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IN VIVO ACTIVITY OF HUMAN RECOMBINANT TISSUE INHIBITOR OF METALLOPROTEINASES (TIMP)

ACTIVITY AGAINST HUMAN STROMELYSIN *IN VITRO* AND IN THE RAT PLEURAL CAVITY

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Abstract—Recombinant human tissue inhibitor of metalloproteinase (rhTIMP) suppressed the ability of native human stromelysin to degrade [^{3}H]transferrin *in vitro*. Maximum inhibition occurred at molar ratios (TIMP:stromelysin) of 2:1 and 1:1. Reduced and alkylated tissue inhibitor of metalloproteinases (TIMP) lost its ability to suppress stromelysin activity. rhTIMP also inhibited stromelysin from degrading proteoglycan monomer *in vitro*. When injected into the rat pleural cavity prior to stromelysin, rhTIMP inhibited the ability of the enzyme to degrade aggregating cartilage proteoglycan monomer. Marked inhibition of stromelysin-mediated proteoglycan degradation *in vivo* occurred at molar ratios (TIMP:enzyme) of 2:1 and 1:1, with less inhibition at molar ratios of 0.5:1 and 0.25:1. Reduction and alkylation prevented rhTIMP from suppressing stromelysin-mediated degradation of proteoglycan monomer *in vivo*. By comparison, an equimolar concentration of the serine proteinase inhibitor, α_1-proteinase inhibitor (α_1-PI), did not inhibit stromelysin activity in the rat pleural cavity. This study demonstrates that rhTIMP is effective in inhibiting native human stromelysin both *in vitro* and *in vivo*.

Tissue inhibitor of metalloproteinases (TIMP†), or a closely related molecule, is produced both *in vitro* and *in vivo* by many cells and tissues including fibroblasts, cartilage, bone, synovial cells, endothelial cells, tumor cells, and alveolar macrophages [1–12]. A TIMP-like molecule has been purified from human skin fibroblast cultures as well as from cartilage, amniotic fluid, calvaria, and human rheumatoid synovial fluid [1–3, 5, 13, 14]. cDNA clones for TIMP have been established recently from human fibroblast libraries [15, 16] and the protein has been expressed in large quantities in C127 cells [15]. Because of its ubiquity in extravascular tissues, TIMP has been proposed as the major inhibitor of the family of extracellular matrix-degrading metalloproteinases including collagenase (EC 3.4.24), stromelysin, and gelatinase [17–19].

A proteoglycan-degrading metalloproteinase, presumably stromelysin, has been reported to be elevated in articular cartilage from patients with osteoarthritis [20]. In addition, gelatinase and at least two other metalloproteinases have been found in either the cytosol [21] or plasma membrane [22] of transformed cells. Thus, these metalloproteinases may be important in homeostatic extracellular matrix turnover as well as in the pathogenesis of rheumatoid arthritis and osteoarthritis [18, 23] and possibly in metastasis [24–26]. Since TIMP modulates the activity of the metalloproteinases, it is likely to influence the pathology of these diseases.

rhTIMP has been shown to inhibit the degradation of collagen films by 1,25-dihydroxyvitamin D-treated mouse osteoblasts [27] and by chondrocytes and endothelial cells stimulated *in vitro* with crude IL-1 [28]. Presumably, when stimulated, these cells synthesize collagenase which is inhibited by the exogenous addition of TIMP. In addition, TIMP has been shown to inhibit migration of invasive tumor cells through amnionic basement membranes and the underlying connective tissue stroma presumably by inhibiting type IV collagenase [24]. Therefore, it is evident that TIMP can inhibit metalloproteinases synthesized in cell culture systems. There is little evidence that recombinant TIMP inhibits metalloproteinases *in vivo*. However, a recent study by Carmichael *et al.* [29] indicated that recombinant TIMP given systemically is efficacious in the treatment of type II collagen-induced arthritis in mice. It is important to demonstrate that TIMP has *in vivo* activity because of its possible therapeutic utility in inflammation and metastasis as well as information that could be derived for developing synthetic inhibitors of these metalloenzymes.

The purpose of the current study was to assess the activity of rhTIMP against transferrin and purified native stromelysin *in vitro* as well as *in vivo* at an extravascular site using proteoglycan monomer as a substrate. The rat pleural cavity is well-suited for pharmacokinetic and *in vivo* biochemical studies because it is easily accessible, and fluids can be

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† Abbreviations: TIMP, tissue inhibitor of metalloproteinases; rhTIMP, recombinant human TIMP; α_1-PI, α_1-proteinase inhibitor; APMA, *p*-aminophenylmercuric acetate; DMMB, dimethylmethylene blue; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, 50 mM phosphate-buffered saline, pH 7.0; SBTI-agarose, soybean trypsin inhibitor-agarose; TCA, trichloroacetic acid; and IL-1, interleukin-1.

quantitatively recovered from it. In addition, numerous synthetic inhibitors can be tested in this model prior to further testing in disease models. The results demonstrate that rhTIMP is effective in inhibiting stromelysin activity both *in vitro* and *in vivo* in the rat pleural cavity. In addition, rhTIMP was most effective at molar ratios near 1:1 and greater, and its activity against stromelysin was abolished by reduction and alkylation.

METHODS

Materials. Reagents were purchased from the following sources: recombinant human TIMP from Celltech, Inc. (Slough, U.K.); Sepharose CL-2B, DEAE-Sephadex, and CM-Cellulose from Pharmacia Inc. (Piscataway, NJ); Green-A Dyematrix from Amicon Division, W. R. Grace & Co. (Danvers, MA); bovine nasal septum from Pel-Freeze (Rogers, AR); transferrin, cesium chloride, guanidine hydrochloride, SBTI-agarose and α_1-PI from the Sigma Chemical Co. (St. Louis, MO); dimethylmethylene blue from Accurate Chemical (Westbury, NY); and trypsin from Fluka, Inc. (Ronkonkoma, NY).

Enzyme. Native stromelysin was isolated from cultures of IL-1-stimulated human gingival fibroblasts [30]. Briefly, prostromelysin was purified from the conditioned medium of these cells using a combination of DEAE-Sephadex, Green-A Dyematrix and CM-Cellulose chromatography. The final enzyme was in 25 mM Tris–HCl, 0.7 M NaCl, 10 mM $CaCl_2$, 0.05% Brij, 0.02% NaN_3, pH 7.5, and was activated upon incubation with trypsin. Trypsin activity was destroyed by incubation with a 100-fold molar excess of SBTI-agarose followed by centrifugation for 5 min at 10,000 *g*. Active enzyme in the supernatant fraction was used for both *in vivo* and *in vitro* experiments. A gel of purified stromelysin is shown in Fig. 1.

Substrates. Human transferrin was reductively methylated as previously described by Okada *et al.* [31]. Briefly, 200 mg of transferrin was treated with 20 mM dithiothreitol and reacted with 1 mCi [^{3}H]iodoacetic acid. The radiolabeled transferrin was separated from unincorporated radioactivity by TCA precipitation in 10% ice-cold TCA. The final specific activity of the transferrin was 2.2×10^5 cpm/nmol.

Proteoglycan monomer was isolated from bovine nasal septum cartilage as described by Hascall and Kimura [32]. Frozen cartilage was minced and extracted with 4 M guanidine hydrochloride in proteinase inhibitor buffer (0.05 M sodium acetate, 0.1 M 6-aminohexanoic acid, 0.01 M EDTA, 0.001 M benzamidine hydrochloride, 0.001 M phenylmethylsulfonyl fluoride, 0.01 M *N*-ethylmaleimide, pH 6.0) for 48 hr at 4°. The extract was brought to an initial starting density of 1.47 g/mL with cesium chloride and centrifuged for 68 hr at 35,000 *g* in a 50.2 Ti rotor. The bottom fourth of the gradient was pooled as the D1 fraction, dialyzed against water and lyophilized. For all experiments, the proteoglycan was resuspended in 25 mM Tris–HCl, 10 mM $CaCl_2$, pH 7.4.

TIMP. Recombinant human TIMP was prepared by Celltech as described by Docherty *et al.* [15]. The protein is glycosylated and migrates as a heterogeneous band on reducing SDS–PAGE gels with an average molecular weight of 30 kD. Reduced and alkylated TIMP was prepared by treatment with 2 mM dithiothreitol and 5 mM iodoacetamide as described by Dean and Woessner [3].

SDS–PAGE. Samples were electrophoresed on a 12% SDS-polyacrylamide gel under conditions described by Laemmli [33]. Gels were stained with silver nitrate and photographed on Type 51 Polaroid film.

Proteoglycan aggregation and gel-filtration chromatography. After incubation in the rat pleural cavity for various times, pleural cavity fluid containing the exogenous proteoglycan and stromelysin was brought to 10 mM with 1,10-phenanthroline for 1 hr at 4°. The proteoglycan was then mixed with 1% hyaluronic acid (w/w) for 3 hr at 4° to induce aggregation. The aggregated material was chromatographed on a Sepharose CL-2B (0.7×100 cm) column eluted with 0.05 M sodium acetate, 0.02% NaN_3, pH 5.8, at a flow rate of 8 mL/hr. Column fractions were assayed using the DMMB assay as previously described [34]. Aggregation of proteoglycan that was digested *in vitro* with stromelysin was also assessed by this method.

Injection into the rat pleural cavity. Female CF-1 rats, 125–150 g, 7–8 weeks of age, were purchased from Taconic Farms, Germantown, NY. They were fed standard autoclavable laboratory chow. They were injected with 0.5 mL of proteoglycan monomer (6 mg) with or without various concentrations of rhTIMP in PBS, immediately followed by 0.5 mL (75 μg) of activated stromelysin (molar ratio of proteoglycan monomer:stromelysin of 2:1). Protein concentrations were determined based on absorbance at 280 nm assuming an extinction coefficient of 1.0 and a molecular weight of 45 kD for stromelysin and 28 kD for TIMP. Pleural cavity fluid was retrieved (six rats per time point) after injection with 2.0 mL PBS, and proteoglycan was quantitated using the DMMB assay. Proteoglycan was mixed with hyaluronic acid as described above, and aggregation was examined by chromatography on Sepharose CL-2B columns.

In all cases, stromelysin activity with or without rhTIMP was examined using [^{3}H]transferrin as a substrate. Five micrograms of trypsin-activated stromelysin (109 pmol) was incubated with various concentrations of rhTIMP for 2 hr at 37° in a total reaction volume of 170 μL in 25 mM Tris–HCl, 10 mM $CaCl_2$, 0.05% Brij, 0.02% NaN_3, pH 7.4. Fifty microliters of the reaction mixture was brought to 100 μL with the above buffer, and 15 μg of radiolabeled transferrin was added to each tube and incubated for 1 hr at 37° (molar ratio of substrate:enzyme of 5.7:1). To determine the amount of degradation, samples were brought to 10% TCA for 1 hr at 4° and centrifuged at 10,000 *g* for 5 min. The amount of TCA-soluble radioactivity was determined by counting in a Packard Tri-Carb liquid scintillation counter.

RESULTS

Inhibition of transferrin degradation by rhTIMP. Prostromelysin had negligible activity against radio-

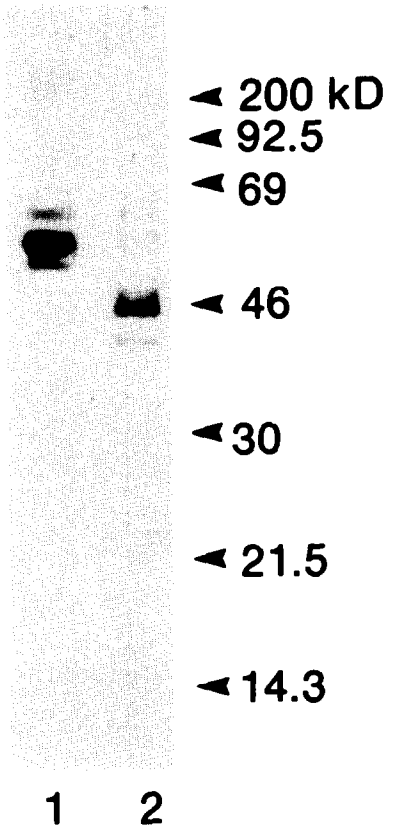

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of prostromelysin and trypsin-activated stromelysin. Both prostromelysin (lane 1) and trypsin-activated stromelysin (lane 2) were electrophoresed through a 12% Laemmli SDS–PAGE and stained with silver nitrate. Positions of molecular weight markers are noted.

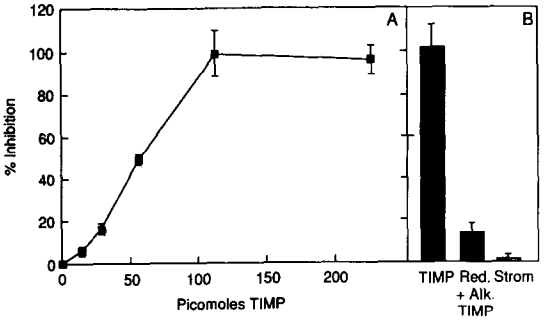

Fig. 2. Inhibition of stromelysin-mediated degradation of transferrin by rhTIMP. (A) Trypsin-activated stromelysin (109 pmol) was incubated with rhTIMP prior to incubation with [^{3}H]transferrin as described in Methods. TCA-soluble radioactivity was determined upon precipitation with 10% TCA. Data are reported as percent inhibition of stromelysin-mediated degradation of transferrin. Incubation of the enzyme with transferrin, without the addition of TIMP, resulted in generation of 9068 ± 608 cpm of radioactivity with an assay background of 605 ± 8 cpm. (B) rhTIMP was treated with dithiothreitol and iodoacetamide as described in Methods. Either untreated (TIMP) or reduced and alkylated TIMP (Red. & Alk. TIMP) at a molar ratio (TIMP : enzyme) of 1 : 1 was incubated with stromelysin and assayed in the transferrin assay. Stromelysin (Strom) alone is also shown.

labeled transferrin. However, when the proenzyme was activated with trypsin, there was significant degradation of transferrin. This activity could not be attributed to residual trypsin since the activated proteinase was treated with SBTI-agarose and a control of SBTI-agarose-treated trypsin had no transferrin-degrading activity (data not shown). Gel electrophoresis indicated that the proenzyme had a molecular weight of 55 and 57 kD and the active form shifted to a molecular weight of 44 and 46 kD (Fig. 1).

To determine if activated stromelysin was inhibited by rhTIMP, the enzyme was mixed with rhTIMP at molar ratios (inhibitor : enzyme) of 2 : 1 to 0.12 : 1 and assayed in the transferrin assay (Fig. 2A). The results indicated that rhTIMP completely inhibited degradation of transferrin at ratios of 1 : 1 and greater. At lower ratios, only partial inhibition of transferrin degradation was observed. When rhTIMP was treated with the reducing agent, dithiothreitol, followed by alkylation with iodoacetamide under conditions previously reported to destroy TIMP activity [3], it lost its ability to inhibit stromelysin-mediated transferrin degradation (Fig. 2B). Together, these data indicate that rhTIMP can inhibit stromelysin-mediated degradation of a large molecular weight substrate and that this effect can be eliminated by reduction and alkylation of TIMP.

Stromelysin-mediated proteoglycan degradation in vitro. When incubated with bovine nasal septum proteoglycan monomer at a molar ratio (substrate : enzyme) of 2 : 1, stromelysin reduced the aggregation of proteoglycan monomer with hyaluronic acid (Fig. 3). During the first 30 min, a portion of the monomer lost its ability to aggregate with hyaluronic acid without significantly altering its molecular size (Fig. 3B). Presumably, this was the result of cleavage of the hyaluronic acid binding region from the intact monomer. By 24 hr (Fig. 3E), however, there was a substantial shift in the molecular mass of this monomer to approximately one-half its original size. Therefore, there appears to be at least two sites of cleavage of the monomer, an initial site of cleavage near the hyaluronic acid binding region and a second site in the middle of the chondroitin sulfate-rich region. Similar observations have been reported by other laboratories [35, 36]. Control samples, using SBTI-inhibited trypsin, resulted in no shift on the gel-filtration columns (Fig. 3F). Therefore, degradation of monomer was not due to residual trypsin activity. Degradation of proteoglycan monomer was inhibited when stromelysin was incubated with rhTIMP at a molar ratio (TIMP : enzyme) of 1 : 1 (Fig. 3G) as shown by complete aggregation of the monomer in the presence of hyaluronic acid. Therefore, rhTIMP can inhibit the degradation of proteoglycan as well as transferrin *in vitro* at approximate stoichiometric concentrations.

Clearance of proteoglycan monomer and stromelysin-digested degradation products from the rat pleural cavity. The rat pleural cavity was chosen for

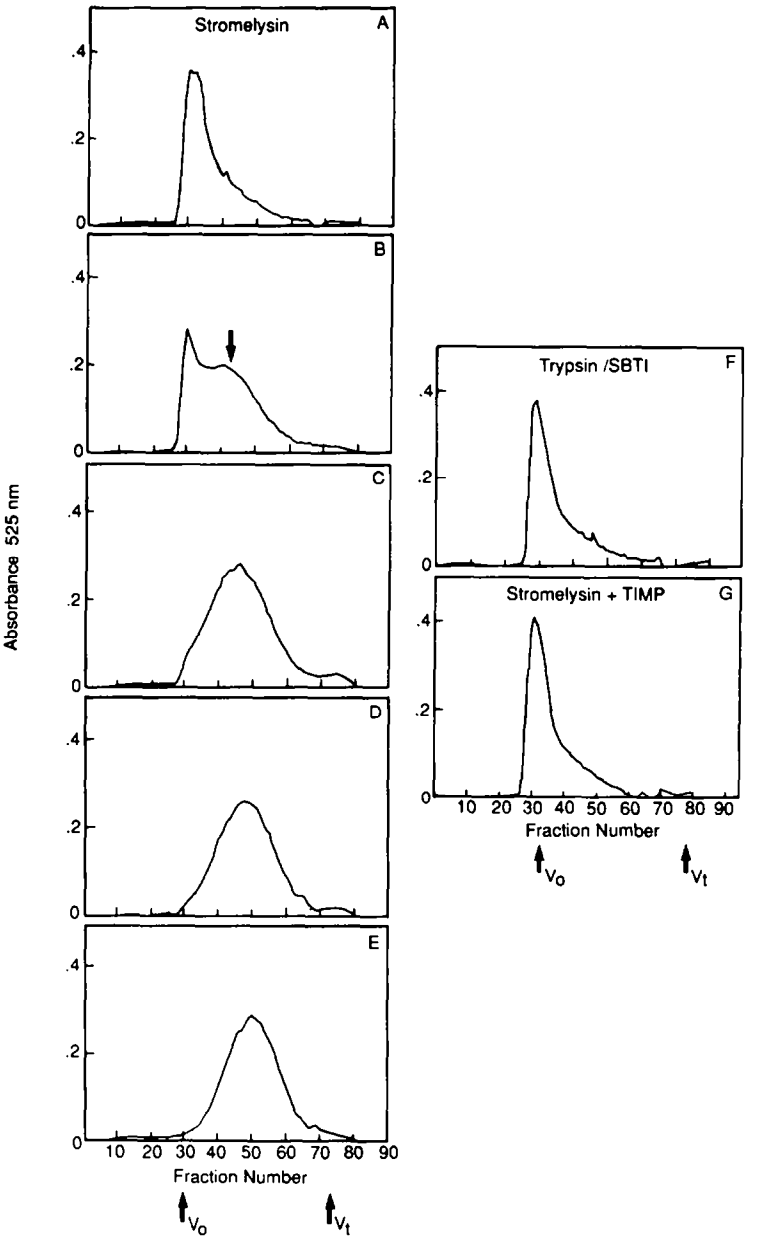

Fig. 3. Gel filtration chromatography of stromelysin-degraded proteoglycan monomer aggregated with hyaluronic acid. Bovine nasal septum proteoglycan monomer was isolated by isopycnic $CsCl_2$ density centrifugation. This monomer was incubated with trypsin-activated stromelysin at a molar ratio (substrate:enzyme) of 2:1 for (A) 0, (B) 0.5, (C) 3, (D) 6, or (E) 24 hr. At each time, the sample was brought to 10 mM with 1,10-phenanthroline. (F) A sample of monomer was incubated with SBTI-agarose-treated trypsin under the conditions used to activate the enzyme to assure that the degradation observed in A–E was due to stromelysin and not to residual trypsin activity. (G) Stromelysin was incubated with TIMP at a molar ratio (TIMP:enzyme) of 1:1, and then incubated with monomer for 3 hr. After incubations, all samples were aggregated with hyaluronic acid and chromatographed on a Sepharose CL-2B column eluted with 50 mM sodium acetate, 0.02% NaN_3, pH 5.8. The void volume (V_0) and total volume (V_t) of the column were determined using blue dextran 2000 and phenol red respectively. The arrow in (B) represents the elution position of non-aggregated monomer.

in vivo studies as a convenient extravascular site from which fluid and cells can be quantitatively recovered. To evaluate whether there was selected loss of intact monomer or stromelysin-mediated breakdown products, we performed clearance studies from the pleural cavity of proteoglycan monomer and stromelysin-digested proteoglycan monomer (24 hr, see Fig. 3E). Clearance half-times of these molecules from the pleural cavity were 3.4 and 4.0 hr, respectively, and are shown in Fig. 4. These results indicate that there would not be selective clearance of stromelysin-mediated degradation products and

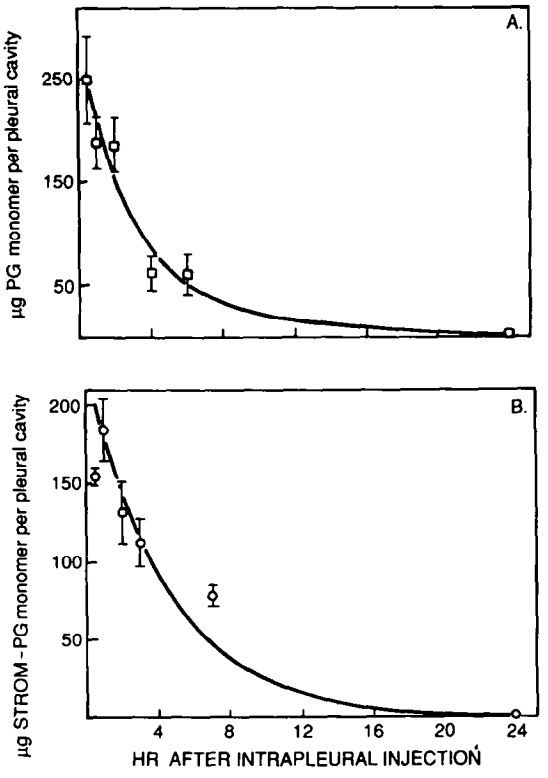

Fig. 4. Clearance of proteoglycan and stromelysin-degraded proteoglycan from the rat pleural cavity. (A) Bovine nasal septum proteoglycan monomer, or (B) stromelysin-degraded monomer was injected into the rat pleural cavity. Over time, the pleural cavities were washed with 2.0 mL of PBS, and the amount of sulfated glycosaminoglycan was quantitated using the DMMB assay. Data were plotted using an HP-45 four parameter curve-fitting program. Each point is the arithmetic mean and SEM of six rats.

that comparisons of the degradation products over 3 hr from the pleural cavity would be valid.

Effects of stromelysin and rhTIMP in the rat pleural cavity. Since intact and digested proteoglycan had similar clearance half-times, digestion of proteoglycan monomer by stromelysin was evaluated *in vivo*. Proteoglycan monomer was injected into the pleural cavity followed immediately by the injection of stromelysin or buffer. Pleural cavity fluids were removed at 0.5, 1, 2 and 3 hr, quantitated by the DMMB method, incubated overnight with 1% hyaluronic acid, and chromatographed on a Sepharose CL-2B column. In all cases, stromelysin caused significant loss in the ability of the proteoglycan to aggregate with hyaluronic acid compared to controls not treated with stromelysin. The 3-hr control and enzyme-treated samples are shown in Fig. 5, panels A and B, respectively.

To determinate if the natural inhibitor of stromelysin, TIMP, could inhibit stromelysin *in vivo*, rhTIMP and proteoglycan monomer were pre-injected into the pleural cavity. Stromelysin was then injected at a final molar ratio (TIMP:enzyme) of 2:1 to 0.25:1. For convenience, the 3-hr point was chosen for these experiments. After 3 hr, the pleural cavity fluid was removed, quantitated by the DMMB method, incubated overnight with 1% hyaluronic acid (w/w), and chromatographed. These data indicate that proteoglycan degradation was inhibited markedly at molar ratios (TIMP:enzyme) of both 1:1 (Fig. 5D) and 2:1 (Fig. 5C). At lower ratios (Fig. 5, E and F), proteoglycan degradation was only partially inhibited. This is consistent with results from the *in vitro* transferrin and proteoglycan degradation assays (Figs. 2 and 3). Therefore, rhTIMP is active against stromelysin *in vitro* and *in vivo* at similar stoichiometric concentrations.

To ensure that the observed effect of rhTIMP was due to activity against stromelysin and not an artifact of injecting a second protein into the pleural cavity, two control experiments were performed. First, rhTIMP was reduced and alkylated as in Fig. 2 and then injected into the pleural cavity. Under these conditions, rhTIMP had no effect at a molar ratio (TIMP:stromelysin) of 1:1 (Fig. 6D), whereas the untreated rhTIMP at the same concentration inhibited proteoglycan degradation (Fig. 6C). In addition, α_1-PI, a serine proteinase inhibitor, was injected at an equimolar ratio and had no effect on stromelysin-mediated degradation of proteoglycan monomer (Fig. 6E). Therefore, the effect of the rhTIMP on proteoglycan degradation *in vivo* is apparently due to its biological activity against stromelysin.

DISCUSSION

In the current study, we have shown that rhTIMP can inhibit stromelysin-mediated degradation of transferrin and proteoglycan monomer *in vitro* and of proteoglycan monomer in the rat pleural cavity. Inhibition was observed at approximate stoichiometric concentrations of enzyme to inhibitor. Stromelysin activity *in vivo* was not inhibited by α_1-proteinase inhibitor. In addition, the biological activity of rhTIMP appeared to be dependent on its tertiary structure, presumably disulfide bonds, as reduction and alkylation abolished its inhibitory activity to stromelysin. Dean and Woessner [3] demonstrated that the inhibitory activity of TIMP to collagenase is abolished by reduction and alkylation.

In a number of cell and organ culture systems as well as in *in vitro* using purified enzymes [4, 6, 8–10, 13, 17, 27, 28, 37, 38], TIMP has been shown to have activity against both collagenase and stromelysin. However, there is a paucity of information on the *in vivo* activity of exogenously-administered TIMP. In the current report, we have shown that TIMP inhibits stromelysin-mediated proteoglycan degradation when injected into the rat pleural cavity, indicating that TIMP can maintain its biological activity at an extravascular site. In other studies from our laboratory,* recombinant human TIMP prevented human

* McDonnell J, Lark MW, Saphos CA and Moore VL, Human stromelysin acts as a proteoglycanase when injected into rabbit joints. Inhibition of stomelysin activity *in vivo* by recombinant human tissue inhibitor of metalloproteinases (TIMP), manuscript in preparation.

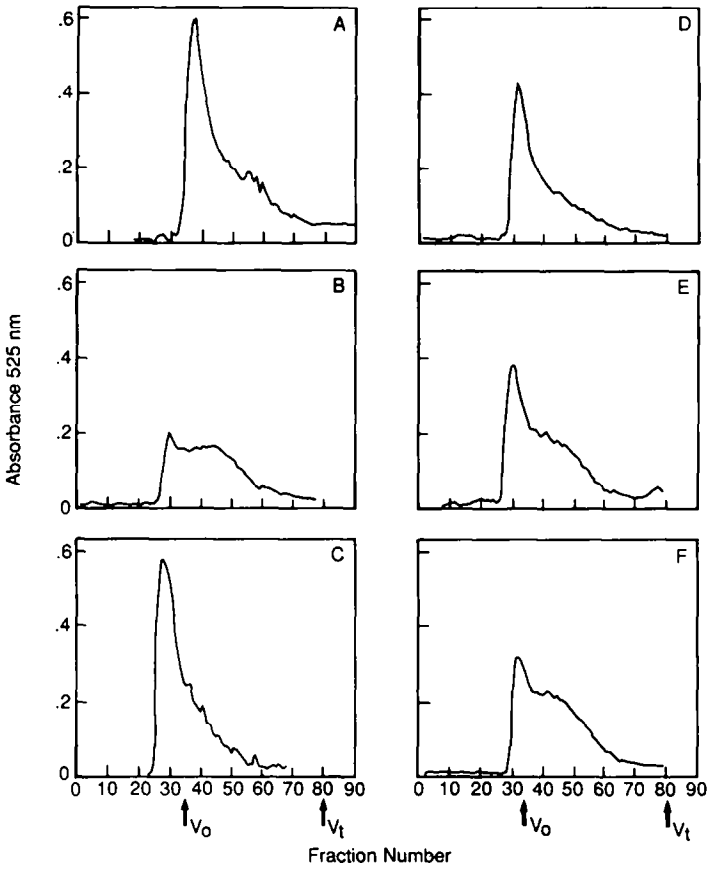

Fig. 5. Stromelysin-mediated proteoglycan degradation in the rat pleural cavity. Proteoglycan monomer was injected into the rat pleural cavity with rhTIMP followed by activated stromelysin at a final molar ratio (substrate:enzyme) of 2:1. The final molar ratio of TIMP:enzyme was (C) 2:1, (D) 1:1, (E) 0.5:1, (F) 0.25:1. (A) Proteoglycan alone or (B) proteoglycan and enzyme without added TIMP were also analyzed. After 3 hr, pleural cavity fluids were removed, quantitated by the DMMB assay, aggregated with hyaluronic acid, and chromatographed on a Sepharose CL-2B column as described in the legend to Fig. 3. Six rats per concentration were analyzed, and representative chromatographs are shown. The V_0 and V_t were determined as described in the legend to Fig. 3.

stromelysin from functioning as a proteoglycanase when both were injected into rabbit joints. This suggests that data obtained in the current study in the pleural cavity can be extrapolated to other extravascular sites, including the synovial cavity.

Although no studies have demonstrated that an exogenous source of TIMP can inhibit metalloproteinase activity in synovium or cartilage, there are other studies which suggest that this molecule is an effective inhibitor of metalloproteinases in these sites. TIMP has been purified from human articular cartilage [3], bovine articular cartilage [8], and from bovine nasal septum cartilage [38]. In addition, McGuire *et al.* [6] reported that normal, rheumatoid and osteoarthritic synovium produce TIMP. However, whereas normal synovium displayed greater TIMP activity, rheumatoid synovium produced more collagenase activity. Somewhat similar data were obtained in studies on the Dumonde–Glynn model of proliferative arthritis [4, 7]. During the development of arthritis, there was an increase in collagenase and a decrease in TIMP production. Moreover, Carmichael *et al.* [29] have reported that recombinant human TIMP has efficacy in type II collagen-induced arthritis in mice. Based on these studies, these investigators suggested that the balance of production of metalloproteinase and TIMP is an important determinant of the degree of cartilage destruction in rheumatoid arthritis and osteoarthritis [7, 18].

There are reports that cartilage damage and loss in rheumatoid arthritis and osteoarthritis may be due to excess metalloproteinase activity [20, 23]. It is possible that stromelysin-like enzymes cleave cartilage proteoglycans near their hyaluronic acid binding region and destroy their ability to aggregate with hyaluronic acid in cartilage. It is known that proteoglycan monomers that cannot aggregate with hyaluronic acid are rapidly lost from cartilage [39]. IL-1 has been implicated as a major regulator of connective tissue breakdown in inflammatory diseases such as rheumatoid arthritis [36, 40–43] by regulating both enzyme and inhibitor levels. IL-1 or IL-1-like molecules from macrophages promote stromelysin transcription [44, 45] and translation into protein [46]. TIMP synthesis may also be affected by IL-1 [42] as well as other molecules such as transforming growth factor-β [47]. IL-1 has also been

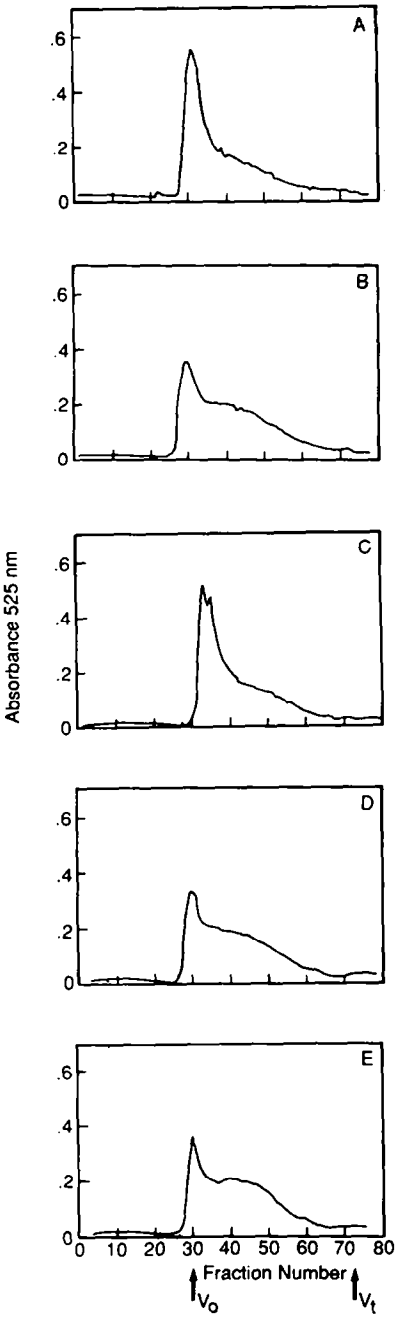

Fig. 6. Effect of reduction and alkylation on the inhibition of stromelysin by rhTIMP in the rat pleural cavity. rhTIMP was treated with dithiothreitol and iodoacetamide as described in Methods. The following combinations were injected into the rat pleural cavity as described in the legend to Fig. 5: (A) proteoglycan monomer, (B) proteoglycan monomer and stromelysin, (C) proteoglycan monomer, TIMP and stromelysin at a final molar ratio (TIMP:enzyme) of 1:1, (D) proteoglycan monomer, reduced and alkylated TIMP, and stromelysin at a final molar ratio (TIMP:enzyme) of 1:1, or (E) proteoglycan monomer, α_1-PI, and stromelysin at a final molar ratio (α_1-PI:stromelysin) of 1:1. The pleural cavity fluids were removed after 3 hr, sulfated proteoglycan was quantitated by the DMMB assay, and the fluids were aggregated with hyaluronic acid and chromatographed on a Sepharose CL-2B column as described in the legend to Fig. 5.

reported to inhibit proteoglycan synthesis, possibly by inhibiting core protein synthesis [36, 48, 49]. Thus, IL-1 may be responsible for cartilage degradation by at least three separate mechanisms: induction of metalloproteinase synthesis, inhibition of proteoglycan synthesis, and regulation of TIMP synthesis. IL-1 may play an important role in rheumatoid arthritis, especially since IL-1-like molecules have been demonstrated in synovial fluid from patients with rheumatoid arthritis [40].

In summary, the current study has shown that rhTIMP can inhibit stromelysin both *in vitro* and *in vivo* as assessed by the ability of TIMP to block stromelysin-mediated degradation of transferrin and proteoglycan. It is possible that TIMP could have some therapeutic benefit in rheumatoid arthritis and osteoarthritis. However, it is more likely that studies of the mechanism of action of TIMP in the inhibition of metalloproteinases could lead to the development of efficacious drugs for these diseases.

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