Volume 263, number 2, 355–357 FEBS 08369 April 1990

Inactivation of human plasma α_1-proteinase inhibitor by human PMN leucocyte collagenase

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Received 27 February 1990; revised version received 12 March 1990

Highly purified human polymorphonuclear leucocyte collagenase cleaved human α-1-proteinase inhibitor (α_1-PI) at the carboxyl site of Phe^{352} (P_7). The inhibitor was thereby rapidly inactivated and generated a primary degradation product as shown by reverse-phase HPLC and N-terminal sequencing. Prolonged incubation of the modified inhibitor with polymorphonuclear leucocyte collagenase led to the generation of a secondary degradation product with additional cleavage at the carboxyl site of Pro^{357} (P_2).

Polymorphonuclear leucocyte collagenase; α_1-Proteinase inhibitor; Proteolytic inactivation

1. INTRODUCTION

Polymorphonuclear (PMN) leucocytes have diverse roles which include the phagocytosis of bacterial microorganisms, the regulation of immune and inflammatory processes and extracellular matrix degradation. Furthermore, PMN leucocytes are believed to play an important role in the development of pulmonary emphysema, which is due to uncontrolled proteolysis of lung tissue in the absence of sufficient quantities of α_1-proteinase inhibitor (α_1-PI). Individuals with genetic α_1-PI deficiency are predisposed to emphysema [1]. The normal function of α_1-PI therefore seems to be the inhibition of PMN leucocyte elastase [2], which normally protects the elastic lung fibers against proteolytic degradation.

It is important to note that, although a genetic defect in α_1-PI production may result in the development of emphysema, this disease occurs frequently in individuals with apparently normal inhibitor levels. For this reason the investigation of oxidative [3,4] or proteolytic [5–13] inactivation of α_1-PI by bacterial or human proteinases becomes an important feature in the regulation of elastic fiber turnover of the lung.

The discovery that a metalloproteinase secreted from PMN leucocytes inactivates α_1-PI [12,13] led to a new understanding of the pathogenesis of pulmonary emphysema. We now conclude that the proteolytic inactivation of α_1-PI is due to PMN leucocyte collagenase.

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2. MATERIALS AND METHODS

α_1-PI was a generous gift from Behringwerke, Marburg, FRG.

2.1. *Purification of PMN leucocyte collagenase, activation and enzyme assay*

Leucocytes were isolated from buffy coat according to the method described in [14]. PMN leucocyte collagenase was purified to homogeneity as recently published [15]. Proenzyme activation was achieved by incubation with 1 mM $HgCl_2$ for 2 h at 37°C. The activity of collagenase was determined by the degradation of the synthetic octapeptide (dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg-OH) as described in [16]. Alternatively, the degradation of soluble type I collagen at 25°C was shown by SDS-PAGE.

2.2. *Proteolytic inactivation of α_1-PI*

1 mg human α_1-PI was incubated with 5 μg activated PMN leucocyte collagenase at 37°C for 4 and 20 h.

2.3. *Separation of α_1-PI degradation products by reverse-phase HPLC*

The degradation products of human α_1-PI inactivated by PMN leucocyte collagenase were subjected to a Bakerbond wide pore C_4-column (4.9 × 250 mm) after 1 h or 24 h incubation time. The separations were performed at a constant flow rate of 1 ml/min using a linear gradient from 0–80% acetonitrile (Fig. 1).

2.4. *Sequence determination*

Aminoterminal sequence determinations of the individual degradation products isolated by reverse-phase HPLC were performed on Immobilon-P membrane (Millipore, Bedford, USA), which was pretreated with 0.5 mg polybrene to ensure binding of peptides and applied to a microsequencer (Model 810, Knauer, Berlin, FRG). The Pth amino-acid separations were performed online on an Applied Biosystems Pth-C_{18}-column (220 × 2.1 mm) at a flow rate of 0.24 ml/min using the buffer system of Hunkapiller [17].

2.5. *SDS-PAGE*

SDS-PAGE was performed according to the method of Laemmli [18]. The proteins were visualized by silver staining as previously described by Heukeshoven and Dernick [19].

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00145793/90/$3.50

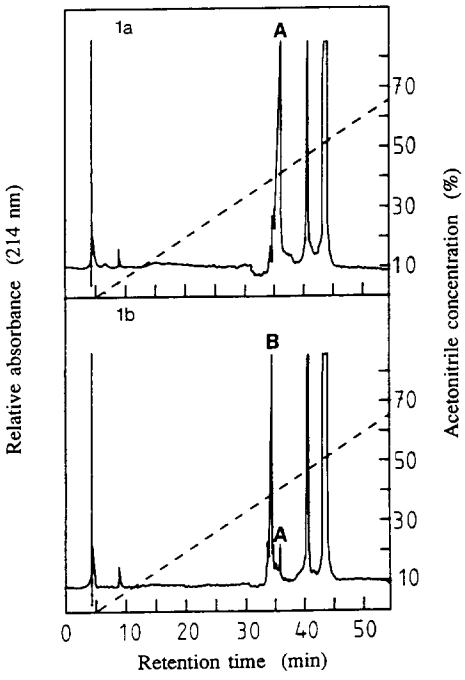

Fig. 1. Reverse-phase HPLC of α_1-PI degradation products. 4 mg α_1-PI was degraded by incubation with 10 μg purified PMN collagenase for 1 h (a) and 24 h (b) at 37°C. A, peptide A (primary degradation product; 1 h at 37°C); B, peptide B (secondary degradation product; 24 h at 37°C).

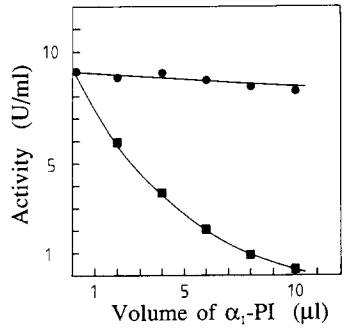

Fig. 2. Proteolytic inactivation of α_1-PI by PMN collagenase. Inactivation of α_1-PI by PMN collagenase after 1 h at 37°C. Inhibition of the inactivation of α_1-PI by PMN collagenase in the presence of 50 mM EDTA. The inhibition of bovine trypsin (12.5 μg) by α_1-PI (2 mg/ml) is shown.

3. RESULTS AND DISCUSSION

The rapid proteolytic inactivation of α_1-PI by highly purified PMN leucocyte collagenase was shown by SDS-PAGE (not shown), reverse-phase HPLC and N-terminal sequence determination. The analysis of the proteolytic inactivation of α_1-PI by PMN leucocyte collagenase showed the generation of a primary degradation product after 1 h at 37°C (Fig. 1a, peptide A). PMN leucocyte collagenase cleaved the Phe^{352}-Leu^{353} peptide bond (P_7–P_6) [20] of the native α_1-PI. The proteolytic cleavage at the carboxyl site of Phe^{352} (P_7) led to the inactivation of the inhibitor (Fig. 2). Inactivation of α_1-PI by PMN leucocyte collagenase was inhibited by EDTA and 1,10-phenanthroline. Further incubation at 37°C resulted in additional cleavage of the Pro^{357}-Met^{358}-(P_2-P_1) peptide bond (Fig. 1b, peptide B).

The serine proteinases, elastase, trypsin and chymotrypsin, which are inhibited by complex formation with α_1-PI, cleave the inhibitor at the Met^{358}-Ser^{359} (P_1-P_1') peptide bond, that corresponds to the reactive site of the inhibitor [21]. The X-ray crystallographic structure of the modified (reactive site cleaved) α_1-PI showed that a dramatic structural change accompanied the cleavage of the reactive site, since Met^{358} (P_1) and Ser^{359} (P_1') were located at opposite sites of the modified inhibitor [22]. The peptide which was generated upon cleavage of the reactive site remained bound to the inhibitor by means of non-covalent interactions.

It was shown during the last two decades that α_1-PI is susceptible to proteolytic inactivation by different proteolytic enzymes of bacterial [5,6] and human origin [9,11–13] (for details: see Table I). The interesting fact that secreted PMN leucocyte collagenase can catalytically cleave and inactivate α_1-PI in the

Table I

Cleavage sites of the proteolytic inactivation of human α_1-PI by different proteolytic enzymes

Residue no. 350					360
Ala-Met-Phe-Leu-Glu-Ala-Ile-Pro-Met-Ser-Ile-Pro-Pro-Glu-Val-Lys-Phe-Asn-Lys-					
↑	↑	↑	↑	↑	
V	IV	III	II	I	

Cleavage site I: Cathepsin L [9], *Serratia marcescens* metalloproteinase [6]
Cleavage site II: *Pseudomonas aeruginosa* [7], macrophage elastase [11], PMN collagenase (secondary product) (this paper)
Cleavage site III: Cathepsin L [9], *Staphylococcus aureus* cysteine and serine proteinase [5]
Cleavage site IV: *Staphylococcus aureus* metalloproteinase [5], secreted PMN metalloproteinase [12,13], PMN collagenase (primary product) (this paper)
Cleavage site V: *Crotalus adamanteus* proteinase II [8]

proteinase-sensitive region may contribute to a persisting activity of PMN leucocyte elastase by lowering the level of intact α_1-PI. Although the physiological significance of α_1-PI inactivation by PMN leucocyte collagenase has yet to be determined, it could possibly play an important role in the extracellular turnover of α_1-PI, since the enzyme is easily secreted upon specific stimuli [23,24] and is optimally active at physiologic pH. It has recently been reported that proteolytically inactivated α_1-PI has chemotactic properties for polymorphonuclear leucocytes [25] and may thus enhance the invasion of cells. This could lead to an additional release of PMN collagenase and PMN elastase which would result in an increased turnover of α_1-PI and lung elastin.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft, special research program SFB 223 project B1. The skillful technical assistance of S. Abke, K. Dunker, A. Groß, T. Kelle and D. Leisner is gratefully acknowledged. The authors thank Mrs G. Delany for the expert linguistic advice.

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