro-Gly-Gly-Pro-Ala, but it degraded soluble recombinant human elastin as well as insoluble elastin. N-terminal amino acid sequencing of two fragments derived from soluble elastin indicated that both resulted from cleavages of Gly-Ala peptide bonds located within similar sequences, Pro-Gly-Val-Gly-Gly-Ala-Xaa (where Xaa is Phe or Gly). In addition, Ala was identified as the predominant N-terminal residue in fragments released by LasA from insoluble elastin. A dose-dependence study of elastase stimulation by LasA indicated that a high molar ratio of LasA to elastase was required for significant enhancement of elastolysis. The present results suggest that LasA is a zinc metalloendopeptidase selective for Gly-Ala peptide bonds within Gly-Gly-Ala sequences in elastin. Substrates that contain no Gly-Gly peptide bonds such as $\beta$-casein appear to be resistant to LasA.

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1 The abbreviations used are: DFP, diisopropyl fluorophosphate; AhP, Aeromonas hydrophila protease; PMSF, phenylmethylsulfonyl fluoride;
and TLCK) had no effect on the staphyloptic activity of LasA, and 1,10-phenanthroline, an inhibitor of LasA staphyloptic activity, did not block β-casein degradation (17). Hence, LasA was proposed to be a “modified” serine protease (14, 17).

Here we present evidence that LasA is a zinc metalloendopeptidase that preferentially cleaves peptide bonds subsequent to Gly-Gly pairs in proteins and peptides. We identify Gly-Gly-Ala sequences as the preferred sites of LasA cleavage in elastin and show that the ability of LasA to enhance elastin digestion by elastase largely depends on the amount of elastase and may require excess amounts of LasA. Finally, we provide evidence suggesting that β-casein digestion by LasA is only due to a contaminating protease.

MATERIALS AND METHODS

Bacterial Strains and Enzyme Purification—LasA and elastase were purified from P. aeruginosa strains FR2D and Habs serotype 1, respectively. Both strains were grown at 37 °C for 24 h in tryptie soy broth without dextrose (Difco), and the enzymes were purified from the respective cell-free conditioned media by ammonium sulfate precipitation and DEAE-cellulose chromatography as described (16, 18). LasA eluted first from the column (peak I) and was followed by three protein peaks designated II, III, and IV. The major constituents in peaks II and III migrated as proteins of about 23 and 28 kDa, respectively. Both strains were grown at 37 °C for 24 h in tryptic soy broth.

Assay of Staphyloptic Activity—Staphyloptic activity was determined spectrophotometrically by monitoring the decrease in absorbance at 595 nm of a heat-killed S. aureus cell suspension (0.3 mg/ml; 0.02 M Tris-HCl, pH 8.5 (buffer A)) (16). The reaction volume was 1 ml, and the amount of purified LasA added was 0.4–0.7 µg (0.05–0.09 units). One unit of activity was defined as the amount of enzyme that causes an Amax decrease of 1 absorbance unit in 1 min. For inhibition studies with this assay, LasA was preincubated (30 min, room temperature) with the specified inhibitor, and then the assay reaction was performed in the presence of the inhibitor.

Assay of Elastolytic Activity—Elastolytic activity was determined with insoluble elastin-Congo red (Sigma) as the substrate. Reaction suspensions (1.1 ml in 50 mM Tris-HCl, 0.5 mM CaCl2, 0.2 mM bovine serum albumin, pH 7.5) containing 10 mg of elastin-Congo red and 0.5 µg of elastase were incubated at 37 °C for 2 h with or without varying amounts of LasA that was first treated with PMSF (0.4 mM) and TLCK (5 mM). Reactions were stopped by adding 0.1 ml of 120 mM EDTA followed by immediate cooling and centrifugation (Beckman Microfuge, 4000 × g) for the determination of elastin solubilization by measuring the absorbance at 495 nm of the clear supernatant.

LasA Digestion of Elastin—Elastin digests with LasA were performed in 0.02 or 0.05 M Tris-HCl, pH 8.5, containing 5 mM TLCK, 0.4 mM PMSF, and 1 mM phosphoramidon (TI buffers). LasA was incubated (30 min, room temperature) with the inhibitors before mixing with the elastin substrate. Soluble recombinant human elastin (tropoelastin, 20 µg in 25 µl of 0.02 M TI buffer) (19) was mixed with LasA (56 ng in 20 µl of 0.02 M TI buffer) and incubated for 2 h at 37 °C. The reaction was terminated by heating in SDS sample buffer (20), and the products were analyzed by SDS-PAGE in a 12% polyacrylamide gel. For LasA digests of insoluble elastin, 5 µg of LasA were preincubated (30 min, room temperature) in 250 µl of 0.05 M TI buffer. 2.5 mg of solid insoluble elastin were then added, and the resulting suspensions were incubated at 37 °C for 8 h. The reaction was stopped by centrifugation (as above) to remove remaining insoluble elastin, and the supernatants were heated in SDS sample buffer. The reaction products were analyzed by SDS-PAGE in a 12% polyacrylamide gel. For LasA digests of insoluble elastin, 5 µg of LasA were preincubated (30 min, room temperature) in 250 µl of 0.05 M TI buffer. 2.5 mg of solid insoluble elastin were then added, and the resulting suspensions were incubated at 37 °C for 8 h. The reaction was stopped by centrifugation (as above) to remove remaining insoluble elastin, and the supernatants were heated in SDS sample buffer. The reaction products were analyzed by SDS-PAGE in a 12% polyacrylamide gel. For LasA digests of insoluble elastin, 5 µg of LasA were preincubated (30 min, room temperature) in 250 µl of 0.05 M TI buffer. 2.5 mg of solid insoluble elastin were then added, and the resulting suspensions were incubated at 37 °C for 8 h. The reaction was stopped by centrifugation (as above) to remove remaining insoluble elastin, and the supernatants were heated in SDS sample buffer. The reaction products were analyzed by SDS-PAGE in a 12% polyacrylamide gel.
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**FIG. 1.** Electrophoretograms showing defined fragments (A) and size distribution of solubilized fragments (B) generated by LasA from soluble human recombinant tropoelastin and insoluble bovine elastin, respectively. Digests were performed in the presence of phosphoramidon (1 mM), PMSF (0.4 mM), and TLCK (5 mM), and the reaction products were analyzed by SDS-PAGE in a 12% (A) or a composite 6/12% gel (see "Materials and Methods" for details) (B). Proteins were visualized by silver (A) or Coomassie Blue (B) staining. A, lane 1, soluble tropoelastin alone; lane 2, tropoelastin plus LasA; lane 3, LasA alone; lane 4, molecular mass standards with respective sizes in kDa shown on the right. En shows the migration position of intact tropoelastin; I–IV indicate migration positions of the respective elastin fragments. B, supernatants obtained after incubation of insoluble elastin and elastin-Congo red with LasA are shown in lanes 1 and 2, respectively. LasA alone and molecular mass standards are shown in lanes 3 and 4, respectively. Molecular mass values are given in kDa on both sides. The arrow shows the border between the 6% (top) and 12% (bottom) portions of the gel.

LasA has been shown to enhance the elastolytic activity of *Pseudomonas* elastase in a dose-dependent manner (11). However, the effect has only been shown with high elastase input (10 μg) and a relatively narrow range of LasA/elastase molar ratios (0.1 to 1.7, 0.5–10 μg of LasA). Here we extended these studies using a low elastase input (0.5 μg) and a broad range of LasA/elastase molar ratios (0.1 to 80, 0.03–24 μg of LasA). To stabilize the enzymes, all incubations were performed in the presence of 0.2 mg/ml bovine serum albumin. Controls to ascertain the stability of elastase and LasA in the presence of each other included mixtures of LasA and elastase (0.5 μg) at representative LasA/elastase molar ratios of 1:1 and 10:1. These mixtures were incubated without elastin. In addition, each enzyme was incubated alone. At the end of the incubation, proteolytic activity of elastase (*versus* azocasein (16, 18)) and staphylolytic activity of LasA (measured in the presence of 0.1 mM phosphoramidon to prevent interference by elastase) were determined, and no loss in activity of either enzyme was noted whether incubated alone or in combination with each other. Thus, degradation of one enzyme by the other in the assays of combined elastolytic activity is unlikely. In the assays of elastolytic activity, LasA alone was about 30-fold less effective than elastase in solubilizing the elastin-Congo red substrate. When added to elastase (Fig. 2), essentially no increase in elastolytic activity was observed at LasA/elastase ratios lower than 1 (LasA input of 0.03–0.3 μg). A 2-fold enhancement was obtained at a LasA/elastase molar ratio of 5 (1.5 μg of LasA), whereas 30- and 80-fold excesses of LasA (9 and 24 μg of LasA) were required to obtain 4- and 12-fold enhancement of elastolysis, respectively. These results confirm the limited elastolytic power of LasA.
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III, are not shown). This raised the possibility that the LasA fraction; similar data, obtained with 1 ng of fraction lane 2 presence ( ) or absence ( ) of protease inhibitors: 1 mM phosphoramidon (PHSN), 0.4 mM PMSF, 5 mM TLCK, and 2 mM 1,10-phenanthroline (PHEN). Blanks: lanes 1, 9, or 18, -casein, LasA, or fraction II alone, respectively. Lane 19, molecular mass standards in kDa indicated on the right. B, SDS-PAGE analysis of -casein digests with fraction II (5 ng). Digests were performed in the absence (lane 2) or presence (lanes 3–8, respectively) of 2 mM PMSF, 5 mM DFP, 5 mM TLCK, 2 mM 1,10-phenanthroline, 3% isopropyl alcohol (a control for digest with PMSF and DFP that contained this amount of isopropyl alcohol), or 1 mM phosphoramidon. Lane 1, -casein alone; -CAS, -casein; F1 and F2, migration positions of -casein fragments. C, SDS-PAGE analysis of -casein digests with fraction II in preparation for N-terminal amino acid sequence analysis. The sequences of the first 10 and 4 residues of fragments from the digest shown in lane 2 were electroblotted to polyvinylidene difluoride and subjected to N-terminal amino acid sequence analysis.

alone and show that, at a low elastase concentration, excessive amounts of LasA are required to significantly increase the rate of solubilization of insoluble amorphous elastin.

**-Casein Is Resistant to LasA**—LasA has been reported to cleave -casein into two distinct fragments by cleavage at the Lys29-Ile30 peptide bond (14, 17). Our LasA preparation, which eluted as fraction I on a DEAE-cellulose column (16), initially exhibited a similar activity toward -casein (Fig. 3A, lane 2). However, the same degradation pattern was observed with DEAE-cellulose fractions II and III, which eluted from the DEAE-cellulose column after LasA and showed very little or no staphylolytic activity (16). Moreover, fractions II and III were more effective in degrading -casein than was the LasA fraction (compare lanes 10 and 14 containing 5 and 2.5 ng of fraction II protein, respectively, with lane 2 containing 50 ng of the LasA fraction; similar data, obtained with 1 ng of fraction III, are not shown). This raised the possibility that the -casein degradation associated with this LasA preparation could reflect the activity of a contaminating protease(s) rather than LasA itself. To explore this possibility, we compared the LasA fraction with fraction II in the hydrolysis of -casein and its inhibition. To achieve approximately the same level of activity toward -casein with both enzyme substrates, the input of protein was 10-fold higher with the LasA fraction than with fraction II (50 and 5 ng, respectively). As shown in Fig. 3A (lanes 3 and 11), -casein cleavage by both protease fractions was totally abolished in the presence of a mixture of inhibitors containing phosphoramidon (PHSN, a specific inhibitor of P. aeruginosa elastase), PMSF (a general serine protease inhibitor), TLCK (an inhibitor of lysine-specific serine proteases), and 1,10-phenanthroline (PHEN, an inhibitor of LasA as well as elastase). Since the observed cleavage of -casein could potentially represent the combined effect of a variety of proteases, we compared subsets of this inhibitor mixture for the ability to block degradation. The inhibition profiles obtained with both the LasA fraction and fraction II under all conditions were the same (Fig. 3A). Inhibition of both was clearly dependent on TLCK, and omitting any of the other three inhibitors did not appear to affect the ability of TLCK to inhibit -casein hydrolysis (lanes 4, 6, and 7 for LasA fraction I and lanes 12, 15, and 16 for fraction II). In the absence of TLCK, the cleavage was essentially the same as that seen in the controls (compare lane 2 with lanes 5 and 8; compare lane 10 with lanes 13 and 17). PMSF at 0.4 mM was not inhibitory and could not substitute for TLCK (lanes 5, 13, and 14). However, in a subsequent experiment in which the effect of each inhibitor on -casein degradation by fraction II was tested independently of the other inhibitors (Fig. 3B), PMSF at 2 mM not only successfully replaced TLCK as an inhibitor of the reaction, but it even appeared to be more effective than TLCK in blocking this activity (Fig. 3B, compare lanes 3 and 5). DFP (5 mM) was also highly inhibitory (Fig. 3B, lane 4), and in agreement with the results of Fig. 3A, neither 1,10-phenanthroline nor phosphoramidon (Fig. 3B, lanes 6 and 8, respectively) alone inhibited fraction II action on -casein. The same results were observed with LasA and fraction III as the active fractions (data not shown). Together, these results exclude -casein as a LasA substrate and suggest that the observed cleavages of -casein arose from the action of a serine protease(s) that was not fully resolved from LasA during purification on DEAE-cellulose. To identify the peptide bonds cleaved by this putative serine protease, -casein fragments 1 and 2 (Fig. 3C), which were generated by fraction II in the presence of 1,10-phenanthroline, phosphoramidon, and PMSF were subjected to automatic N-terminal amino acid sequence analysis. The sequences of the first 10 and 4 residues of fragments 1 and 2 were found to be XELEELNVP and IEXF, respectively (X, unidentified residue). These sequences correspond to residues 1–10 and 30–33 in -casein (23). Thus, fragment 1 represents an N-terminal -casein peptide that most likely resulted from cleavage(s) of a peptide bond(s) within the C-terminal portion of the -casein molecule, whereas fragment II apparently resulted from cleavage of the Lys29-Ile30 of -casein, the same as that previously reported for the LasA cleavage site (14, 17).

**DISCUSSION**

Previous conflicting results regarding the classification and cleavage specificity of LasA (14, 17) led us to the present study of these issues. To assign LasA into one of the four known classes of proteases, serine, aspartic, thiol, or metalloendopeptidases (24), we examined the inhibition properties of the enzyme (Table I). The assay of staphylolytic activity that we used for this purpose is sensitive and specific. Although the rate of staphylosis by LasA may be increased by up to 2.5-fold in the presence of P. aeruginosa elastase or alkaline protease (observed by us (15) and others (17)), neither elastase nor alkaline protease alone show staphylolytic activity. In addition, for significant enhancement of LasA activity on S. aureus cells, relatively large amounts of elastase or alkaline protease are required. As suggested from results with -casein as the substrate, our purified LasA preparation apparently contained traces of contaminating proteases. These, however, are below the levels that may affect LasA action on the staphylococcal cells. Thus, the assay of staphylolytic activity as used here is reliable. Since LasA staphylolytic activity was inhibited by two...
zinc chelators (1,10-phenanthroline and tetraethylenepentamine) but not by any of the other class-specific inhibitors, we conclude that LasA is a zinc-dependent metalloendopeptidase. This conclusion is further supported by the inhibitory effect of excess zinc ions (Table 1), a property characteristic of other zinc-dependent peptidases (reviewed in Ref. 25).

The amino acid sequence of LasA shows approximately 40% identity with those of L. enzymogenes and A. lyticus β-lytic endopeptidases (4, 16, 26, 27). In addition, the sequence of the first 40 amino acid residues of a recently described Aeromonas hydrophila zinc-dependent endopeptidase (AhP) (28) is 46 and 69% identical with those of L. enzymogenes β-lytic endopeptidase and LasA, respectively. Thus, LasA, AhP, and the β-lytic endopeptidases of L. enzymogenes and A. lyticus are closely related. The β-lytic endopeptidase of L. enzymogenes (26) and AhP (28) were shown to contain 1 zinc atom/mol. This supports LasA as a zinc metalloendopeptidase and is consistent with the recent demonstration that efficient production of LasA by P. aeruginosa requires zinc ions (29). LasA as well as the Lysozyme and Achromobacter β-lytic endopeptidases (15, 26, 27) does not contain the HEXXH zinc-binding motif typical of most zinc proteinases. An HXH motif found in A. lyticus β-lytic endopeptidase (27) and shared with both the Lysozyme enzyme and LasA (positions 120–122) was proposed as a potential zinc ligand (27). Substitution in LasA of His-120 blocks LasA activity (15), suggesting that His-120 may be one of the zinc ligands in LasA.

As a zinc metalloendopeptidase, LasA action on substrates other than S. aureus is expected to be blocked by zinc chelators but not by inhibitors of the other classes of proteases. Our results with soluble tropoelastin and insoluble elastin as substrates were fully consistent in this regard. While LasA action on elastin was inhibited by 1,10-phenanthroline (data not shown), it was not blocked by phosphoramidon nor was it affected by DFP, PMSF, or TLCK. Our identification of the preferred LasA cleavage sites in elastin as the Gly-Ala peptide bonds within the Gly-Gly-Ala sequences surrounds by apolar sequences is in accordance with previous reports of LasA staphylocytic protease specificity of cleavage, i.e. hydrolysis of pentaglycine, hexaglycine, or oligopeptides containing at least 2 glycine residues in a row (6, 7, 16, 17). That Gly-Gly pairs subsequent to Gly-Gly pairs, in particular those followed by Ala and located within apolar environments. However, no such sequences exist in β-casein. Furthermore, the only 4 Gly residues in β-casein are followed by Pro, Glu, or Val (23), and these sites are unlikely to be sensitive to LasA. In addition, the Gly-Ile bond of β-casein is situated within a highly charged environment containing a Glu, a phosphorylated Ser, and 2 Lys residues in close vicinity (23). Charged residues should interfere with LasA action, as was shown directly for AhP (28), which is a protease similar to LasA. Yet our initial studies did show that the LasA preparation could cleave β-casein with inhibition by TLCK, PMSF, and DFP. This would suggest that the active entity is a lysine-specific serine proteinase rather than a zinc metalloendopeptidase such as LasA. Further investigation revealed that fractions II and III of the DEAE-cellulose fractionation of P. aeruginosa extracellular proteins, which contained only traces of LasA, were even more effective than the LasA fraction in eliciting this cleavage. A 30-kDa lysine-specific endopeptidase from P. aeruginosa has been described that cleaves peptide bonds involving the carboxyl side of internal lysine residues and is inhibited by TLCK (5). Our fraction III contained a protein of about 28 kDa molecular mass as a major component (16), and this may be the lysyl-specific protease that was responsible for the observed β-casein cleaving activity in LasA preparations.

Several studies have demonstrated a synergism between P. aeruginosa elastase and LasA protease in the efficient degradation of elastin. Although purified LasA alone does not show high elastolytic activity, culture filtrates of lasA mutants (e.g. PAO-E64 and FRD2128) show much less elastolytic activity (30–90%) when compared with their respective wild type strains (8, 10, 11, 16). Peters and Galloway (11) examined this further with purified components and showed that the elastolytic activity of elastase (10 μg input) increased 25-fold in the presence of purified LasA (10 μg input), which is a LasA elastase molar ratio of ~1.7. We also used purified enzymes to characterize the LasA-dependent enhancement of elastolytic activity but at a 20-fold lower elastase input as this amount is more in the range used in our standard assays of elastolytic activity. Under such conditions, LasA appears to be much less effective in potentiating elastase activity. Although the degree of enhancement of elastolysis was still dependent on LasA concentration, a LasA/elastase molar ratio of ~30 was required to increase elastolysis by 4-fold, and a molar ratio of ~80 increased the rate of elastolysis by 12-fold. This quantitative difference between situations with high and low elastase concentrations demonstrates that the synergistic effect of LasA and elastase in the solubilization of insoluble, amorphous elastin substrates is better pronounced in the presence of high elastase concentrations. This may be explained by the complementary specificities of LasA and elastase, the insoluble nature of the elastin substrate, and the fact that only soluble degradation products are apparent in the assay. The limited cleavages by LasA, either alone or in the presence of small amounts of elastase, may only yield a limited number of soluble elastin fragments. On the other hand, in the presence of excess elastase many insoluble elastin fragments may be produced in which LasA cleavage sites are likely more accessible to LasA than in the intact elastin substrate, and thus, even small
amounts of LasA may largely increase the rate of elastin solubilization. Colonies of lasA mutants on elastin-containing agar plates are dramatically deficient in elastin clearing when compared with wild type strains (8, 10), which suggests that the relative concentration of elastase is high under these conditions. We conclude that elastase potentiates LasA enhancement of the elastolytic potential of P. aeruginosa.

The limited elastolytic power of LasA suggests that elastin may not be the primary or only substrate for LasA action. Collagen, another glycine-rich connective tissue protein that we examined as a potential substrate for LasA, was also a poor substrate. Only a minor fraction of the α-chains was partially cleaved and no more than three smaller fragments were generated even after prolonged incubations (data not shown). These limited cleavages could occur at sequences such as Gly-Ala-Ala-Gly found at several sites within the α1(I) and α2(I) collagen chains (33). LasA was reported to effectively hydrolyze two biologically active peptides containing internal Gly-Gly or Gly-Gly-Gly sequences (17). Although consistent with LasA specificity, cleavage of these peptides (serac, a stimulator of sea urchin spermatozoa, and a sleep-inducing peptide, respectively) has no immediate relevance to P. aeruginosa infections. LasA, however, is a potent staphylolytic protease. By virtue of its action on the cell wall peptidoglycan of S. aureus cells, LasA may provide P. aeruginosa with a selective advantage against S. aureus cells during colonization at the infection site.

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