Volume 290, number 1,2, 99–102 FEBS 10172 September 1991

ADONIS 0014579391008846

Inactivation of human plasma C1-inhibitor by human PMN leucocyte matrix metalloproteinases

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Received 18 July 1991

Highly purified human polymorphonuclear (PMN) leucocyte matrix metalloproteinases, collagenase and gelatinase, cleaved human plasma C1-inhibitor at the carboxyl site of Ala^{439} (P_6). This led to a concomitant loss of C1-inhibitor activity. An additional cleavage site, at the carboxyl site of Ser^{441} (P_4), was observed during PMN leucocyte gelatinase-induced inactivation, and a minor fragment of the plasma C1-inhibitor was generated.

Polymorphonuclear leucocyte collagenase; Polymorphonuclear leucocyte gelatinase; C1-inhibitor; Proteolytic inactivation

1. INTRODUCTION

C1-inhibitor inactivates proteinases of the complement (C1r, C1s), fibrinolytic (plasmin), kinin-generation (kallikrein) and coagulation (factor XIa, XIIa) system by the formation of equimolar stoichiometric complexes between inhibitor and target proteinase [1,2]. Like in other members of the serine proteinase inhibitor (serpin) superfamily, the reactive site of C1-inhibitor is located in an exposed loop near the C-terminus of the molecule [3–5]. Limited proteolysis of C1-inhibitor by chymotrypsin-like proteinases results in N-terminal modification of the molecule without change in inhibitor capacity [5–7]. Serin proteinases which are not inhibited by C1-inhibitor can cleave within the reactive site loop resulting in a loss of inhibitory activity [6,8,9]. This process is accompanied by a conformational rearrangement and an increase in heat stability. The physiological role of C1-inhibitor has been demonstrated by its involvement in type I and type II hereditary angiooedema [10–12]. It has recently been shown, that human PMN leucocytes are able to inactivate C1-inhibitor by limited proteolysis [6]. This is due to proteolytic cleavage of PMN leucocyte elastase on the N- and C-terminal part of the inhibitor. Since the regulation of the complement system is critically dependent on a balance between C1r, C1s and their inhibitor, proteolytic inactivation of C1-inhibitor by PMN leucocytes may result in complement activation. The influence of PMN leucocyte metalloproteinases on the inhibitory activity of C1-inhibitor is described in this report. The inactivation of C1-inhibitor by PMN leucocyte collagenase and gelatinase is demonstrated, and we discuss the possible role of PMN leucocyte metalloproteinases as antagonists of human plasma C1-inhibitor.

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2. MATERIALS AND METHODS

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

PMN leucocyte procollagenase was purified to homogeneity exactly as recently published [13]. PMN leucocyte progelatinase was purified according to the method described by Wilhelm et al. for the homologous enzyme from simian virus 40 transformed lung fibroblasts [14]. It was demonstrated by N-terminal sequence determination that both enzyme preparations were homogeneous, showing the N-terminal sequences of PMN leucocyte procollagenase and PMN leucocyte progelatinase. Activation of both proenzymes was achieved by treatment with 1 mM $HgCl_2$ for 2 h at 37°C. The enzymatic activity of PMN leucocyte collagenase and PMN leucocyte gelatinase was determined by the degradation of the synthesic octapeptide (DNp-Pro-Gln-Gly-Ile-Ala-Gly-Gln-^{D}Arg-OH) as described by Masui [15].

2.2. *Proteolytic inactivation of C1-inhibitor*

2 mg human C1-inhibitor were incubated with 5 μg of either active PMN leucocyte collagenase or PMN leucocyte gelatinase at 37°C for 20 h.

2.3. *Purification of C1-inhibitor degradation products by reverse-phase HPLC*

Fragments from the PMN leucocyte collagenase and PMN leucocyte gelatinase digests of C1-inhibitor were separated by reverse-phase HPLC on a Bakerbond wide pore C_{18}-column (4.9 × 250 mm) at a constant flow rate of 0.8 ml/min using a linear gradient from 0–80% acetonitrile.

2.4. *Sequence determination*

N-terminal sequence determinations of the individual degradation products were performed by automated Edman degradation using a microsequencer (Model 810, Knauer, Berlin). Pth amino acids were separated on an Applied Biosystems Pth-C_{18}-column (220 × 2.1 mm) at a flow rate of 0.24 ml/min as recently published [16].

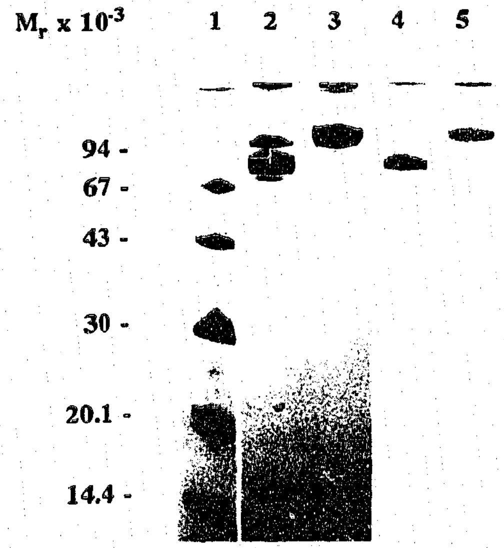


Fig. 1. Cleavage of C1-inhibitor by PMN leucocyte collagenase and gelatinase. 10% SDS-PAGE unreduced. The protein bands were stained with silver. Lane 1, molecular mass markers; lane 2, 1 mg C1-inhibitor incubated with buffer at 37°C for 24 h; lane 3, 1 mg C1-inhibitor incubated with 5 μg active collagenase at 37°C for 24 h; lane 4, 1 mg C1-inhibitor incubated with 5 μg active collagenase in the presence of EDTA; lane 5, 1 mg C1-inhibitor incubated with 5 μg active gelatinase at 37°C for 24 h; lane 6, 1 mg C1-inhibitor incubated with 5 μg active gelatinase in the presence of EDTA.

2.5. *SDS-PAGE*

The molecular masses of C1-inhibitor and degradation products were analysed by SDS-PAGE as described by Laemmli [17]. The proteins were stained with silver according to the protocol of Heukeshoeven and Dernick [18].

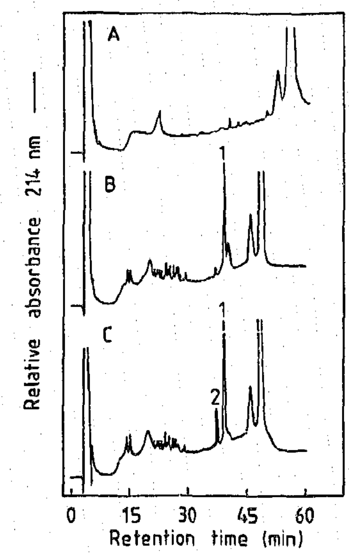
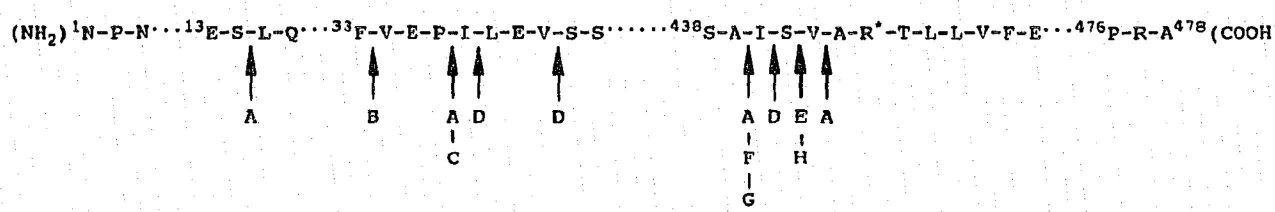


Fig. 2. Reverse-phase HPLC of C1-inhibitor and C1-inhibitor degradation products (A) intact C1-inhibitor; (B) active collagenase digest of C1-inhibitor; 1, cleavage fragment Ile^{440} N-terminus; (C) active gelatinase digest of C1-inhibitor; 1, cleavage fragment Ile^{440} N-terminus; 2, cleavage fragment Val^{442} N-terminus.

3. RESULTS AND DISCUSSION

The rapid proteolytic fragmentation of C1-inhibitor by highly purified PMN leucocyte collagenase and gelatinase was shown by SDS-PAGE (Fig. 1), reverse-phase HPLC (Fig. 2) and N-terminal sequence determination (Fig. 3). The native C1-inhibitor was proteolytically cleaved by PMN leucocyte collagenase and gelatinase at the Ala^{439}–Ile^{440} peptide bond. This proteolytic cleavage at the carboxyl site of Ala^{439} (P_6), five residues pre-



A, Bitis arientans venom [6]

B, Human skin chymase, human cathepsin G, bovine chymotrypsin [7]

C, Bungarus fasciatus venom [6]

D, Human PMN elastase [6]

E, Pseudomonas aeruginosa [6]

F, Human PMN collagenase [this report]

G, Human PMN gelatinase (main product) [this report]

H, Human PMN gelatinase (minor product) [this report]

*, reactive site [5]

Fig. 3. Proteolytic modification of human C1-inhibitor by various proteolytic enzymes as indicated by arrows.

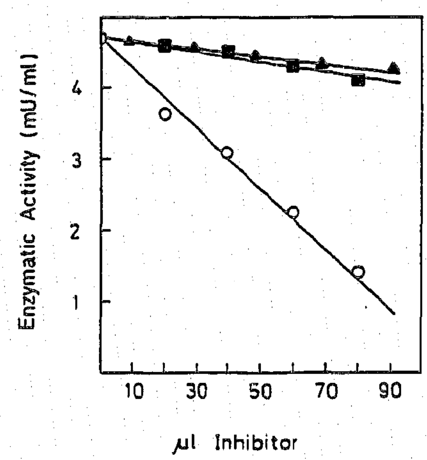


Fig. 4. Demonstration of the loss of inhibitory capacity of C1-inhibitor after proteolysis by PMN leucocyte collagenase and gelatinase. (■), inactivation of C1-inhibitor by collagenase after 24 h at 37°C; (▲), inactivation of C1-inhibitor by gelatinase after 24 h at 37°C; (○), intact C1-inhibitor after 24 h incubation at 37°C in the presence of buffer. The inhibition of human plasmin (10 μg) by C1-inhibitor (1 mg/ml) is shown.

ceding the reactive site, led to the concomitant inactivation of the inhibitor (Fig. 4). A further minor fragment was generated by proteolytic cleavage of the Ser^{441}–Val^{442} peptide bond by PMN leucocyte gelatinase within the protein core of the C1-inhibitor. Inactivation of C1-inhibitor by PMN leucocyte collagenase and gelatinase was inhibited by EDTA or 1,10-phenanthroline (not shown) indicating metalloproteinase specific cleavage.

The fragmentation of C1-inhibitor by different proteolytic enzymes of bacterial [9], reptilian [6] and human origin [6–8] has been investigated over the last ten years. The N-terminal and C-terminal region of the inhibitor is sensitive to proteolytic attack. However, the loss of inhibitory activity is only observed when cleavages occur within the reactive site region of the molecule, which is located at the C-terminus [5]. This is followed by a significant conformational change in the exposed reactive site loop of the native inhibitor, which has been demonstrated by crystallisation of the inactivated homologous serpin α_1-PI [19]. Recently, Huber and Carrell [20] showed that the structural model of α_1-PI can generally act as a template for other members of the serpin family.

Inactivation of C1-inhibitor by proteinases derived from potentially pathogenic organisms or by human PMN leucocyte elastase would lead to an imbalance of the complement system. This would potentiate pathological proteolysis at sites of inflammatory reactions. As it was recently shown that PMN leucocyte collagenase and gelatinase are regular plasma components [21], we investigated the ability of these matrix metalloproteinases to digest plasma components [22]. Both PMN leucocyte matrix metalloproteinases, collagenase and gelatinase, can catalytically cleave C1-inhibitor within the reactive site loop and thus may contribute to complement activation during inflammation. These enzymes could possibly play an important role in the turnover of C1-inhibitor, since they are easily secreted upon specific stimuli and are optimally active at physiological pH [23,24]. PMN leucocyte metalloproteinases may behave as the main antagonists of α_1-PI [22] or C1-inhibitor. Proteolysis of these inhibitors results in the potentiation of inflammatory processes, which are regulated by these members of the serpin family. However, the physiological significance of C1-inhibitor inactivation by PMN leucocyte matrix metalloproteinases has yet to be determined.

Acknowledgements: This work was supported by DFG, special research programme SFB 223 project B2. The authors thank Dr Dickneite, Behringwerke, Marburg, Germany for the generous gift of C1-inhibitor. The skilful technical assistance of K. Etzold and S. Rottmann is greatfully acknowledged. The authors thank Mrs G. Delany for linguistic advice.

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