Inactivation of tissue inhibitor of metalloproteinases by neutrophil elastase and other serine proteinases

Yasunori Okada, Shoji Watanabe*, Isao Nakanishi, Jun-ichi Kishi[+], Taro Hayakawa[+],
Wieslaw Watorek[°], James Travis[°] and Hideaki Nagase[†]

Department of Pathology and *Orthopedic Surgery, School of Medicine, Kanazawa University, 13-1 Takara-machi,
Kanazawa 920, [+]Department of Biochemistry, School of Dentistry, Aichi-Gakuin University, Suemori-dori, Chikusaku,
Nagoya 464, Japan, [°]Department of Biochemistry, University of Georgia, Athens, GA 30602 and [†]Department of
Biochemistry, University of Kansas Medical Center, Kansas City, KS 66103, USA

Tissue inhibitor of metalloproteinases (TIMP) from cultured bovine dental pulp inhibits human rheumatoid synovial matrix metalloproteinase 3 (MMP-3) with a stoichiometry of 1:1 on a molar basis. Among the serine proteinases examined, human neutrophil elastase, trypsin and α-chymotrypsin destroyed the inhibitory activity of TIMP against MMP-3 by degrading the inhibitor molecule into small fragments. In contrast, the inhibitory activity of TIMP was not significantly reduced by the actions of cathepsin G, pancreatic elastase and plasmin. These data indicate that neutrophils which infiltrate tissues in various inflammatory conditions may play an important role in regulating TIMP activity in vivo through the action of neutrophil elastase.

Tissue inhibitor of metalloproteinases; Neutrophil elastase; Metalloproteinase; Extracellular matrix

1. INTRODUCTION

Human rheumatoid synovial cells in culture secrete at least three distinct metalloproteinases, all of which are capable of degrading extracellular matrix components [1]. We have referred to them as matrix metalloproteinases (MMPs): MMP-1 corresponds to collagenase [2], MMP-2 to gelatinase [3,4] and MMP-3 to a third enzyme which digests proteoglycans, type IV collagen, laminin and fibronectin [1]. The properties of MMP-3 purified from human rheumatoid synovial cells in culture are essentially identical to those described for the metalloproteinase purified from culture media of rabbit bone explants called 'proteoglycanase' [5] and of rabbit synovial fibroblasts termed 'stromelysin' [6]. On the other hand, connective tissue cells, including rheumatoid synovial

cells, also produce a specific inhibitor to these MMPs called tissue inhibitor of metalloproteinases (TIMP) [7,8]. Thus, the balance between the activities of tissue metalloproteinases and TIMP is thought to be an important determinant of whether MMPs participate in extracellular matrix breakdown in vivo. Here, we have examined the effect of serine proteinases on a metalloproteinase inhibitor from bovine dental pulp which has properties typical of TIMP. We report that its inhibitory activity against human MMP-3 is destroyed by human neutrophil elastase as well as trypsin and α-chymotrypsin.

2. MATERIALS AND METHODS

2.1. Materials

TIMP was purified from the culture medium of bovine dental pulp as reported [9]. Two active forms of MMP-3 (M_r 28000 and 45000) were purified from the medium of rheumatoid synovial cells treated with rabbit macrophage-conditioned medium [1]. Both the inhibitor and MMP-3 were homogeneous according to SDS-polyacrylamide gel electrophoresis (SDS-

Correspondence address: Y. Okada, Department of Pathology,
School of Medicine, Kanazawa University, 13-1 Takara-machi,
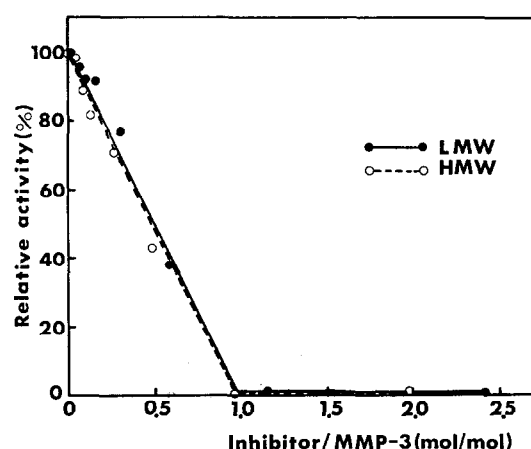Kanazawa 920, Japan

Fig.1. Inhibition of MMP-3 by the inhibitor from bovine dental pulp. A fixed amount of active low-M_r MMP-3 (M_r 28000) or high-M_r MMP-3 (M_r 45000) was incubated with inhibitor at various molar ratios and residual activity of MMP-3 then assayed at 37°C for 1.5 h using [^{3}H]Cm-Tf as substrate.
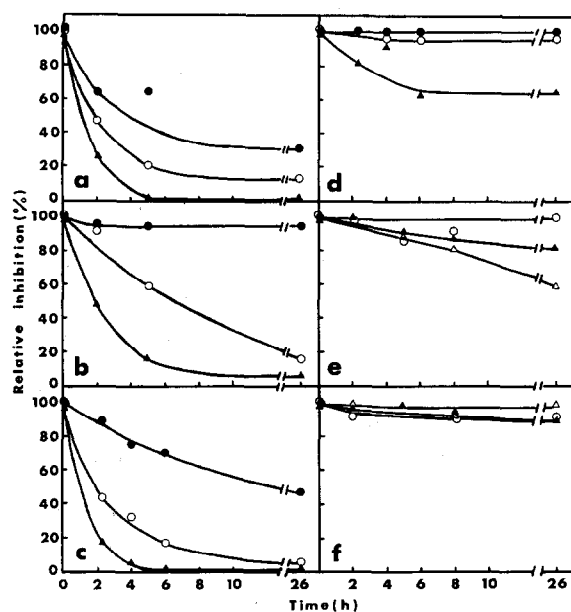

Fig.2. Effects of serine proteinases on TIMP activity against MMP-3. TIMP was incubated with (a) bovine trypsin, (b) bovine α-chymotrypsin, (c) human neutrophil elastase, (d) human neutrophil cathepsin G, (e) porcine pancreatic elastase, and (f) human plasmin at various concentrations (●, 1; ○, 10; ▲, 50; △, 100 μg/ml). After inactivating the serine proteinases, a 1.1 molar excess of MMP-3 was added to mixtures and residual enzymic activity of MMP-3 assayed. The inhibitory activity of TIMP obtained from samples incubated in the absence of serine proteinases was taken as 100% activity.
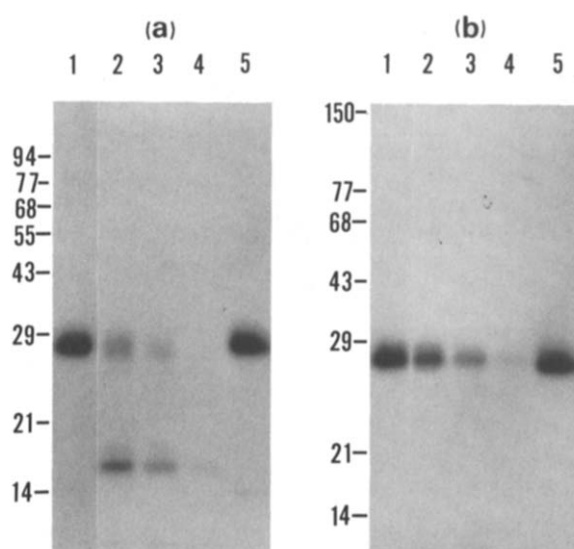

Fig.3. Degradation of TIMP by human neutrophil elastase. A mixture of unlabeled and ^{125}I-labeled TIMP was incubated with neutrophil elastase (10 μg/ml). Samples were subjected on SDS-PAGE (12.5% total acrylamide) in (a) the presence and (b) the absence of 2-mercaptoethanol and the gels autoradiographed. Lanes: 1,5, TIMP incubated without enzyme for 0 and 24 h; 2–4, TIMP treated with the enzyme for 4, 9 and 24 h, respectively. M_r values ($\times$ 10^{-3}) given to the left of the gels.
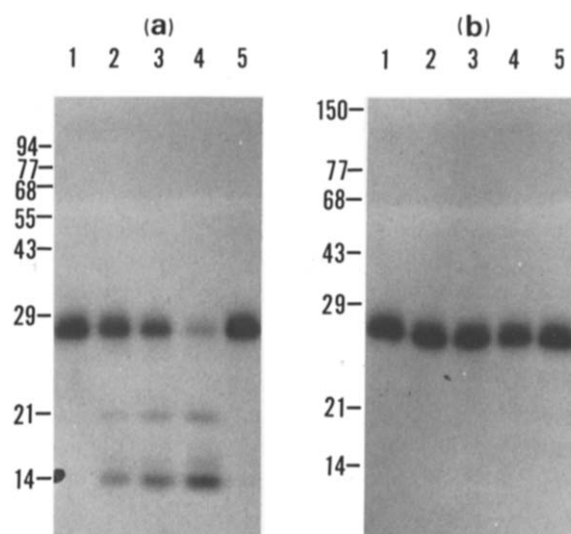

Fig.4. Digestion of TIMP by human neutrophil cathepsin G. A mixture of unlabeled and ^{125}I-labeled TIMP was treated with neutrophil cathepsin G (10 μg/ml) and analyzed by SDS-PAGE as described in the legend to fig.3. M_r values ($\times$ 10^{-3}) given to the left.

PAGE). MMP-1 free from MMP-2 and MMP-3 was obtained by removing each contaminating component by chromatography on affi-gel-gelatin and anti-(MMP-3) IgG-Sepharose, respectively. MMP-2 free from MMP-1 and MMP-3 was prepared using immunoadsorbent columns of anti-(MMP-1)IgG and anti-(MMP-3)IgG coupled to Sepharose. Bovine Tos-Phe-CH₂-Cl-treated trypsin, α-chymotrypsin, human plasminogen, streptokinase, bovine thrombin, porcine pancreatic elastase and diisopropyl fluorophosphate were obtained from Sigma. Plasma kallikrein was purified according to Nagase and Barrett [10]. Plasminogen was activated to plasmin by streptokinase. Human leukocyte elastase [11] and cathepsin G [12] were purified as described.

2.2. Inhibition of MMP-1, -2 and -3 activities by bovine dental pulp inhibitor

The activities of MMP-1–3 were assayed as in [1] using [¹⁴C]collagen, [¹⁴C]gelatin and [³H]carboxymethylated transferrin ([³H]Cm-Tf) as substrates for MMP-1, MMP-2 and MMP-3, respectively. The inhibitory activity of the bovine dental pulp inhibitor against metalloproteinases was determined by measuring the residual enzymic activity after incubating enzyme with inhibitor for 1 h at 23°C.

2.3. Degradation of TIMP by serine proteinases

Inactivation of TIMP by serine proteinases was investigated as follows: TIMP (68 ng) in 10 μl of 50 mM Tris-HCl, pH 7.6, 0.15 M NaCl, 10 mM Ca²⁺, 0.02% NaN₃ was initially reacted with an equal volume of trypsin (1, 10, 50 μg/ml), α-chymotrypsin (1, 10, 50 μg/ml), human neutrophil elastase (1, 10, 50 μg/ml), human neutrophil cathepsin G (1, 10, 50 μg/ml), porcine pancreatic elastase (10, 50, 100 μg/ml), human plasma plasmin (10, 50, 100 μg/ml), human plasma kallikrein (1, 10 μg/ml) or bovine thrombin (10, 50, 100 μg/ml) at 37°C for 2–26 h. After blocking the activity of the serine proteinases with 3.3 mM diisopropyl fluorophosphate, a 1.1 molar excess of MMP-3 (M_r 28000, 75 ng) was added to the mixtures and incubation performed for 1 h at 23°C. The residual enzymic activity of MMP-3 was determined by incubation with [³H]Cm-Tf for 1.5 h at 37°C.

2.4. Electrophoretic analyses of M_r changes of TIMP

TIMP (11 μg) was radioiodinated according to Fraker and Speck [13]. A mixture containing unlabeled TIMP (60 ng) and ¹²⁵I-labeled inhibitor (8 ng) was treated with trypsin (1, 10 μg/ml), α-chymotrypsin (1, 10 μg/ml), human neutrophil elastase (1, 10, 50 μg/ml), human neutrophil cathepsin G (10, 50, 100 μg/ml), porcine pancreatic elastase (10, 50, 100 μg/ml), human plasma plasmin (10, 50, 100 μg/ml), human plasma kallikrein (1, 10, 20 μg/ml) or bovine thrombin (10, 50, 100 μg/ml) at 37°C for 4–24 h. After the incubation, the serine proteinases were inactivated using 4 mM diisopropyl fluorophosphate. Proteins in the samples were resolved by SDS-PAGE using 12.5% polyacrylamide gels with or without reduction with 2-mercaptoethanol. Gels were dried and autoradiographed.

3. RESULTS AND DISCUSSION

The inhibitor from bovine dental pulp showed dose-dependent inhibition of MMP-1 and MMP-2 from human rheumatoid synovial cells (not shown). Fig.1 illustrates the 1:1 molar stoichiometric inhibition of two active forms of MMP-3. The same 1:1 ratio of stoichiometric inhibition has been reported for collagenase and TIMP purified from culture medium of rabbit bone [7] and human skin fibroblasts [14]. The bovine dental pulp inhibitor used here was considered to be TIMP according to the following criteria: (i) it is a sialoglycoprotein with M_r 28000 and is heat-stable, but is destroyed by reduction and alkylation [9,15]; (ii) it inhibits MMP-1–3 from human rheumatoid synovial cells (this study) as well as various other animal collagenases [16]; (iii) monoclonal antibodies raised against the inhibitor show cross-reactivity to TIMP purified from human gingival fibroblasts [17]; and (iv) it contains six disulfide linkages [9] and the sequence of 45 amino acids at the NH₂-terminus (unpublished) is 91% identical to that of human TIMP deduced from its cDNA [18].

The inhibitory activity of TIMP against MMP-3 was almost completely abolished by reacting the inhibitor with relatively low concentrations of trypsin, α-chymotrypsin and neutrophil elastase (fig.2). Cathepsin G and pancreatic elastase showed some degradative effects on the inhibitor but higher concentrations were required for partial inactivation (fig.2). However, plasmin (fig.2), plasma kallikrein and thrombin had no effect even at 100 μg/ml (not shown).

TIMP treated with human neutrophil elastase (10 μg/ml) was converted into forms with M_r 17000 and 16000. These were further degraded into small fragments on prolonged incubation (fig.3). However, under non-reducing conditions, the polypeptides of M_r 17000 and 16000 were not detected, but there was a time-dependent disappearance of the band corresponding to native TIMP (fig.3). These results indicate that the polypeptides of M_r 17000 and 16000 correspond to disulfide-linked fragments of nicked TIMP of M_r 28000; after prolonged incubation the inhibitor was degraded into smaller inactive fragments. Similar findings were made with trypsin and α-chymotrypsin except that the two fragments generated were of M_r 18000 and 14000.

Human neutrophil cathepsin G (fig.4), porcine pancreatic elastase and human plasma plasmin

also processed TIMP and produced two major polypeptides of M_r 18000–19000 and 13000–14000 (not shown). However, the unreduced samples showed no evidence of the disappearance of TIMP of M_r 28000 (fig.4) and the decrease in inhibitory activity was minimal (fig.2). These observations suggest that in some cases TIMP retains its inhibitory activity even after limited proteolysis if the processed polypeptides remain linked via disulfide bonds, presumably in such a way as to retain the correct conformation of the inhibitor molecule.

It has been reported that the inhibitory activity of TIMP isolated from culture media of bone explants and fibroblasts [7,19] or from extracts of articular cartilage [20] is sensitive to trypsin. The present study has shown for the first time that human neutrophil elastase and α-chymotrypsin can also abolish the metalloproteinase inhibitory capacity of TIMP. It is clear from our study that these serine proteinases inactivate TIMP by achieving its proteolytic degradation. Similar proteolytic inactivation has also been reported for plasma serine proteinase inhibitors, for example, α_1-proteinase inhibitor by *Pseudomonas aeruginosa* elastase [21] and antithrombin by neutrophil elastase [22].

Matrix metalloproteinases are able to digest most extracellular matrix components including collagen types I, II, III, IV, V and X, proteoglycans, laminin and fibronectin [1–6,23]. We have observed, using indirect immunofluorescence, that MMP-1 and MMP-3 are synthesized and secreted by hyperplastic synovial lining (intima) cells of human rheumatoid synovium (in preparation). It is envisaged that for activated metalloproteinases to carry out a destructive function in rheumatoid joints, they must be present in sufficient quantities to exhaust the inhibitory activity of TIMP present in articular cartilage [20] and secreted from synovial cells [24]. Our present results suggest that neutrophils which have penetrated the joint space during the inflammatory stage of rheumatoid arthritis [25] may indirectly participate in joint destruction. The potentiation of metalloproteinase activity through inactivation of TIMP, as proposed here, does not preclude the possibility of direct proteolysis of extracellular matrix components by elastase and other neutrophil enzymes.

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