Volume 249, number 2, 353–356 FEB 07223 June 1989

Activation of matrix metalloproteinase 3 (stromelysin) and matrix metalloproteinase 2 ('gelatinase') by human neutrophil elastase and cathepsin G

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Received 18 April 1989

The ability of human neutrophil elastase and cathepsin G to activate matrix metalloproteinase 3 (MMP-3 = stromelysin) and MMP-2 ('gelatinase') purified from human rheumatoid synovial fibroblasts in culture was examined. The zymogen of MMP-3 (proMMP-3) was activated to full activity with elastase and cathepsin G by limited proteolysis of the molecule into two active forms of $M_r \sim 45000$ and $M_r \sim 25000$. In contrast, proMMP-2 was not activated at all by these neutrophil serine proteinases, although it was degraded into small fragments. These data suggest that neutrophil elastase and cathepsin G may play an important role in the activation of proMMP-3 in vivo in various inflammatory conditions, but proMMP-2 may be activated in different ways.

Metalloproteinase; Activation; Neutrophil enzyme; Elastase; Cathepsin G; Extracellular matrix

1. INTRODUCTION

Rheumatoid synovial fibroblasts in culture secrete three distinct matrix metalloproteinases (MMPs): MMP-1 corresponds to collagenase (EC 3.4.24.7) [1], MMP-2 to 'gelatinase' and type IV collagenase [2–5] and MMP-3 to stromelysin [6–8]. Collagenase digests type I, II, III and X [9] collagens. MMP-2 is thought to be involved in the degradation of collagen by digesting gelatin derived from collagen molecules cleaved by the action of collagenase [2], although the ability of the enzyme to digest type IV and type V collagens has been pointed out [4,5]. MMP-3 has a broad range of activities to extracellular macromolecules; it degrades proteoglycans, type IV collagen, laminin, fibronectin and gelatin, and removes N-terminal propeptides of type I procollagen [6–8]. We have recently demonstrated that MMP-3 also digests type IX collagen which has an important role in maintaining the structural integrity of cartilage [10]. The synthesis and secretion of the proteinase by synovial lining cells in rheumatoid synovium have been shown by immunohistochemical studies [11].

MMP-3 and MMP-2 as well as collagenase are, however, secreted in inactive proenzymes (proMMPs) which are then activated extracellularly [4,5,12–14]. Thus, their activation is a key process for them to participate in the degradation of extracellular matrix components in vivo. We report here that proMMP-3 is activated by human neutrophil elastase and cathepsin G by limited proteolysis, but proMMP-2 is not.

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2. MATERIALS AND METHODS

2.1. *Materials*

ProMMP-3 was purified from the culture medium of rheumatoid synovial cells treated with rabbit macrophage-conditioned medium as reported [14]. ProMMP-2 was also isolated from the above-mentioned culture medium (Okada, Y. et al., manuscript in preparation). Both proMMP-3 and proMMP-2 were homogeneous according to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Human neutrophil

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00145793/89/$3.50

elastase [15] and cathepsin G [16] were generous gifts from Dr J. Travis, Department of Biochemistry, University of Georgia, Athens, GA, USA. Diisopropyl fluorophosphate and 4-aminophenylmercuric acetate ($NH_2PhHgAc$) were obtained from Sigma.

2.2. *Activation of proMMP-3 and proMMP-2 by human neutrophil elastase and cathepsin G*

The activation of proMMP-3 and proMMP-2 by neutrophil serine proteinases was investigated as follows: proMMP-3 (180 ng) and proMMP-2 (50 ng) in 10 μl of 50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 10 mM Ca^{2+}, 0.02% NaN_3, 0.05% Brij 35 were reacted with an equal volume of human neutrophil elastase (0.1, 1, 10 μg/ml) or cathepsin G (0.1, 1, 10 μg/ml) at 37°C for 5 min–24 h. After blocking the activity of the serine proteinases with 3.0 mM diisopropyl fluorophosphate (for 30 min at 23°C), the activities of MMP-3 and MMP-2 were assayed by incubation for 1.5 h at 37°C using [^{3}H]carboxymethylated transferrin and [^{14}C]gelatin as substrates for MMP-3 and MMP-2, respectively [8]. The activation rate of proMMP-3 and proMMP-2 was determined in comparison with the full activities obtained from the samples incubated before assays with 1.5 mM $NH_2PhHgAc$ for 24 h at 37°C for proMMP-3 and with 1.0 mM $NH_2PhHgAc$ for 10 min at 37°C for proMMP-2.

2.3. *Electrophoretic analyses of* M_r *changes of proMMP-3 and proMMP-2*

ProMMP-3 (2.7 μg) and proMMP-2 (3.6 μg) were radioiodinated according to Fraker and Speck [17]. Mixtures containing unlabeled proMMP-3 (162 ng) and ^{125}I-labeled proMMP-3 (10 ng), and unlabeled proMMP-2 (50 ng) and ^{125}I-labeled proMMP-2 (11 ng) were treated with human neutrophil elastase (10 μg/ml) or cathepsin G (10 μg/ml) for 10 min–22 h at 37°C. After the incubation, the proteinase activities were inactivated using 4.5 mM diisopropyl fluorophosphate and 40 mM EDTA. Proteins in the samples were resolved by SDS-PAGE using 10% polyacrylamide gels with reduction with 2-mercaptoethanol. Gels were dried and autoradiographed.

3. RESULTS AND DISCUSSION

Incubation of proMMP-3 with human neutrophil elastase at a concentration of 10 μg/ml resulted in almost full activation in 2 h at 37°C and the MMP-3 activity remained stable after a 24 h incubation (fig.1a). At a lower concentration of elastase (1 μg/ml), proMMP-3 was gradually activated up to 75% of the full activity, but the enzyme at the concentration of 0.1 μg/ml did not significantly activate proMMP-3 compared with a buffer control which showed minimal spontaneous activation (15% of full activity) after 24 h at 37°C (fig.1a). Analyses of ^{125}I-labeled proMMP-3 after the reaction with neutrophil elastase (10 μg/ml) showed that proMMP-3 of M_r 57000 was pro-

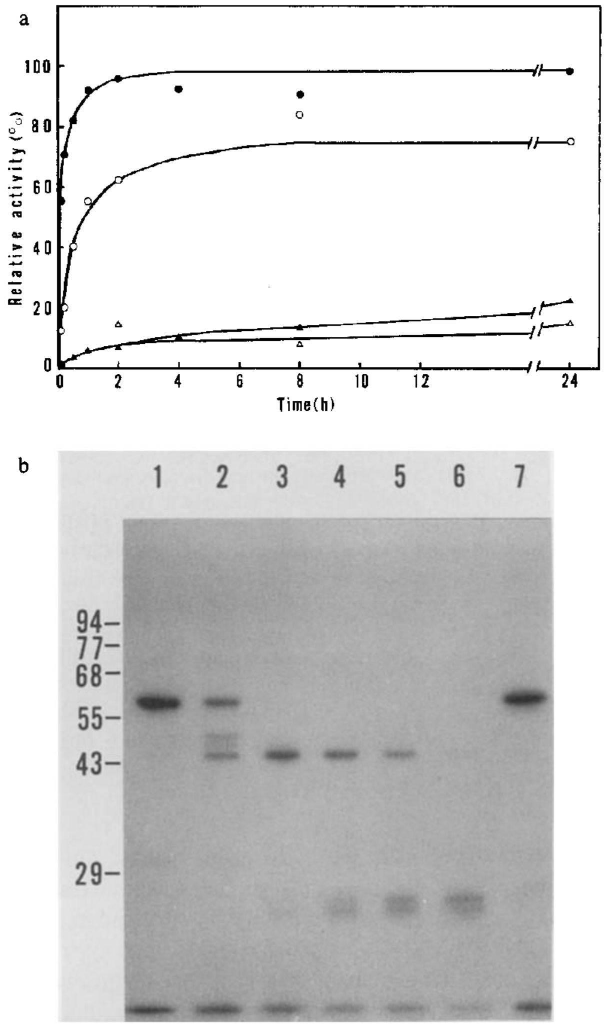

Fig.1. Activation of proMMP-3 by human neutrophil elastase. (a) ProMMP-3 was incubated with elastase at various concentrations (△, 0; ▲, 0.1; ○, 1; ●, 10 μg/ml). After inactivating the serine proteinase, the activity of MMP-3 was assayed. The activity of MMP-3 obtained from samples incubated with 1.5 mM $NH_2PhHgAc$ for 24 h at 37°C was taken as 100% activity. (b) A mixture of unlabeled and ^{125}I-labeled proMMP-3 was incubated with neutrophil elastase (10 μg/ml). Samples were subjected on SDS-PAGE (10% total acrylamide) under the reduction and the gels autoradiographed. Lanes: 1,7, proMMP-3 incubated without enzyme for 0 and 22 h; 2–6, proMMP-3 treated with the enzyme for 10 min, 1, 4, 8 and 22 h, respectively. M_r values ($\times 10^{-3}$) given to the left of the gel.

cessed to a polypeptide of M_r 45000 with an intermediate form of M_r 49000 and into a doublet of M_r 25000 and 23000 (fig.1b). The lower intensity of radioactivity of activated fragments is probably due to the smaller amount of ^{125}I-labeling in these regions.

Neutrophil cathepsin G (10 μg/ml) also activated proMMP-3 to full activity of the enzyme, although the time course of the activation was rather gradual. It required a 22 h incubation at 37°C for full activity (fig.2a). At a concentration of 1 μg/ml, only partial activation of proMMP-3 (up to 35%) was observed but no significant activation was observed at a concentration of 0.1 μg/ml even after a 22 h incubation at 37°C (fig.2a). SDS-polyacrylamide gel electrophoresis showed that neutrophil cathepsin G (10 μg/ml) converted proMMP-3 into a polypeptide of M_r 46000 and then into a major fragment of M_r 26000 (fig.2b).

In contrast to the effective activation of proMMP-3 by neutrophil elastase and cathepsin G, proMMP-2 was not activated at all by these neutrophil serine proteinases at any concentrations (0.1, 1 and 10 μg/ml). When ^{125}I-labeled proMMP-2 was incubated with elastase (10 μg/ml) at 37°C, the zymogen of M_r 74000 was degraded into major fragments of M_r 45000, 40000 and 18000. Similar findings were made with neutrophil cathepsin G (10 μg/ml) except that the smaller fragments generated were of M_r 43000, 24000, 19000 and 17000.

It has been reported that the zymogens of collagenase and MMP-3 are activated either by direct limited proteolysis by endopeptidases including trypsin, α-chymotrypsin, plasma kallikrein, plasmin, thermolysin and cathepsin B [12–14,18,19], or, alternatively, by mercurial compounds such as $NH_2PhHgAc$ that may cause certain conformational changes in the molecule [12–14]. The present study has shown for the first time that proMMP-3 can be activated with elastase and cathepsin G from human neutrophils. It is clear from our study that these serine proteinases activate proMMP-3 by achieving its limited proteolysis. The comparison of the enzymic activity and the fragments produced by these serine proteinases leads us to the conclusion that the polypeptides of M_r ~45000 and ~25000 are responsible for the activity. A similar finding has been obtained in the case of proMMP-3 activation by plasmin [14]. These serine proteinases activate proMMP-3 to active forms with lower M_r values by the removal of N-terminal and C-terminal fragments from the precursor, since the amino acid sequence thought to be involved in binding a zinc atom is located approximately in the middle of proMMP-3 [20].

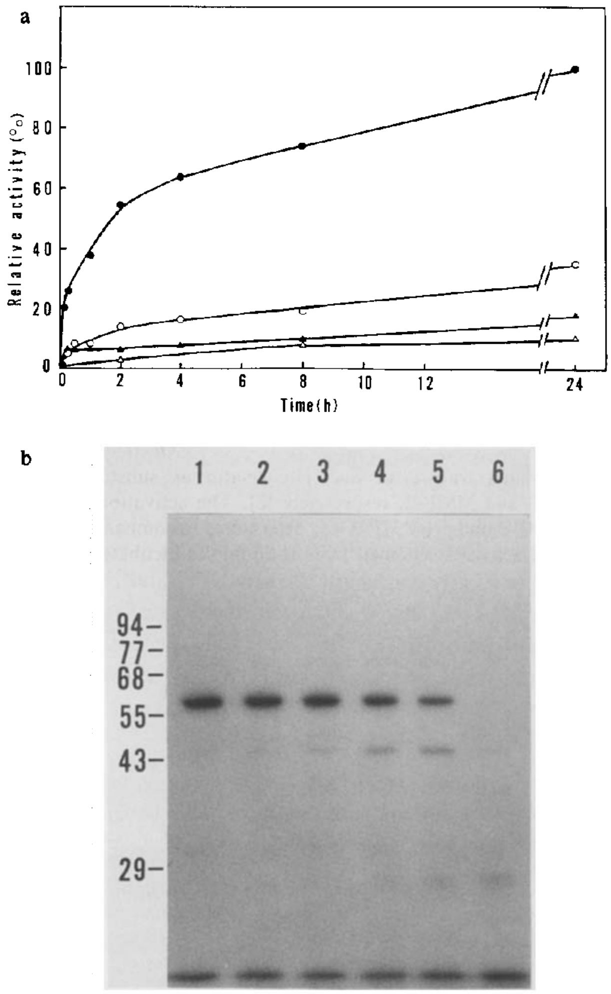

Fig.2. Activation of proMMP-3 by human neutrophil cathepsin G. (a) ProMMP-3 was incubated with cathepsin G at different concentrations (△, 0; ▲, 0.1; ○, 1; ●, 10 μg/ml). Activation rate of proMMP-3 was measured as described in the legend to fig.1a. (b) A mixture of unlabeled and ^{125}I-labeled proMMP-3 was treated with neutrophil cathepsin G (10 μg/ml) and analyzed by SDS-PAGE as described in the legend to fig.1b. Lanes: 1, proMMP-3 incubated without enzyme for 0 h; 2–6, proMMP-3 treated with the enzyme for 10 min, 1, 4, 8 and 24 h, respectively. M_r values ($\times 10^{-3}$) given to the left.

Plasmin has been considered to be a good candidate as an activator of proMMP-3 or procollagenase in vivo [14,18,19]. The present study, however, indicates that neutrophil elastase and cathepsin G are also good activators of proMMP-3. Tissue inhibitor of metalloproteinases (TIMP), a specific inhibitor of MMPs, is destroyed completely by neutrophil elastase and partially by cathepsin G, but not by plasmin [21]. Taken together, both elastase and cathepsin G which can be supplied from neutrophils infiltrated in rheumatoid joint cavity [22] may play important roles in regulation of MMP-3 activity by both activation of the zymogen and inactivation of TIMP.

It is of interest that proMMP-2 is not activated with neutrophil elastase or cathepsin G. The enzyme is readily activated with $NH_2PhHgAc$ [4,5] probably by changes in the molecular conformation as reported in the activation of procollagenase and proMMP-3 [12–14]. However, the data that serine proteinases such as trypsin [4,5] as well as neutrophil elastase and cathepsin G reported here cannot activate proMMP-2 suggest that different mechanisms are involved in the activation of this metalloproteinase. Further work is necessary to elucidate the activation mechanisms of proMMP-2 in vivo.

Acknowledgements: We are grateful to Dr Hideaki Nagase of the Department of Biochemistry and Molecular Biology, University of Kansas Medical School for his critical reading of this manuscript.

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