Pergamon

Biochemical Pharmacology, Vol. 48, No. 4, pp. 651–657, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00227-4

SPECIFIC CLEAVAGE OF SECRETORY LEUKOPROTEASE INHIBITOR BY NEUTROPHIL ELASTASE AND SALIVA

KEN-ICHI MASUDA,* TETSUYA SUGA, AKIKO TAKEUCHI, MASAHARU KANESAKI, ATSUSHI IMAIZUMI and YOJI SUZUKI

Institute for Biomedical Research, Teijin Limited, Hino, Tokyo 191, Japan

(*Received* 26 *October* 1993; *accepted* 9 *May* 1994)

Abstract—In an attempt to explore the process of naturally occurring secretory leukoprotease inhibitor (SLPI) fragmentation, the cleavage profile of SLPI, which had been prepared by recombinant techniques, was investigated biochemically. Restricted fragments of SLPI were detected using SDS–PAGE after treatment with human neutrophil elastase (NE) or normal saliva and sequenced at their cleavage sites. Among these restricted fragments, two species of nearly half-length SLPIs that contained the C-terminal domain, (Arg^{58}–Ala^{107})SLPI and (Arg^{59}–Ala^{107})SLPI, were detected. They were both as active at inhibiting NE as the parent SLPI. These results suggest that functional SLPI derivatives may be generated physiologically in the respiratory tract under inflammatory and healthy conditions.

Key words: anti-protease; domain; fragmentation; inhibitory specificity; neutrophil protease; respiratory tract

SLPI†, also called antileukoprotease, is a non-glycosylated serine protease inhibitor of 107 amino acids [1–3]. It inhibits a wide range of proteases, including elastase, cathepsin G, chymotrypsin and trypsin [1, 2]. X-ray crystallographic analysis has shown that SLPI comprises two separate domains of similar architecture [4]. It is found in a variety of fluids, including saliva [5], bronchial mucus [6], tears [7], cervical mucus [8] and seminal plasma [9]. In the respiratory tract, SLPI is produced by serous cells of tracheal and bronchial submucosal glands and by non-ciliated bronchiolar epithelial cells, which have been identified as goblet and clara cells [10–12]. Recent studies have also shown the secretion of SLPI by type II pneumocyte cell lines [13]. The exact physiological function of SLPI has yet to be elucidated fully, but its major role is thought to be protection of the airway epithelial surface from attack by NE [14–16] which has been found in pulmonary lavage fluid and sputum from patients with inflammatory respiratory diseases [17, 18].

Investigations into the relationships between the anti-protease activities of SLPI and its domain structures have demonstrated that the inhibitory activity is located in the second (C-terminal) domain [19–21]. We also reported recently that a recombinant half-sized SLPI containing the C-terminal domain, (Asn^{55}–Ala^{107})SLPI, is as active an inhibitor as SLPI itself of NE and cathepsin G [22, 23]. It would be very interesting to determine whether similar active SLPI fragments are present in the respiratory tract under physiological and pathological conditions.

The occurrence of inactivation and fragmentation of SLPI in the respiratory tract has been suggested by the following observations: (a) active oxygen species released by neutrophils can oxidize methionine residues of SLPI and decrease its inhibitory capacity [24]; (b) western blotting analysis demonstrated that SLPI in pulmonary lavage fluids from patients with cystic fibrosis was fragmented [16]; and (c) SLPI was found to be cleaved and inactivated by proteinases from *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are found frequently in patients with respiratory infections [25]. However, the precise mechanism responsible for SLPI fragmentation in the respiratory tract is not well understood.

In this study, we investigated restricted cleavage of SLPI by NE and human normal saliva. Among the degradation products, two half-length SLPIs containing the C-terminal domain (Arg^{58}–Ala^{107})SLPI and (Arg^{59}–Ala^{107})SLPI, were detected. Both restricted fragments were prepared using recombinant technology and showed anti-NE activities equally as strong as that of intact SLPI. These results suggest that these half-sized SLPI derivatives are naturally occurring functional entities in the respiratory tract.

MATERIALS AND METHODS

Enzyme and substrate.

Human neutrophil elastase (NE) and cathepsin G were purchased from Elastin Products Co (Owensville, MO, U.S.A.). Bovine pancreatic trypsin and chymotrypsin, porcine pancreatic elastase, human plasma thrombin and plasmin were from Sigma Chemical Co. (St Louis, MO, U.S.A.) and human plasma kallikrein was obtained from Protogen AG (Läufelfingen, Switzerland). The following substrates were used: methoxy succinyl-Ala-Ala-Pro-Val-4-nitroanilide (MeO-Suc-Ala-Ala-

* Corresponding author. Tel. (81) 425-86-8133; FAX (81) 425-87-5515.

† Abbreviations: SLPI, secretory leukoprotease inhibitor; NE, human neutrophil elastase; PEG-6000, polyethylene glycol 6000; IC_{50}, 50% inhibitory concentration; K_i, inhibition constant.

Pro-Val-pNA) for NE, benzoyl-Arg-4-nitroanilide (Bz-Arg-pNA) for trypsin, succinyl-Ala-Ala-Pro-Phe-4-nitroanilide (Suc-Ala-Ala-Pro-Phe-pNA) for chymotrypsin and succinyl-Phe-Pro-Phe-4-nitroanilide (Suc-Phe-Pro-Phe-pNA) for cathepsin G were obtained from Bachem Feinchemikalien AG (Bubendorf, Switzerland), D-Phe-pipecolyl-Arg-4-nitroanilide (D-Phe-Pip-Arg-pNA) for thrombin, D-Val-Leu-Lys-4-nitroanilide (D-Val-Leu-Lys-pNA) for plasmin and D-Pro-Phe-Arg-4-nitroanilide (D-Pro-Phe-Arg-pNA) for kallikrein were purchased from Kabi Vitrum (Stockholm, Sweden) and succinyl-Ala-Ala-Ala-4-nitroanilide (Suc-Ala-Ala-Ala-pNA) for porcine elastase was purchased from Nacalai Tesque Co (Kyoto, Japan).

Recombinant SLPI.

The DNA fragments encoding SLPI [1] were synthesized chemically using appropriate codons from *Escherichia coli*. The human growth hormone gene was fused to the SLPI gene to optimize the expression size via a DNA sequence encoding Leu-Val-Pro-Arg, which can be cleaved by thrombin [22, 26]. The expression vector was constructed and introduced into *E. coli* HB101; the transformed cell selected by ampicillin was cultured and the fusion protein was obtained as an inclusion body. The fusion protein was dissolved and cleaved with thrombin, and the active inhibitor was purified by chromatography after refolding. The inhibition constant (K_i value) of the recombinant SLPI against elastase was comparable with that of native SLPI, 1.87×10^{-10}, which was reported by Smith and Johnson [3].

Incubation of SLPI with NE

The SLPI was incubated with NE at 37° in 0.1 M (2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonicacid (HEPES), containing 1.0 M NaCl, 0.1% (w/v) polyethylene glycol 6000 (PEG-6000), pH 7.5, at the indicated molar ratios in Fig. 1. At the required time-points, 50 μL aliquots were removed and stored at −20° until required for SDS–PAGE analysis.

Incubation of SLPI with human normal saliva

The SLPI was dissolved (1 mg/mL) in human normal saliva, which was filtered through a 0.22 μm filter (Milex GS, Nihon Millipore Ltd., Tokyo, Japan) to remove bacteria, and incubated at 37°. At the required time-points in Fig. 2, 50 μL aliquots were removed and stored at −20° until required for SDS–PAGE analysis.

Sodium dodecyl sulfate polyacrylamide-gel electrophoresis (SDS–PAGE)

The SDS–PAGE analysis was carried out as described by Schägger and Jagow [27]. The samples were diluted 1:1 with 50 mM Tris–HCl (pH 6.8), containing 4% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, 12% (v/v) glycerol, heated at 100° for 3 min and applied to the gel. Electrophoresis was carried out on a 14% (w/v) gel at 30 mA, after which the gels were stained with Coomassie brilliant blue.

Amino acid sequencing

Direct protein sequencing from an Immobilon-P transfer membrane (Nihon Millipore Ltd., Tokyo, Japan) was carried out, as described by Andersen [28]. The proteins separated by SDS–PAGE were electroblotted from the gels onto a transfer membrane at 500 mA for 30 min using an electroblotting system MilliBlot-SDE (Nihon Millipore Ltd., Tokyo, Japan). The protein bands were visualized with Coomassie brilliant blue, cut from the membrane and sequenced directly using an Applied Biosystems Model 477A protein sequencer.

Inhibition assay

Fifty per cent inhibitory concentration (IC_{50} value). The reaction buffer used throughout this experiment was 0.1 M HEPES (pH 7.5), containing 0.5 M NaCl. The required inhibitor solution was preincubated for 10 min at 37° in a total volume of 160 μL with appropriate amounts of the required enzyme. The reaction was started by adding 20 μL substrate and carried out at 37° for 20 min. The absorbance of the reaction mixture at 405 nm was measured. The residual enzyme activities were plotted for various concentrations of the inhibitor and the IC_{50} value was determined.

Inhibition constants (K_i value). The substrates used were 0.3 mM MeO-Suc-Ala-Ala-Pro-Val-pNA for NE and 1.0 mM Bz-Arg-pNA for bovine pancreatic trypsin, and the reaction buffer used for both enzymes was 0.1 M HEPES, containing 1.0 M NaCl, 0.1% (w/v) PEG-6000, pH 7.5. The required inhibitor solution was preincubated for 1 hr at 37° (total volume of 160 μL) with appropriate amounts of the required enzyme, 20 nM elastase and 200 nM trypsin. The reactions were started by adding 20 μL substrate and carried out at 37° for 20 min. The absorbance of the reaction mixture at 405 nm was recorded and the inhibition constants were determined using the method of Henderson [29].

RESULTS

Detection of SLPI fragments

The proteolytic cleavage of SLPI after incubation with NE and human normal saliva was analysed using SDS–PAGE. Little or no cleavage of SLPI was observed with NE/SLPI molar ratios of 0.0625, 0.125, 0.25, 0.5 and 1.0 after incubation at 37° for 1 hr, but SLPI was fragmented to 11.0- and 5.5-kDa proteins when NE was in excess of SLPI at NE/SLPI molar ratios of 2.0 and 4.0 [Fig. 1(A)]. The SLPI was cleaved to an 11.0-kDa protein first at a NE/SLPI molar ratio of 2.0 and then gradually migrated to a 5.5-kDa [Fig. 1(B)]. The 5.5-kDa protein was resistant to further degradation when incubated for 8 days in the presence of NE. Specific cleavage of SLPI by human normal saliva was observed, but no exopeptidase activity was detected in the saliva. Fragments were detected, 10.0-, 7.0-, 5.5- and 3.0-kDa, after incubation for 28 days (Fig. 2). When SLPI was incubated with saline under the same conditions (control) for 28 days it remained in its intact form, and native SLPI in saliva was undetectable by SDS–PAGE (data not shown).

Determination of the cleavage site

After incubating SLPI with NE or saliva, the resulting fragments were separated by SDS–PAGE,

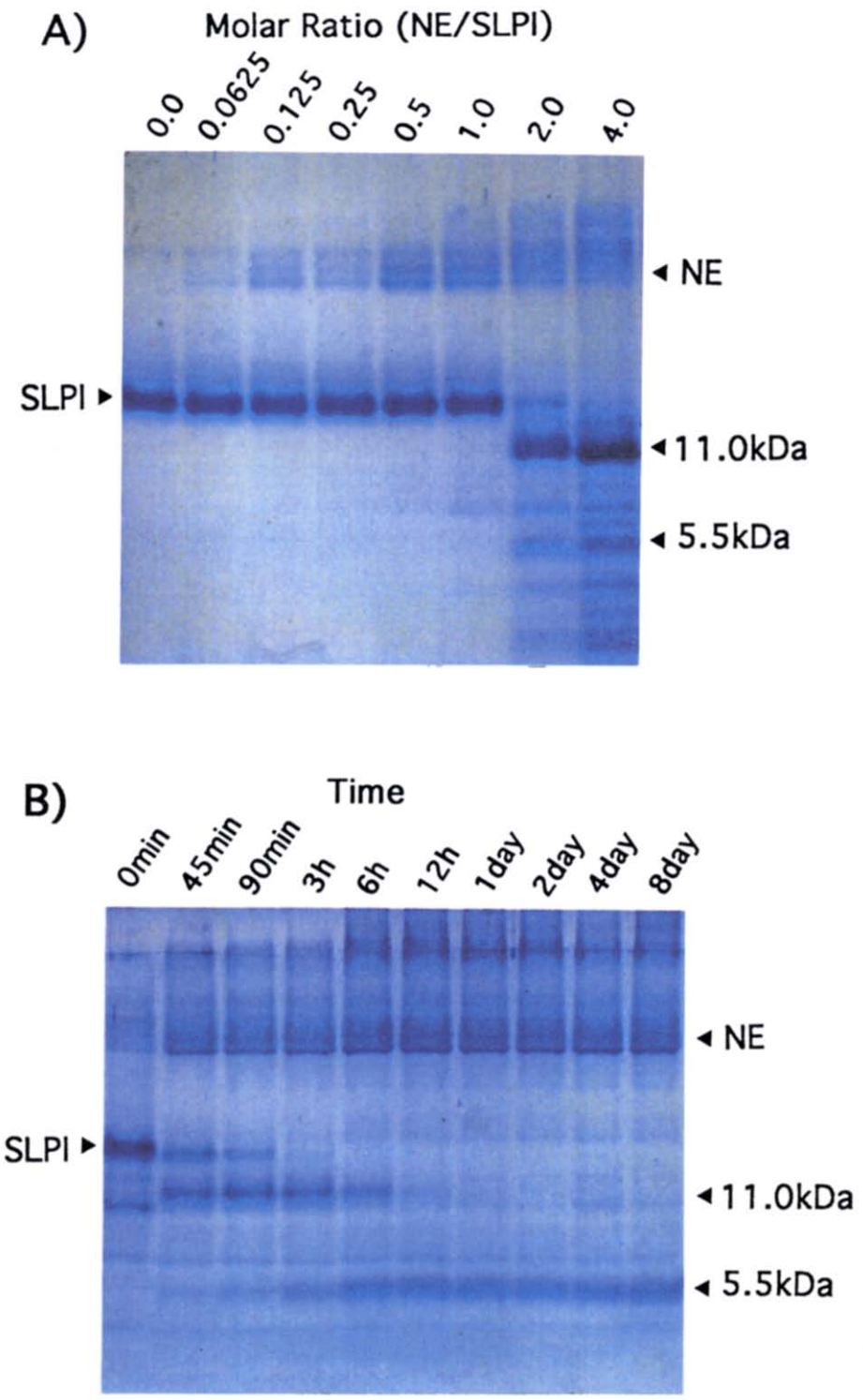

Fig. 1. SDS–PAGE analysis of the fragmentation of SLPI with NE. (A) SLPI was incubated with NE at the indicated molar ratios (NE/SLPI) at 37° for 1 hr. (B) SLPI was incubated at a molar ratio of 2.0 (NE/SLPI) for the indicated times.

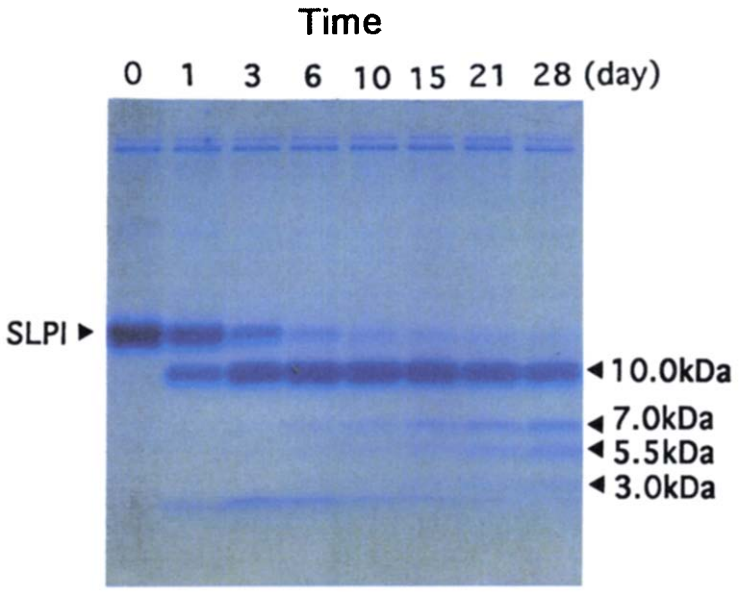

Fig. 2. SDS–PAGE analysis of the fragmentation of SLPI in human normal saliva. SLPI was incubated in saliva at concentration of 1 mg/mL. At the indicated times, aliquots were removed and subjected to electrophoresis.

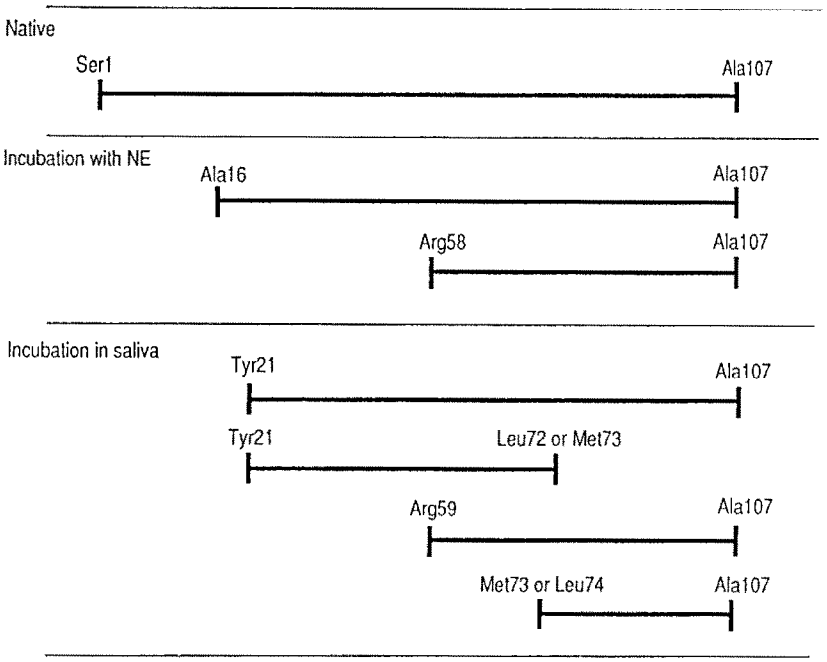

Fig. 3. Restricted fragments of SLPI after incubation with NE or human normal saliva.

electroblotted onto a transfer membrane and sequenced directly using a protein sequencer. Determination of the N-terminal amino acid sequences of 11.0- and 5.5-kDa proteins generated by NE at a NE/SLPI molar ratio of 2.0 showed that SLPI was cleaved at two different sites, Ser^{15}–Ala^{16} and Thr^{57}–Arg^{58}. Therefore, we concluded that the 11.0- and 5.5-kDa fragments corresponded to (Ala^{16}–Ala^{107})SLPI and (Arg^{58}–Ala^{107})SLPI, respectively. With respect to the fragmentation order, SDS–PAGE and N-terminal sequence analyses demonstrated that cleavage occurred first mainly between Ser^{15}–Ala^{16} followed by cleavage at Thr^{57}–Arg^{58}. After digestion with NE for 12 hr, SLPI was converted completely

Table 1. Fifty per cent inhibitory concentrations (IC_{50}) of SLPI and half-length SLPI against serine proteases

Enzyme (concentration; M)	50% inhibitory concentration (M)		
	SLPI	(Arg^{58}–Ala^{107})SLPI	(Arg^{59}–Ala^{107})SLPI
NE (2.0×10^{-8})	1.0×10^{-8}	1.3×10^{-8}	1.4×10^{-8}
Porcin pancreatic elastase (1.0×10^{-6})	3.7×10^{-5}	3.3×10^{-5}	3.7×10^{-5}
Human cathepsin G (2.0×10^{-8})	5.5×10^{-9}	8.0×10^{-9}	8.0×10^{-9}
Bovine pancreatic chymotrypsin (1.0×10^{-8})	1.0×10^{-8}	1.0×10^{-8}	1.1×10^{-8}
Bovine pancreatic trypsin (2.0×10^{-7})	3.3×10^{-7}	2.0×10^{-6}	2.0×10^{-6}
Human plasma thrombin (3.0×10^{-9})	$>1.0 \times 10^{-5}$	$>1.0 \times 10^{-5}$	$>1.0 \times 10^{-5}$
Human plasma plasmin (1.0×10^{-7})	$>1.0 \times 10^{-5}$	$>1.0 \times 10^{-5}$	$>1.0 \times 10^{-5}$
Human plasma kallikrein (1.0×10^{-7})	$>1.0 \times 10^{-5}$	$>1.0 \times 10^{-5}$	$>1.0 \times 10^{-5}$

Assay conditions; 0.1 M HEPES (pH 7.5), 0.5 M NaCl, 37°.
Chromogenic substrates; NE 0.3 mM MeO-Suc-Ala-Ala-Pro-Val-pNA; P.P.Elastase, 1.0 mM Suc-Ala-Ala-Ala-pNA; Cathepsin G, 1.0 mM Suc-Phe-Pro-Phe-pNA; Chymotrypsin, 1.0 mM Suc-Ala-Ala-Pro-Phe-pNA; Trypsin, 1.0 mM Bz-Arg-pNA; Thrombin, 0.2 mM D-Phe-Pip-Arg-pNA; Plasmin, 0.8 mM D-Val-Leu-Lys-pNA; Kallikrein, 2.0 mM D-Pro-Phe-Arg-pNA.

Table 2. Inhibition constants (K_i values) of SLPI and half-length SLPI against NE and bovine pancreatic trypsin

Enzyme	K_i values (M)		
	SLPI	(Arg^{58}–Ala^{107})SLPI	(Arg^{59}–Ala^{107})SLPI
NE	0.8×10^{-10}	0.9×10^{-10}	0.8×10^{-10}
Bovine pancreatic trypsin	3.3×10^{-8}	1.1×10^{-6}	1.1×10^{-6}

to a half-sized SLPI containing the C-terminal domain (Arg^{58}–Ala^{107})SLPI, which was not fragmented after incubation for a further 8 days. The N-terminal sequences of the fragments generated by incubation with saliva showed that cleavage of SLPI occurred at the Arg^{20}–Tyr^{21}, Arg^{58}–Arg^{59}, Leu^{72}–Met^{73} and Met^{73}–Leu^{74} peptide bonds. Among these degraded fragments, the 5.5-kDa protein was identified as the half-sized SLPI (Arg^{59}–Ala^{107})SLPI. All the fragments of SLPI generated by NE and saliva that were detected are shown schematically in Fig. 3. Although SLPI was fragmented partially by incubation with NE and saliva, the C-terminal domain structure remained intact under all the experimental conditions used.

Inhibitory activities of (Arg^{58}–Ala^{107})SLPI and (Arg^{59}–Ala^{107})SLPI

The genes encoding the amino acids 58–107 and 59–107 of SLPI were synthesized chemically and (Arg^{58}–Ala^{107})SLPI and (Arg^{59}–Ala^{107})SLPI were prepared using a thrombin-cleavable fused protein expression system [22], described in Materials and Methods. The inhibitory activities (IC_{50} values) of SLPI and the half-length SLPIs against several serine proteases were determined. The inhibitory activities of (Arg^{58}–Ala^{107})SLPI and (Arg^{59}–Ala^{107})SLPI against elastase, cathepsin G and chymotrypsin were as strong as that of intact SLPI; both half-length SLPIs showed less trypsin inhibitory activity than intact SLPI and none of the inhibitors showed any inhibitory activity against thrombin, plasmin or kallikrein (Table 1). The inhibition constants (K_i values) of the full- and half-length SLPIs against NE and bovine pancreatic trypsin were determined using the method of Henderson [29] (Table 2). The K_i values of (Arg^{58}–Ala^{107})SLPI and (Arg^{59}–Ala^{107})SLPI for NE were comparable with that of intact SLPI, but their trypsin inhibitory activities were reduced compared with SLPI itself. These half-length SLPIs were more specific for NE than trypsin compared with full-length SLPI.

DISCUSSION

The SLPI is generally considered to play a defensive role in the respiratory epithelial surface as a physiological inhibitor of neutrophil protease and it has been suggested that fragmentation of SLPI occurs in inflamed airways. *P. aeruginosa* elastase has been reported to cleave the active center of SLPI [25], which is believed to be located in Leu^{72}–Met^{73}–Leu^{74} [1]. *In vitro* studies on cleavage by NE showed that SLPI remained intact when exposed to equimolar amounts of NE alone, whereas a combination of NE and oxidants caused fragmentation to an 8-kDa protein [16]. However, little is known about the process of degradation of SLPI and the inhibitory activities of its fragments in the inflamed respiratory tract.

Our results demonstrate that NE could not cleave SLPI at a NE/SLPI molar ratio of 1.0, which may be because the complex is stable, whereas it was

fragmented by excess NE at at least two specific sites in the molecule, Ser^{15}–Ala^{16} and Thr^{57}–Arg^{58}. SLPI should form stable complexes with NE during the incubation, but these were undetectable on the gels (Fig. 1) because they were dissociated under SDS–PAGE conditions. No cleavage at the active site was observed and a fragment containing the C-terminal domain (Arg^{58}–Ala^{107})SLPI, was generated. It is noteworthy that this molecule was resistant to further degradation by NE. These results suggest that excess NE arising from an imbalance between proteases and anti-proteases may cause degradation not only of the matrix, but also of protease inhibitors in the lung. Therefore, we speculate that the C-terminal domain structure of SLPI remains even when the amount of NE is in excess of that of anti-proteases in the inflammatory regions and (Arg^{58}–Ala^{107})SLPI may exist in the respiratory tract under physiological conditions.

In order to ascertain whether similar fragmentation occurred in human normal saliva, we investigated the degradation of SLPI incubated with saliva, which had weak proteolytic activity with cleavage occurring at the following four different sites, at least, Arg^{20}–Tyr^{21}, Arg^{58}–Arg^{59}, Leu^{72}–Met^{73} and Met^{73}–Leu^{74}. Cleavage was detected after a 24 hr incubation, although SLPI was in excess of the unknown enzymes in saliva compared with normal conditions *in vivo*. Therefore, native SLPI may be fragmented even under healthy conditions in less than 24 hr and the half-sized SLPI (Arg^{59}–Ala^{107})SLPI, is generated as a minor entity.

In order to characterize these phenomena more precisely, the inhibitory activities of (Arg^{58}–Ala^{107})SLPI and (Arg^{59}–Ala^{107})SLPI were investigated. Both fragments were as active against NE as, but had less trypsin inhibitory activity than, full-length SLPI. It has been reported that the recombinant C-terminal domains of SLPI retain elastase inhibitory activity [19, 20, 22], but the physiological generation of similar active fragments in the respiratory tract has not been mentioned. Our results suggest that SLPI derivatives containing the C-terminal domain may still act as elastase-specific inhibitors, even after SLPI fragmentation in the inflamed airway.

In conclusion (Arg^{58}–Ala^{107})SLPI and (Arg^{59}–Ala^{107})SLPI were identified as naturally occurring SLPI fragments that retained the full inhibitory activity of intact SLPI against NE. Thus, the half-length SLPI containing the C-terminal domain would appear to be an inhibitor which plays a defensive role to protect the pulmonary structures against attack by NE.

REFERENCES

1. Thompson RC and Ohlsson K, Isolation, properties, and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. *Proc Natl Acad Sci USA* **83**: 6692–6696, 1986.
2. Seemüller U, Arnhold M, Fritz H, Wiedenmann K, Machleidt W, Heinzel R, Appelhans H, Gassen HG and Lottspeich F, The acid-stable proteinase inhibitor of human mucus secretions (HUSI-I, antileukoprotease). *FEBS Lett* **199**: 43–48, 1986.
3. Smith CE and Johnson A, Human bronchial leukocyte protease inhibitor. *Biochem J* **225**: 463–472, 1985.
4. Grütter MG, Fendrich G, Huber R and Bode W, The 2.5Å X-ray crystal structure of the acid-stable proteinase inhibitor from human mucous secretions analysed in its complex with bovine chymotrypsin. *EMBO J* **7**: 345–351, 1988.
5. Ohlsson M, Rosengren M, Tegner H and Ohlsson K, Quantification of granulocyte elastase inhibitors in human mixed saliva and pure parotid secretion. *Hoppe-Seyler's Z Physiol Chem* **364**: 1323–1328, 1983.
6. Ohlsson K, Tegner H and Akesson U, Isolation and partial characterization of a low molecular weight acid-stable protease inhibitor from human bronchial secretion. *Hoppe-Seyler's Z Physiol Chem* **358**: 583–589, 1977.
7. Kuppers F, Protease inhibitor in human tears. *Biochim Biophys Acta* **229**: 845–849, 1971.
8. Wallner O and Fritz H, Characterization of an acid-stable proteinase inhibitor in human cervical mucus. *Hoppe-Seyler's Z Physiol Chem* **355**: 709–715, 1974.
9. Schiessler H, Arnhold M, Ohlsson K and Fritz H, Inhibitors of acrosin and granulocyte proteinases from human genital tract secretions. *Hoppe-Seyler's Z Physiol Chem* **357**: 1251–1260, 1976.
10. Kramps JA, Franken C, Meijer CJLM and Dijkman JH, Localization of low molecular weight protease inhibitor in serous secretory cells of the respiratory tract. *J Histochem Cytochem* **29**: 712–719, 1981.
11. Willems LNA, Kramps JA, Stijnen T, Fleuren GJ, Franken C and Dijkman JH, Evaluation of antileukoprotease in surgical specimens. *Eur J Respir Dis* **69**: 242–247, 1986.
12. Kramps JA, Boekhorst AHTT, Fransen JAM, Ginsel LA and Dijkman JH, Antileukoprotease is associated with elastin fibers in the extracellular matrix of the human lung. *Am Rev Respir Dis* **140**: 471–476, 1989.
13. Sallenave JM, Silva A, Marsden E and Ryle AP, Secretion of mucus proteinase inhibitor and elafin by clara cell and type II pneumocyte cell lines. *Am J Respir Cell Mol Biol* **8**: 126–133, 1993.
14. Hubbard RC and Crystal RG, Antiproteases. In: *The Lung, Scientific Foundations* (Eds. Crystal RG, West JB, Barnes F, Weibel ER and Cherniack NS), pp. 1775–1788. Raven Press, New York, 1991.
15. Water RD, Williams LNA, Van Muijen GNP, Franken C, Fransen JAM, Dijkman JH and Kramps JA, Ultrastructural localization of bronchial anti-leukoprotease in central and peripheral human airways by a gold-labeling technique using monoclonal antibodies. *Am Rev Respir Dis* **133**: 882–890, 1986.
16. Vogelmeier C, Hubbard RC, Fells GA, Schnebli HP, Thompson RC, Fritz H and Crystal RG, Anti-neutrophil elastase defense of the normal human respiratory epithelial surface provided by the secretory leukoprotease inhibitor. *J Clin Invest* **87**: 482–488, 1991.
17. Lee CT, Fein AM, Lippmann M, Holtzman H, Kimbel P and Weinbaum G, Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory-distress syndrome. *N Eng J Med* **304**: 192–196, 1981.
18. Stockley RT and Burnett D, Alpha 1-antitrypsin and leukocyte elastase in infected and noninfected sputum. *Am Rev Respir Dis* **120**: 1081–1086, 1979.
19. Stetler GL, Forsyth C, Gleason T, Wilson J and Thompson RC, Secretion of active, full- and half-length human secretory leukocyte protease inhibitor by *Saccharomyces cerevisiae*. *Biotechnology* **7**: 55–60, 1989.
20. Meckelein B, Nikiforov T, Clemen A and Appelhans H, The location of inhibitory specificities in human mucus proteinase inhibitor (MPI): separate expression of the COOH-terminal domain yields an active inhibitor

of three different proteinases. *Prot Engng* **3**: 215–220, 1990.

21. Kramps JA, van Twisk C, Appelhans H, Meckelein B, Nikiforov T and Dijkman JH, Proteinase inhibitory activities of antileukoprotease are represented by its second COOH-terminal domain. *Biochim Biophys Acta* **1038**: 178–185, 1990.
22. Masuda K, Imaizumi A, Kamimura T, Watanabe K, Sugiyama T, Suzuki Y and Ohtsuka E, Expression of the C-terminal domain of secretory leukoprotease inhibitor in *E. coli* as a thrombin-cleavable fusion protein. *Am Rev Respir Dis* **145**: A-200, 1992.
23. Renesto P, Balloy V, Kamimura T, Masuda K, Imaizumi A and Chignard M, Inhibition by recombinant SLPI and half-SLPI (Asn55–Ala107) of elastase and cathepsin G activities: consequence for neutrophil–platelet cooperation. *Br J Pharmacol* **108**, 1100–1106, 1993.
24. Kramps JA, Twisk C, Klasen EC and Dijkman JH, Interaction among stimulated human polymorphonuclear leukocytes, released elastase and bronchial antileukoprotease. *Clin Sci* **75**: 53–62, 1988.
25. Sponer M, Nick HP and Schnebli HP, Different susceptibility of elastase inhibitors to inactivation by proteinase from *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Biol Chem Hoppe-Seyler* **372**: 963–970, 1991.
26. Ikehara M, Ohtsuka E, Tokunaga T, Taniyama Y, Iwai S, Kitano K, Miyamoto S, Ohgi T, Sakuragawa Y, Fujiyama K, Ikari T, Kobayashi M, Miyake T, Shibahara S, Ono A, Ueda T, Tanaka T, Baba H, Miki T, Sakurai A, Oishi T, Chisaka O and Matsubara K, Synthesis of a gene for human growth hormone and its expression in *Escherichia coli*. *Proc Natl Acad Sci USA* **81**: 5956–5960, 1984.
27. Schägger H and Jagow GV, Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* **166**: 368–379 1987.
28. Andersen JK, Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J Biochem Biophys Methods* **10**: 203–209, 1984.
29. Henderson PJF, A linear equation that describes the steady-state kinetics of enzymes and subcellular particles interacting with tightly bound inhibitors. *Biochem J* **127**: 321–333, 1972.