Structural and functional characterization of elastases from horse neutrophils

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In order better to understand the pathophysiology of the equine form of emphysema, two elastolytic enzymes from horse neutrophils, referred to as proteinases 2A and 2B, have been extensively characterized and compared with the human neutrophil proteinases, proteinase-3 and elastase. Specificity studies using both the oxidized insulin B-chain and synthetic peptides revealed that cleavage of peptide bonds with P1 alanine or valine residues was preferred. Further characterization of the two horse elastases by N-terminal sequence and reactive-site analyses indicated that proteinases 2A and 2B have considerable sequence similarity to each other, to proteinase-3 from human neutrophils (proteinase 2A), to human neutrophil elastase (proteinase 2B) and to a lesser extent to pig pancreatic elastase. Horse and

human elastases differed somewhat in their interaction with some natural protein proteinase inhibitors. For example, in contrast with its action on human neutrophil elastase, aprotinin did not inhibit either of the horse proteinases. However, the Val¹⁵, α -aminobutyric acid-15 (Abu¹⁵), α -aminovaleric acid-15 (Nva¹⁵) and Ala¹⁵ reactive-site variants of aprotinin were good inhibitors of proteinase 2B ($K_i < 10^{-9}$ M) but only weak inhibitors of proteinase 2A ($K_i > 10^{-7}$ M). In summary, despite these differences, the horse neutrophil elastases were found to resemble closely their human counterparts, thus implicating them in the pathological degradation of connective tissue in chronic lung diseases in the equine species.

INTRODUCTION

The human neutrophil serine proteinases, elastase (HNE), cathepsin G and proteinase-3 (PR-3), are thought to be involved in such physiological activities as neutrophil migration through basement membranes (Henson and Johnston, 1987), platelet activation (Selak, 1992), oxygen-independent bactericidal activity (Campanelli et al., 1990a) and digestion of micro-organisms and other material within the phagolysosome (Janoff, 1985). In the healthy state, their activities are precisely controlled; however, tissue destruction occurs when the enzymes released from neutrophils overwhelm the capacity of the natural inhibitors present within the connective tissue (Weiss, 1989). The action of neutrophil proteinases has been implicated in the development of emphysema, adult respiratory distress syndrome (Laurell and Eriksson, 1963; Cohen, 1983) and reperfusion damage of small blood vessels after ischaemia (Smedly et al., 1986), as well as rheumatoid arthritis and glomerulonephritis (Weiss, 1989). Granule-associated serine proteinases are also the targets of autoantibody formation and have been detected in the sera of patients suffering from chronic inflammatory diseases (Kallenberg et al., 1991; Jennette et al., 1991).

As a result of their pathophysiological importance, the human neutrophil serine proteinases have been very well characterized compared with similar enzymes present in the granular fraction of neutrophils from other mammalian species (Bieth, 1986). However, there are few reports implicating such enzymes as pathogenic factors in degenerative and inflammatory diseases associated with abnormal tissue injury in mammals. The exception is the horse, which is known to be the only animal (besides man) to suffer from spontaneous chronic lung disease (Gillespie and Tyler, 1969; Gerber, 1973). Although in this case

the elastolytic serine proteinases of neutrophil origin are expected to be involved in connective tissue damage of the lung (von Fellenberg et al., 1985), little is known about their structure and function. In the present report, we describe the specificity of action of two horse elastases, referred to as proteinases 2A and 2B, towards insulin B-chain, synthetic substrates and protein proteinase inhibitors. Moreover, the N-terminal and amino acid sequence around the reactive-site serine residue, including the residues lining the primary binding site (S1), have also been determined. The data obtained clearly indicate that proteinases 2A and 2B are homologous with human neutrophil PR-3 and elastase respectively. To our knowledge, this is the first report directly indicating that elastolytic proteinases with similar characteristics occur in both human and other mammalian neutrophils. By analogy with humans, this also implicates equine elastases as a pathogenic factor in horse emphysema.

MATERIALS AND METHODS

Materials

Horse (*Equus caballus*) leucocyte elastases 2A and 2B and HNE were purified by previously published procedures (Dubin et al., 1976; Baugh and Travis, 1976). Aprotinin and its reactive-site variants were a gift from Dr. H. Tschesche (University of Bielefeld, Bielefeld, Germany). Boc-Val-ONp was from Novabiochem (La Jolla, CA, U.S.A.), [1,3-³H]di-isopropyl phosphorofluoridate (specific radioactivity 6.5 Ci/mmol) was from Amersham (Amersham, Bucks., U.K.), CNBr, guanidinium chloride, trifluoroacetic acid and citraconic anhydride were from Pierce (Rockford, IL, U.S.A.), oxidized insulin B-chain was from Boehringer-Mannheim (Vienna, Austria) and acetonitrile and h.p.l.c.-grade water were from Backer (Philipsburg, NJ, U.S.A.).

Abbreviations used: PR-3, human neutrophil proteinase-3; HNE, human neutrophil elastase; PPE, pig pancreatic elastase; α_1 -PI, α_1 -proteinase inhibitor; Ac-, acetyl; Bz, benzoyl; -ONp, *p*-nitrophenyl ester; -NA, 4-nitroanilide; MeO-, methoxy; Suc-, succinyl; Boc-, *t*-butyloxycarbonyl; Abu, α -aminobutyric acid; Nva, α -aminovaleric acid (norvaline).

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All other enzymes and chemicals of at least analytical grade were from Sigma (St. Louis, MO, U.S.A.).

Active-site titration

Pig pancreatic trypsin was active-site-titrated with *p*-nitrophenyl guanidobenzoate (Chase and Shaw, 1970) and used to standardize a solution of human α_1 -proteinase inhibitor (α_1 -PI). Human and horse neutrophil elastases were titrated against α_1 -PI as a secondary standard.

Substrate kinetics

Enzymic hydrolysis of peptide *p*-nitrophenyl esters or 4-nitroanilide substrates catalysed by horse neutrophil elastases was measured in 0.1 M Hepes buffer, pH 7.5, containing 0.5 M NaCl and 10% (v/v) methanol (*p*-nitrophenyl ester substrates) or dimethyl sulphoxide (4-nitroanilide substrates) at 25 °C. The initial rates were measured at 405 nm ($\epsilon_{405} = 9200 \text{ M}^{-1} \cdot \text{cm}^{-1}$ and $18300 \text{ M}^{-1} \cdot \text{s}^{-1}$ for 4-nitroanilide and *p*-nitrophenol respectively) using 20 μl of a stock enzyme solution added to a cuvette containing 480 μl of buffer containing the appropriate substrate concentration. The kinetic constants k_{cat} , K_m and k_{cat}/K_m were obtained from Lineweaver–Burk plots based on triplicate rate determinations at six separate substrate concentrations in the range 0.05–5 mM. The data were processed by non-linear least-squares regression using either Marquardt–Levenberg or Gauss–Newton algorithms by using a computer program kindly provided by Dr. S. P. J. Brooks (Brooks, 1992).

Measurement of inhibition constants

The inhibition constants (K_i) of horse neutrophil elastases with aprotinin and the inhibitor reactive-site variants were determined as described by Bieth (1980).

Enzyme active-site radiolabelling and fragmentation

Elastases 2A and 2B (20 nmol) were labelled at the reactive-site serine by incubation with 30 μCi of [^3H]di-isopropyl phosphorofluoridate in 0.1 M Tris/HCl, pH 8.0, for 30 min at 25 °C. The protein was denatured in 6 M guanidinium chloride, reduced with dithiothreitol and S-carboxymethylated. The derivatized protein was subjected to fragmentation by digestion with CNBr or Tos-Phe-CH₂Cl-treated bovine trypsin as previously described (Dubin et al., 1992).

Digestion of insulin by horse neutrophil proteinases

Oxidized insulin B-chain (125 μg , 34 nmol) was incubated with 0.1 nmol of elastase 2A or 2B at 37 °C in a final volume of 250 μl of 50 mM NaHCO₃. Samples (50 μl) were withdrawn at various intervals (10–360 min) and the reaction was terminated with 1 μl of trifluoroacetic acid.

Peptide purification

The peptides from elastase 2A or 2B digestion of insulin were fractionated by reversed-phase h.p.l.c. using a Vydac Protein C18-10 column (4 mm $\times$ 300 mm) equilibrated with 0.1% trifluoroacetic acid. Peptides were eluted with 60 ml of a linear gradient of acetonitrile from 0 to 80%, containing 0.08% trifluoroacetic acid (flow rate 1 ml/min). Eluted peptides were monitored at 220 nm and collected manually. The ^3H radioactivities were determined using a dioxane-based Bray scintillation mixture in a Beckman liquid-scintillation counter. Individual peptides were repurified by h.p.l.c. on the same column and subjected to amino acid sequence and composition analysis.

Amino acid analysis

Amino acid analysis of purified peptides was performed on a Beckman System 6300 high-performance amino acid analyser with a Hewlett–Packard 3390A integrator. Automated Edman degradation was performed with an Applied Biosystems 470A gas-phase sequencer, using the program designated by the manufacturer.

RESULTS

Amino acid sequence

Previously, it has been shown that horse leucocyte granules contain two serine proteinases referred to as elastases 2A and 2B (Dubin et al., 1976). Figure 1 shows alignment of the N-terminal sequence, a section of the internal part of the polypeptide chain and the substrate-binding regions of each of these enzymes with the corresponding regions of homologous proteinases from human neutrophils. The comparison reveals that, although the enzymes are closely related to each other, horse elastase 2A exhibits a stronger similarity to PR-3 (79% sequence identity) than to elastase 2B (71% identity) or HNE (68% identity). Correspondingly, proteinase 2B is more homologous to HNE (86% identity) than to proteinase 2A or PR-3 (71% identity). The reciprocal similarity between proteinase 2A and PR-3 and between elastase 2B and HNE is especially manifested at the N-terminus. Within the first 34 residues, elastase 2A differs from PR-3 in five positions and elastase 2B differs from HNE in only three positions. Moreover, compared with HNE and elastase 2B, the former enzymes have two amino acid insertions between Gly³⁸ and Gly³⁹, and a single insertion between Gly³⁹ and His⁴⁰ (Figure 1). These insertions may affect the S1' and S2' subsite geometry and appear to account for the reactivity differences

Figure 1 Alignment of the partial amino acid sequence of horse neutrophil elastases (2A and 2B) with HNE and PR-3

The single-letter amino acid code and chymotrypsin numbering system is used for comparison. Identical residues in neighbouring sequences are indicated by (:) and conserved residues by (:). A gap (-) in HNE (and elastase 2B) was introduced on the basis of topological criteria (Bode et al., 1989). The catalytic serine residue (Ser¹⁹⁵) is indicated by an asterisk. Amino acid residues lining the primary binding pocket S1 are shown in bold type. Amino acid sequences of PR-3 and HNE are from Campanelli et al. (1990b) and Sinha et al. (1987) respectively.

Table 1 Amino acid composition of peptides derived from insulin B-chain by elastases 2A and 2B

I, Amino acid residue estimated by amino acid analysis; II, amino acid suggested from insulin B-chain sequence. Numbers in square brackets indicate the elution time (min) of the given peptide.

Amino acid	Amino acid composition									
	a [17.55]		b [17.9]		c [18.6]		d [20.0]		e [21.3]	
	I	II	I	II	I	II	I	II	I	II
Asx	1.21 (1)	1	1.05 (1)	1	0.07 (0)	1	—	—	0.14 (0)	—
Thr	—	—	—	—	1.00 (1)	1	—	—	0.77 (1)	1
Ser	1.06 (1)	1	1.00 (1)	1	0.23 (0)	—	—	—	0.13 (0)	—
Glx	1.92 (2)	2	1.43 (1)	1	0.56 (1)	1	—	—	1.45 (1)	1
Pro	—	—	—	—	0.91 (1)	1	—	—	0.71 (1)	1
Gly	1.83 (2)	1	1.34 (1)	1	2.25 (2)	2	—	—	2.26 (2)	2
Ala	1.00 (1)	1	0.28 (0)	—	1.00 (1)	1	—	—	1.00 (1)	1
Cys(O ₃ H)	—	1	—	1	—	1	—	—	—	1
Val	1.64 (2)	2	1.77 (2)	2	0.15 (0)	—	1.00 (1)	1	0.90 (1)	1
Ile	0.22 (0)	—	—	—	0.06 (0)	—	—	—	0.03 (0)	—
Leu	2.49 (2)	2	2.25 (2)	2	0.17 (0)	—	2.58 (3)	2	2.24 (2)	2
Tyr	0.10 (0)	—	—	—	0.76 (1)	1	0.80 (1)	1	1.10 (1)	2
Phe	1.34 (1)	1	0.97 (1)	1	1.80 (2)	2	1.10 (1)	—	1.56 (2)	2
His	1.41 (1)	2	1.88 (2)	2	—	—	—	—	—	—
Lys	0.29 (0)	—	—	—	0.88 (1)	1	—	—	—	—
Arg	—	—	—	—	0.85 (1)	1	—	—	0.72 (1)	1
Suggested fragment	1–14		1–12		19–30		15–18		15–30

Figure 2 Specificity of action of horse neutrophil elastases 2A and 2B on insulin B-chain compared with HNE, PR-3 and porcine pancreatic elastase (PPE)

The single and double-arrows indicate the minor and major cleavage sites of insulin B-chain respectively. Data for PR-3 were obtained from Rao et al. (1991), those for HNE from Blow (1977) and those for PPE from Narayanan and Anwar (1969).

between PR-3 and HNE toward acylaminoisocoumarins (Kam et al., 1992).

Action on Insulin B-chain

To gain insight into which peptide bonds are susceptible to scission by horse neutrophil elastases, five peptides produced by the action of proteinases 2A and 2B on the oxidized insulin B-chain were purified and characterized. The pattern of reversed-phase h.p.l.c. fractionation of peptides together with their amino acid compositions (Table 1) indicate that, for both proteinases, the initial cleavage sites were Ala¹⁴-Leu¹⁵ and Val¹⁸-Cys¹⁹ bonds. On the basis of the peptide recovery, it appears that cleavage of the Val¹²-Glu¹³ bond was a secondary event resulting from further cleavage of the primary peptide (Phe¹-Ala¹⁴) (Figure 2). These results suggest that both horse elastases have, unexpectedly, the same specificity on the insulin B-chain and preferentially attack peptide bonds with small aliphatic amino acids (Val, Ala) at the P1 site.

Action on synthetic substrates

Horse neutrophil proteinases have previously been shown to have strong elastinolytic activity at physiological pH and ionic

strength. Whereas proteinase 2B displayed severalfold higher elastinolytic activity than HNE, proteinase 2A degraded elastin at half the rate of the human enzyme (Potempa et al., 1986). Therefore the finding that both enzymes have precisely the same specificity on insulin was unexpected. To address this discrepancy, the amidolytic and esterolytic activity of both horse enzymes was assessed using chromogenic substrates. The results, compared with available data for PR-3 and HNE, are summarized in Table 2. Both enzymes hydrolysed *p*-nitrophenyl esters of alanine and valine very efficiently. In this respect they were similar to HNE and PR-3; however, the catalytic efficiency of the latter was substantially lower than that of HNE or the horse enzymes. Neither horse enzyme showed activity towards protected amino acid ester or *p*-nitroanilide substrates containing either aromatic (Bz-Tyr-NA, Suc-Phe-NA) or hydrophobic (Boc-Leu-ONp, Boc-Met-ONp, Boc-Leu-NA) amino acids at the P1 site, similarly to PR-3 (Rao et al., 1991). However, in contrast with their activity on single-amino acid substrates, the horse proteinases differed significantly in catalytic potency towards tri- and tetra-peptide substrates. Elastase 2A hydrolysed Suc-Ala-Ala-Ala-NA ($k_{\text{cat.}}/K_m = 90 \text{ M}^{-1} \cdot \text{s}^{-1}$) and Suc-Ala-Ala-Val-NA ($k_{\text{cat.}}/K_m = 570 \text{ M}^{-1} \cdot \text{s}^{-1}$) both of which were not cleaved by its human counterpart PR-3 (Rao et al., 1991; Kam et al., 1992). On the

Table 2 Comparison of kinetic constants for hydrolysis of some *p*-nitrophenyl ester of protected amino acids and *p*-nitroanilide peptide substrates by horse and human neutrophil elastases

Kinetic constants were measured in 0.1 M Hepes buffer, pH 7.5, containing 0.5 M NaCl and 10% (v/v) methanol (*p*-nitrophenyl ester substrates) or dimethyl sulphoxide (4-nitroanilide substrates) at 25 °C. The units for k_{cat} , K_m and k_{cat}/K_m are s^{-1} , mM and $M^{-1} \cdot s^{-1}$, respectively. NR, no reaction was observed with 5 μ g of elastases. d.n.a., data not available.

Substrate (P5-P4-P3-P2-P1*)	Kinetic constant measured	Values for kinetic constants			
		2A	PR-3†	2B	HNE
Boc-Ala-ONp	k_{cat}	26.1	10.0	12.0	23
	K_m	0.27	0.66	0.12	80
	k_{cat}/K_m	97 000	15 150	99 000	300 000
Boc-Val-ONp	k_{cat}	4.5	2.3	2.5	2.7
	K_m	0.04	0.42	0.02	5.6
	k_{cat}/K_m	98 000	5600	120 000	480 000
Suc-Ala-Ala-Ala-NA‡	k_{cat}	2.5	NR	3.4	0.7
	K_m	27.8	—	3.2	3.3
	k_{cat}/K_m	90	NR	1060	210
Suc-Ala-Ala-Val-NA‡	k_{cat}	1.6	NR	6.2	3.1
	K_m	2.8	—	3.0	1.1
	k_{cat}/K_m	570	NR	2060	2820
MeO-Suc-Ala-Ala-Pro-Val-NA‡	k_{cat}	9.5	0.82	17.9	10.8
	K_m	0.3	0.27	0.6	0.2
	k_{cat}/K_m	31 600	3050	29 800	54 000
MeO-Suc-Ala-Ala-Pro-Met-NA§	k_{cat}	NR	d.n.a.	NR	0.72
	K_m	—	d.n.a.	—	2.4
	k_{cat}/K_m	NR	d.n.a.	NR	300

* The nomenclature of Schechter and Berger (1967) is used to designate the individual amino acid residues (P2, P1, P1', P2', etc.) of a peptide substrate and the corresponding subsites (S2, S1, S1', S2', etc.) of the enzyme. The scissile bond is the P1—P1' peptide bond.

† Data obtained from Rao et al. (1991).

‡ For horse enzymes and HNE, data were obtained from Dubin et al. (1986).

§ For HNE, data were from Nakajima et al. (1979).

|| For *N*-carbobenzoxy amino acid *p*-nitrophenyl esters (Stein, 1985).

Table 3 Inhibition constants K_i of the enzyme–inhibitor complex obtained for human (HNE) and horse (2A and 2B) neutrophil elastases and native aprotinin or its reactive-site variants

HNE was used to determine the activity of aprotinin reactive-site variants. All titrations were carried out in 0.1 M Hepes buffer, pH 7.5, containing 0.5 M NaCl with preincubation of the proteinase inhibitor mixture for 30 min at room temperature and using MeO-Suc-Ala-Ala-Pro-Val-NA (final concentration 0.5 mM) as substrate to assay residual enzyme activity. n.i., No inhibition of the enzyme up to 100 molar excess of the inhibitor in the reaction mixture. o-o, Carbon atoms of amino acid at the P1 site.

Aprotinin reactive-site variants		Inhibition constant K_i (nM)		
Xaa ¹⁵	Side chain	HNE	2A	2B
Lys	o-o-o-o-o-NH ₂	3000*	n.i.	n.i.
Ala	o-o	1.8	n.i.	1.8
Abu	o-o-o	0.2	370	0.4
Nva	o-o-o-o	0.2	220	1.1
Val	$ \begin{array}{c} \text{O} \\ \diagup \\ \text{O}-\text{O} \\ \diagdown \\ \text{O} \end{array} $	0.3	120	0.4

* Data obtained from Brinkmann et al. (1991).

other hand, MeO-Suc-Ala-Ala-Pro-Met-NA, a substrate of HNE ($k_{cat}/K_m = 300 M^{-1} \cdot s^{-1}$) (Nakajima et al., 1979), was not hydrolysed by horse elastase 2A or 2B. Besides some individual differences in turnover rate, the hydrolysis of synthetic peptides confirms that horse and human leucocyte elastases prefer long

substrates containing proline at the P2 site with small aliphatic residues (alanine and valine) at the P1 site.

Interaction with aprotinin and its reactive-site variants

To explore further the differences in the specificity between proteinases 2A and 2B, the interaction of each enzyme with aprotinin and aprotinin reactive-site variants was investigated. Although unmodified aprotinin did not inhibit horse elastases, its variants (Val¹⁵ = Abu¹⁵ > Nva¹⁵ > Ala¹⁵) were efficient inhibitors of proteinase 2B with inhibition constant K_i values in the nanomolar range (from 0.4 to 1.8 nM) (Table 3). In this regard, elastase 2B closely resembles HNE. Apart from the Nva¹⁵ inhibitor being a 5-fold better inhibitor of human than horse elastase, the rest were equally effective against both enzymes. In sharp contrast with elastase 2B, none of the variants was a good inhibitor of elastase 2A (K_i value in micromolar range). Moreover, the Ala¹⁵ variant had no inhibitory activity against this proteinase (Table 3), increasing the difference in specificity between the two horse enzymes.

DISCUSSION

Three serine proteinases with elastase-like activity on synthetic substrates have been purified from the granular fraction of horse neutrophils (Dubin et al., 1976; Potempa, 1982), the enzymes being designated proteinase 1, 2A and 2B. All have been examined in some detail (Ardelt et al., 1976; Koj et al., 1976; Dubin et al., 1986), with the latter two being shown to possess strong elastinolytic activity. Thus, by analogy with HNE, they were implicated as possible aetiology factors in chronic lung disease in

the horse (Potempa et al., 1986). To facilitate further the comparison between human and horse neutrophil proteinases, we have determined the partial primary structure of elastases 2A and 2B. The sequences reported here reveal that the former enzyme is homologous to PR-3 and the latter to HNE. However, horse elastase 2A and human PR-3 differ markedly in their cleavage specificity on insulin B-chain (Figure 2) and in catalytic efficiency towards synthetic substrates (Table 2). In addition, they differ in reactivity with one of the natural protein proteinase inhibitors, eglin C. Whereas eglin C is a very efficient inhibitor of elastase 2A (K_i 0.5 nM) (Potempa et al., 1985), it is only a weak inhibitor of PR-3 (Rao et al., 1991). The differences are probably attributable to variations in the amino acid residues lining the primary (S1) and secondary binding pockets. Indeed, comparison of the active-site regions of PR-3 and elastase 2A (Figure 1) indicates that, despite a strong similarity between these two enzymes, there is a difference in at least one residue in the primary binding site. Asp²¹³ which is located at the bottom of the S1 subsite in PR-3 (Bode et al., 1989; Brubaker et al., 1992) is replaced by Phe in elastase 2A. This change may explain the differences in the enzyme activities of the two enzymes.

Remarkably, the two enzymes degrade elastin at the same rate, being about half as fast as degradation by HNE (Potempa et al., 1986; Kao et al., 1988). This may be significant, as PR-3 was recently described as a human neutrophil proteinase with elastinolytic activity which, at high concentration, is able to cause extensive tissue damage and emphysema in animal models (Kao et al., 1988). Besides connective tissue degradation, PR-3 also appears to regulate growth and differentiation of the myelomonocyte lineage (Boris et al., 1989; Jenne et al., 1990), possess antimicrobial activity independent of its enzyme activity (Gabay et al., 1989; Campanelli et al., 1990a) and serve as an antigen recognized by Wegener's granulomatosis autoantibodies (Goldschmeding et al., 1989; Niles et al., 1989; Jannette et al., 1990). Whether these are also properties of horse elastase 2A remains to be established.

In comparison with HNE, elastase 2B cleaves Suc-Ala-Ala-Ala-NA more effectively, which is consistent with the primary cleavage of the Ala¹⁴-Leu¹⁵ peptide bond in the insulin B-chain. The enzymes also differ significantly in their affinity for eglin C (P1 residue leucine). Whereas HNE forms a very tight complex with this inhibitor ($K_i < 10^{-9}$ M) (Seemüller et al., 1986), the latter seems to have a much lower affinity for elastase 2B ($K_i > 10^{-7}$ M) (Potempa et al., 1985). On the other hand, except for a variant inhibitor with Nva at the P1 residue, HNE and elastase 2B show surprisingly similar reactivity with Ala¹⁵, Abu¹⁵ and Val¹⁵ variants of aprotinin (Table 3). The lower affinity of the Nva¹⁵ aprotinin variant for elastase 2B compared with HNE, along with the fact that eglin C is only a poor inhibitor of the horse proteinase, indicates that the primary specificity pocket of elastase 2B is shallower and/or slightly smaller than the S1 pocket of HNE.

Horse neutrophil elastase 2B possesses a 7-fold higher elastinolytic activity (Potempa et al., 1986) than HNE, which is comparable with that of PPE, one of the most potent elastin-degrading proteinases of mammalian origin. This difference in catalytic efficiency can probably be explained by the architecture of extended substrate-binding sites, particularly the primary specificity site (S1), the variations in the external loops and the surface distribution of charged residues. Unfortunately, the limited sequence data available make it impossible to make such a comparison. Of the residues forming the central core of the binding site (i.e. peptide segments 189 to Ser¹⁹⁵, 213–216, 226–228, residue 41) (Bode et al., 1989), only the first segment (residues 189–195) can be directly compared in this study.

Nonetheless, substitutions of amino acid residues such as Val¹⁹⁰ by Ile¹⁹⁰ in this region could explain differences in enzyme reactivity with substrates and natural inhibitors.

To our knowledge this is the first report that not only details the properties of neutrophil proteinases from a non-primate mammal, but also indicates the presence of a PR-3-related enzyme in this species. Whether this latter enzyme plays a major role in the development of lung disease remains to be established. However, as the general properties of the human and horse elastinolytic enzymes are comparable, despite some structural differences, the equine species may make an excellent model for the preparation and testing of proteinase inhibitors to counteract the effects of elastinolytic proteinases during the development of obstructive pulmonary disease in man.

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