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Pharmacological activity of the C-terminal and N-terminal domains of secretory leukoprotease inhibitor *in vitro*

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1 In order to characterize the physiological functions of the domain structure of secretory leukoprotease inhibitor (SLPI), the biological capacities of half-length SLPIs, (Ser1-Pro54)SLPI and (Asn55-Ala107)SLPI, were investigated and compared with those of full-length SLPI.

2 The activities of these inhibitors against several serine proteases were determined using synthetic chromogenic substrates. The inhibitory capacity of the C-terminal domain, (Asn55-Ala107)SLPI, was as strong as that of full-length SLPI against human neutrophil elastase (NE), cathepsin G and chymotrypsin. It possessed less trypsin inhibitory activity than intact SLPI. For the N-terminal domain of SLPI, (Ser1-Pro54)SLPI, no inhibitory activity could be detected against the serine proteases tested in this study.

3 The inhibitory activity of (Asn55-Ala107)SLPI against the proteolysis of the natural substrates elastin and collagen by NE was comparable with that of full-SLPI (elastin, $IC_{50}=907\pm31$ nM for SLPI, 767 ± 33 nM for (Asn55-Ala107)SLPI; collagen, $IC_{50}=862\pm36$ nM for SLPI, 727 ± 47 nM for (Asn55-Ala107)SLPI).

4 The binding affinities of full- and half-length SLPIs for heparin were measured by affinity column chromatography. Full-length SLPI showed high affinity for heparin while the binding capacities of both half-length SLPIs were lower. (Concentration of NaCl for elution, 0.45 M for SLPI, 0.24 M for (Ser1-Pro54)SLPI, 0.27 M for (Asn55-Ala107)SLPI).

5 The effects of full-SLPI and (Asn55-Ala107)SLPI on blood coagulation were measured using the activated partial thromboplastin time (APTT). Full-length SLPI prolonged clotting time dose-dependently (1.25, 2.5 and 5.0 μM), whereas (Asn55-Ala107)SLPI had no effect even at the highest concentration.

6 In conclusion, the C-terminal domain of SLPI is a promising candidate for the treatment of inflammatory diseases in which participation of neutrophil proteases has been suggested.

Keywords: Secretory leukoprotease inhibitor (SLPI); domain structure; neutrophil protease; anti-protease; respiratory disease

Introduction

An imbalance between neutrophil proteases and anti-proteases is a possible mechanism for the origin of destructive lung diseases (Cohen *et al.*, 1983; Janoff, 1985). Neutrophil elastase (NE) is a lysosomal serine protease which is thought to be responsible for lung tissue degradation (Campbell *et al.*, 1987). NE attacks the extracellular matrix of the respiratory tract and degrades elastin (Boudier *et al.*, 1981), collagen (Mainardi *et al.*, 1980), fibronectin (McDonald *et al.*, 1980) and proteoglycans (Keiser *et al.*, 1976). Studies of the role of proteases in pulmonary diseases such as emphysema and cystic fibrosis have focused on NE (Snider *et al.*, 1984; Meyer *et al.*, 1991). The possible participation of cathepsin G in inflammation has also been reported. Cathepsin G enhances the activity of NE (Boudier *et al.*, 1981) and degrades cartilage matrix proteoglycan (Janusz & Doherty, 1991). Human plasma α_1-proteinase inhibitor, also known as α_1-antitrypsin, is a physiological inhibitor of these proteases (Beatty *et al.*, 1980) and its exogenous administration has been proposed as a therapeutic weapon to arrest or delay tissue destruction (Wewers *et al.*, 1987; Crystal *et al.*, 1989).

Secretory leukoprotease inhibitor (SLPI), also known as antileukoprotease, is another promising candidate for the treatment of diseases in which NE and cathepsin G could be involved (Travis & Fritz, 1991). It is a 107-residue, non-glycosylated serine protease inhibitor which inhibits a wide-range of proteases including NE, cathepsin G, chymotrypsin and trypsin (Smith & Johnson, 1985; Thompson & Ohlson, 1986). It has been reported to be widely distributed in submucosal glands (Franken *et al.*, 1989). In the respiratory tract, SLPI is produced by serous glandular cells in the central airways and by clara and goblet cells in the peripheral airways (Kramps *et al.*, 1981; De Water *et al.*, 1986). Recent studies have also demonstrated secretion of SLPI by type II pneumocyte cell lines (Sallenave *et al.*, 1993). The exact physiological function of SLPI is not clear, but its major role is thought to be protection of the airway epithelial surface from attack by neutrophil proteases (Vogelmeier *et al.*, 1991; Piccioni *et al.*, 1992). X-ray crystallographic analysis has shown that SLPI comprises two separate domains of similar architecture (Grütter *et al.*, 1988). Investigations into the relationship between the anti-protease activity of SLPI and its domain structures have demonstrated that inhibitory activity against NE, chymotrypsin and trypsin is located in the C-terminal domain (Stetler *et al.*, 1989; Meckelein *et al.*, 1990; Kramps *et al.*, 1990). We have also found recently that a recombinant half-sized SLPI containing the C-terminal domain, (Asn55-Ala107)SLPI, is as active an inhibitor as full-length SLPI against NE and cathepsin G (Masuda *et al.*, 1992; Renesto *et al.*, 1993). However, the inhibitory activity of the N-terminal domain has not been investigated quantitatively and the exact functions of the two domains have yet to be fully elucidated. It would be interesting to determine the physiological role of each domain.

In this study, we first investigated the inhibitory activities of (Ser1-Pro54)SLPI, (Asn55-Ala107)SLPI and full-length SLPI against several serine proteases using various synthetic chromogenic substrates. The inhibitory capacity of the activity-

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retaining half-length SLPI, (Asn55-Ala107)SLPI, against the proteolysis of natural substrates by NE was further tested and compared with that of full-length SLPI. We then investigated the heparin-binding affinities of these inhibitors and examined the effects of (Asn55-Ala107)SLPI and intact SLPI on blood coagulation *in vitro* in order to explore the possibility of intravenous administration.

Methods

Enzymes and substrates

Human neutrophil elastase (NE) and cathepsin G were purchased from Elastin Products Co. (Owensville, MO, U.S.A.). Bovine pancreatic trypsin and chymotrypsin, as well as human plasma thrombin and plasmin, were from Sigma Chemical Co. (St Louis, MO, U.S.A.). Human plasma kallikrein was obtained from Protogen AG (Laufelfingen, Switzerland) and bovine factor Xa (FXa) was purchased from New England Biolabs Inc. (Beverly, MA, U.S.A.). The following synthetic substrates were used: methoxy succinyl-Ala-Ala-Pro-Val-4-nitroanilide (MeO-Suc-Ala-Ala-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), while 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, D-Pro-Phe-Arg-4-nitroanilide (D-Pro-Phe-Arg-pNA) for kallikrein and benzoyl-Ile-Glu-Gly-Arg-4-nitroanilide (Bz-Ile-Glu-Gly-Arg-pNA) for FXa were purchased from Kabi Vitrum (Stockholm, Sweden).

Production of recombinant full- and half-length SLPIs

DNA fragments encoding the amino acids 1-107, 1-54 and 55-107 of SLPI (Thompson & Ohlson, 1986) were synthesized chemically using appropriate codons from *E.coli*. The human growth hormone gene was fused to the genes for full- and half-length SLPIs to optimize the expression size via a DNA sequence encoding Leu-Val-Pro-Arg, which can be cleaved by thrombin (Ikehara *et al.*, 1984; Masuda *et al.*, 1992). The expression vectors were constructed and introduced into *E. coli* HB101, the transformed cells were selected by ampicillin and cultured; then the fusion proteins were obtained as inclusion bodies. The fusion proteins were dissolved and cleaved with thrombin, and the full- and half-length SLPIs were purified by chromatography after refolding.

Inhibition assay using synthetic chromogenic substrates

(1) *Fifty percent inhibitory concentration* (IC_{50}). 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°C 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 for 20 min at 37°C. The light absorbance of the reaction mixture at 405 nm was measured. Residual enzyme activities were plotted for various concentrations of inhibitor and the IC_{50} was determined.

(2) *Inhibition constants* (K_i values). The substrates 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 h at 25°C (total volume 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 for 20 min at 25°C. The absorbance of the reaction mixture at 405 nm was recorded and the inhibition constants were determined (Henderson, 1972).

Proteolysis of elastin

Elastolysis was monitored by measuring the absorbance at 280 nm. The absorption of the soluble peptides resulting from the enzymatic digestion of elastin is due to desmosin and isodesmosin (Boudier *et al.*, 1981). The required inhibitor solution (0.9 ml) in 50 mM Tris buffer, pH 8.0, was added to 3.0 mg bovine ligament elastin (Sigma Chemical Company, St Louis, Missouri, U.S.A.). Elastolysis was started by adding 100 µl NE (final concentration, 0.5 µM) and the suspension was incubated for 2 h at 37°C; 400 µl of the continuously stirred suspension was removed and added to 15 µl 3 M acetate buffer, pH 4.5, in order to stop the reaction. After centrifuging at 4000 *g* for 15 min, the absorbances of the supernatants were read at 280 nm. Results were expressed as the percentage of enzymatic activity in controls without inhibitor.

Proteolysis of collagen

The required inhibitor solution (180 µl) in 50 mM Tris buffer, pH 7.5, containing 100 mM NaCl and 2.5 mM $CaCl_2$, was added to 50 µg bovine type I collagen labelled with fluorescein 5-isothiocyanate (FITC) (COSMO BIO Corp., Tokyo, Japan). The degradation of collagen was started by adding 20 µl NE (final concentration, 1 µM) and the reaction mixture was incubated for 3 h at 37°C; 200 µl of 50 mM Tris buffer, pH 9.5, containing 50% (v/v) ethanol was added in order to stop the reaction and precipitate intact collagen. After centrifugation at 4000 *g* for 15 min, the fluorescence of the supernatants, including denatured collagen, was detected with emission at 520 nm and excitation at 495 nm. Results were expressed as the percentage of enzymatic activity in controls without inhibitor.

Heparin-affinity chromatography

A mixture of full- and half-length SLPIs (20 µg) dissolved in 20 mM Tris buffer (pH 7.5) was applied to a TSK-gel Heparin-5PW column (7.5 mm × 7.5 cm, TOSOH Corp., Tokyo, Japan) and elution was carried out over 30 min with a gradient of 0–0.7 M NaCl in 20 mM Tris buffer, pH 7.5, at a flow rate of 1.0 ml min^{-1}. The effluent was monitored by measuring the absorbance at 215 nm.

Blood coagulation assay

Blood coagulation was assayed by a modification of the activated partial thromboplastin time (APTT) test (Fulcher & Zimmerman, 1982). The required inhibitor solution (20 µl) was preincubated with 100 µl activated partial thromboplastin (Sysmex SAP-20,Toa Medical Electronics Co., Kobe, Japan), 100 µl normal plasma and 100 µl FVIII-deficient plasma (George King Bio-Medical, Overland Park, KS, U.S.A.) for 2 min at 37°C. The coagulation assay was started by adding 100 µl 20 mM $CaCl_2$. The final concentrations of inhibitors were 1.25, 2.5 and 5.0 µM. The time required for clot formation was recorded using a blood coagulation analyser (Sysmex CA-100, Toa Medical Electronics Co., Kobe, Japan).

Results

Inhibition of enzymatic activity of serine proteases

We first evaluated the capacity of half-length SLPIs to inhibit the enzymatic activities of purified serine proteases, using synthetic chromogenic substrates. The inhibitory activity (IC_{50}) of (Asn55-Ala107)SLPI against elastase, cathepsin G and chymotrypsin was as strong as that of intact SLPI, but it

Table 1 Fifty percent inhibitory concentrations (IC_{50}) of SLPI and (Asn55-Ala107)SLPI against serine proteases

Enzyme (concentration; M)	*50% inhibitory concentration (M)* SLPI	(Asn55-Ala107)SLPI
NE (2.0×10^{-8})	1.0×10^{-8}	1.1×10^{-8}
Human cathepsin G (2.0×10^{-8})	5.5×10^{-9}	7.2×10^{-9}
Bovine pancreatic chymotrypsin (1.0×10^{-8})	1.0×10^{-8}	1.2×10^{-8}
Bovine pancreatic trypsin (2.0×10^{-7})	3.3×10^{-7}	2.3×10^{-6}
Human plasma thrombin (3.0×10^{-9})	$> 1.0 \times 10^{-5}$	$> 1.0 \times 10^{-5}$
Human plasma plasmin (1.0×10^{-7})	$> 1.0 \times 10^{-5}$	$> 1.0 \times 10^{-5}$
Human plasma kallikrein (1.0×10^{-8})	$> 1.0 \times 10^{-5}$	$> 1.0 \times 10^{-5}$
Bovine plasma FXa (1.0×10^{-8})	$> 1.0 \times 10^{-5}$	$> 1.0 \times 10^{-5}$

Assay conditions; 0.1 M HEPES (pH 7.5), 0.5 M NaCl, 37°C
Chromogenic substrates: NE, 0.3 mM MeO-Suc-Ala-Ala-Pro-Val-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; FXa, 0.5 mM Bz-Ile-Glu-Gly-Arg-pNA.

Table 2 Inhibition constants (K_i values) of SLPI and (Asn55-Ala107)SLPI against neutrophil elastase (NE) and bovine pancreatic trypsin

Enzyme	K_i *value (M)* SLPI	(Asn55-Ala107)SLPI
NE	0.8×10^{-10}	1.2×10^{-10}
Bovine pancreatic trypsin	3.3×10^{-8}	1.0×10^{-6}

Assay conditions; 0.1 M HEPES (pH 7.5), 1.0 M NaCl, 0.1% (w/v) PEG-6000, 25°C
Chromogenic substrates: NE, 0.3 mM MeO-Suc-Ala-Ala-Pro-Val-pNA; trypsin, 1.0 mM Bz-Arg-pNA.

possessed less trypsin inhibitory activity than intact SLPI, and neither of the inhibitors showed any inhibitory activity against thrombin, plasmin, kallikrein or FXa (Table 1). No inhibitory activity of the N-terminal domain, (Serl-Pro54)SLPI (final concentration, 10 μM), could be detected against the serine proteases shown in Table 1. The inhibition constants (K_i values) of (Asn55-Ala107)SLPI and SLPI against NE and bovine pancreatic trypsin were determined. The K_i value of (Asn55-Ala107)SLPI for NE was comparable with that of intact SLPI but its trypsin inhibitory activity was reduced (Table 2). The C-terminal domain was more specific for NE than trypsin, compared with SLPI itself.

Inhibition of the proteolysis of elastin and collagen by NE

Since (Serl-Pro54)SLPI did not inhibit enzymatic activity, we investigated only the effects of SLPI and (Asn55-Ala107)SLPI on the proteolysis of the natural substrates, bovine elastin and type I collagen. All experiments were carried out without preincubation of NE and the inhibitors. Proteolysis was started by adding NE to the mixture of inhibitor and substrate described in Methods. SLPI effectively inhibited the degradation of elastin (Figure 1a) and collagen (Figure 2a), the active concentrations ranged from 0.5–2.0 μM. The IC_{50} of SLPI was 907±31 nM for elastin and 862±36 nM for collagen. The same effect was obtained with (Asn55-Ala107)SLPI, which inhibited the proteolysis of elastin (Figure 1b) and collagen (Figure 2b), IC_{50} values being 767±33 nM and 727±47 nM, respectively. The C-terminal domain of SLPI was therefore as active in inhibiting the proteolysis of natural substrates as the parent SLPI.

Heparin-binding affinities of full- and half-length SLPIs

The binding capacities of full- and half-length SLPIs for heparin were investigated by heparin-conjugate affinity chromatography, and the elution pattern of the inhibitors is shown in Figure 3. This chromatography showed that (Serl-Pro54)SLPI, (Asn55-Ala107)SLPI and SLPI bind to the matrix in 20 mM Tris buffer, pH 7.5, and elute with 0.24, 0.27 and 0.45 M NaCl, respectively. SLPI showed high affinity for heparin as has been

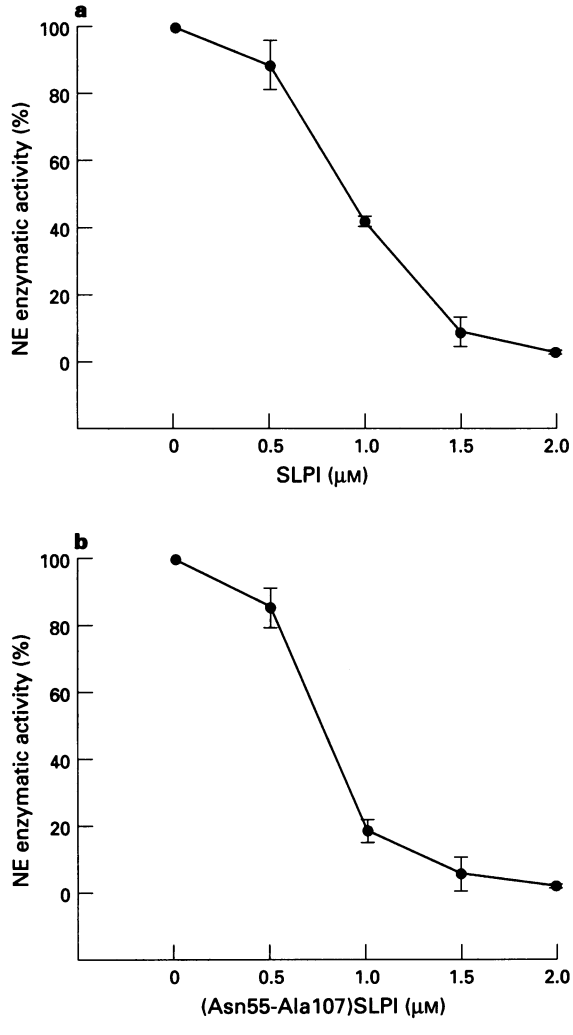

Figure 1 Inhibition of the proteolysis of bovine ligament elastin by SLPI (a) and (Asn55-Ala107)SLPI (b). Increasing concentrations of inhibitors were incubated for 2 h at 37°C in 50 mM Tris buffer (pH 8.0) in the presence of elastin (3 mg) and 0.5 μM neutrophil elastase (NE). Enzymatic activity was measured by reading the absorbance of the supernatant at 280 nm. Results are expressed as the percentage activity compared with controls without inhibitor. Each point is the mean±s.d. of 3 separate experiments.

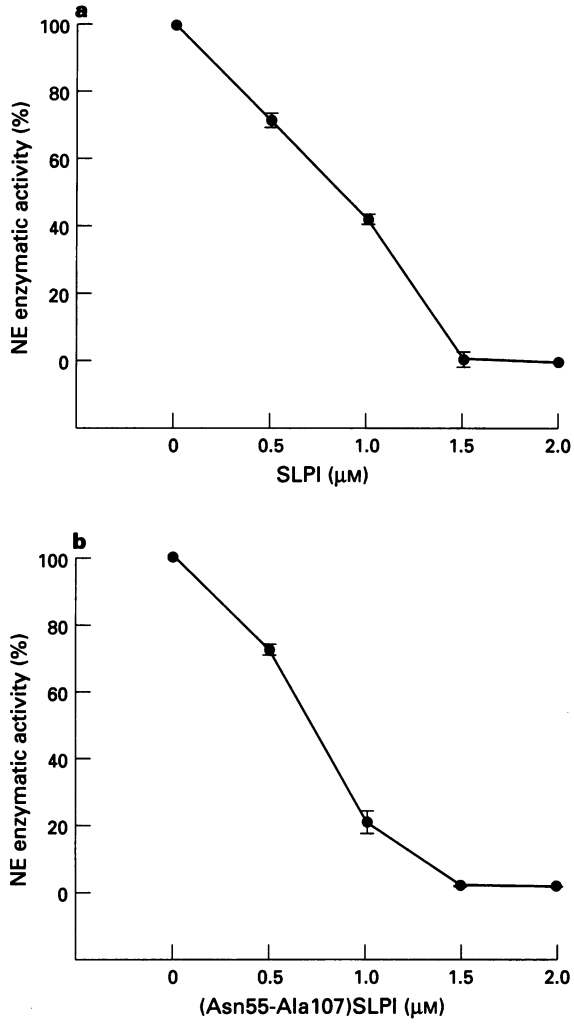

Figure 2 Inhibition of the proteolysis of bovine type I collagen by SLPI (a) and (Asn55-Ala107)SLPI (b). Increasing concentrations of inhibitors were incubated for 3 h at 37°C in 50 mM Tris buffer (pH 7.5) in the presence of FITC-labelled collagen (50 μg) and 1.0 μM neutrophil elastase (NE). Enzymatic activity was measured by reading the fluorescence of the supernatant with emission at 520 nm and excitation at 495 nm. Results are expressed as the percentage activity compared with controls without inhibitor. Each point is the mean ± s.d. of 3 separate experiments.

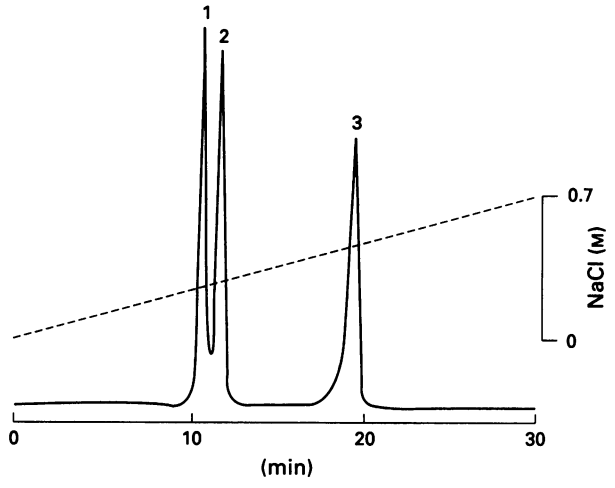

Figure 3 Heparin-affinity chromatography of full- and half-length SLPIs. A mixture of inhibitors dissolved in 20 mM Tris buffer (pH 7.5) was loaded onto a TSK-gel Heparin-5PW column. Elution was carried out over 30 min with a gradient of 0–0.7 M NaCl in 20 mM Tris buffer (pH 7.5) and the effluent was monitored at 215 nm. Peaks 1, 2 and 3 correspond to (Serl-Pro54)SLPI, (Asn55-Ala107)SLPI and SLPI, respectively.

Table 3 Effects of SLPI and (Asn55-Ala107)SLPI on blood coagulation

	APTT (s)
Control (no inhibitor)	105.1 ± 1.0
SLPI (1.25 μM)	162.0 ± 10.0*
SLPI (2.5 μM)	220.2 ± 10.4*
SLPI (5.0 μM)	361.7 ± 80.2*
(Asn55-Ala107)SLPI (1.25 μM)	102.9 ± 1.3
(Asn55-Ala107)SLPI (2.5 μM)	103.3 ± 3.4
(Asn55-Ala107)SLPI (5.0 μM)	107.7 ± 4.2

Inhibitors were preincubated for 2 min at 37°C with activated partial thromboplastin and human plasma (total volume 320 μl). The clotting time was measured after adding 100 μl 20 mM $CaCl_2$. Results are expressed as means ± s.d. of 3 distinct experiments. *$P < 0.05$ significant versus control.

previously described (Faller *et al.*, 1992). Both half-length SLPIs had reduced binding affinity for heparin compared with intact SLPI.

Effects of (Asn55-Ala107)SLPI and SLPI on blood coagulation

The effects of (Asn55-Ala107)SLPI and SLPI on blood coagulation were measured by the APTT test and the results are shown in Table 3. A dose-dependent inhibitory effect on coagulation was observed and APTT was significantly prolonged with 1.25, 2.5 and 5.0 μM SLPI (final concentrations). On the other hand, (Asn55-Ala107)SLPI showed no effect on APTT under the same conditions.

Discussion

SLPI is generally considered to play a defensive role at the respiratory epithelial surface and has a variety of physiological functions that protect the respiratory tissue matrix. These include: (a) inhibitory activity against a wide range of proteases (Smith & Johnson, 1985; Thompson & Ohlsson, 1986); (b) association with elastin fibres in the extracellular matrix of the human lung (Kramps *et al.*, 1989) and inhibition of proteolysis of elastin and fibronectin at the neutrophil-matrix interface (Rice & Weiss, 1990); (c) augmentation of glutathione, a major component of anti-oxidants, in lung epithelial lining fluid (Gillissen *et al.*, 1993) and (d) heparin-induced activation (Faller *et al.*, 1992). It is interesting to speculate upon the relationships between these biological functions and domain structure; however, little is known about the exact roles of the N-and C-terminal domains.

It has been postulated, in view of sequence similarities with the active site of Kazal inhibitors, that the N-terminal domain is active against trypsin whereas the C-terminal domain is active against elastase (Thompson & Ohlsson, 1986). However, further investigations into the relationship between SLPI's domain structure and its anti-protease activity have demonstrated that the inhibitory activities against NE, chymotrypsin and trypsin are all located in the C-terminal domain (Stetler *et al.*, 1989; Kramps *et al.*, 1990). Only weak activity against trypsin has been reported for the recombinant N-terminal domain, which was not completely purified (Meckelein *et al.*, 1990), but its activity has not been studied quantitatively. Our results demonstrate that the C-terminal domain, (Asn55-Ala107)SLPI, is as active in inhibiting NE, cathepsin G and chymotrypsin as the parent SLPI, and shows less trypsin inhibitory activity than intact SLPI. Indeed, the inhibitory ca-

pacity of (Asn55-Ala107)SLPI against the proteolysis of the natural substrates elastin and collagen by NE was as strong as that of full-SLPI. No inhibitory activity of the N-terminal domain, (Serl-Pro54)SLPI, could be detected even at concentrations 100 times greater than those of the proteases. These data suggest that all active inhibitory sites are located in the C-terminal domain and that the N-terminal domain plays only a co-operative role in inhibiting trypsin.

The binding capacity of SLPI for elastin and heparin is important for physiological and therapeutic reasons, but the binding region has not been clearly defined. We have demonstrated that the heparin-binding affinities of both the half-sized SLPIs, (Serl-Pro54)SLPI and (Asn55-Ala107)SLPI, are reduced compared with intact SLPI. We therefore speculate that the three-dimensional structure is critical in conferring high affinity for heparin and this finding may suggest considerations for the pharmacokinetics of SLPI derivatives.

Recombinant SLPI is being tested as a potential anti-elastase drug for the treatment of destructive lung diseases (Lucey *et al.*, 1990; Rudolphus *et al.*, 1991). It has been reported that aerosol administration of SLPI to sheep enhances the anti-NE capacity of the lung (Vogelmeier *et al.*, 1990). However, intravenous administration of the anti-protease might be more effective than inhalation for the treatment of severe destructive lung diseases in which airway obstruction occurs. In such patients, hypersecretion of mucus containing an increased number of neutrophils is observed. We have demonstrated that full-length SLPI prolongs blood coagulation *in vitro*, whereas (Asn55-Ala107)SLPI produced no effect on clotting time under the same conditions. We speculate that SLPI might have effects against trypsin-like proteases which function in the coagulation cascade although it is unclear which plasma protease is inhibited by SLPI. Our results suggest that (Asn55-Ala107)SLPI has no effect on APTT in this study because it has less trypsin inhibitory activity than intact SLPI. We suggest on the basis of these results that the C-terminal domain might be more desirable than SLPI for intravenous administration as an anti-elastase therapy.

In conclusion, (Asn55-Ala107)SLPI is a more specific inhibitor of NE and cathepsin G than of trypsin, compared with SLPI, and is as active in inhibiting the proteolysis of elastin and collagen by NE as intact SLPI. Furthermore, (Asn55-Ala107)SLPI had no effect on clotting time while SLPI inhibits blood coagulation. Thus, the C-terminal domain of SLPI could be a promising candidate, and could be administered via a variety of routes, for the treatment of conditions in which neutrophil proteases are involved.

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