Inhibition of Autoproteolytic Activation of Interstitial Procollagenase by Recombinant Metalloproteinase Inhibitor MI/TIMP-2*

Yves A. DeClerck§, Tsuey-Dawn Yeant§, Hsieng S. Lu§, Jerry Ting¶, and Keith E. Langley¶

From the Division of Hematology/Oncology, Children’s Hospital of Los Angeles and the University of Southern California, Los Angeles, California 90027 and ¶Amgen, Inc., Thousand Oaks, California 91320

The purification and cloning of a novel metalloproteinase inhibitor (MI or TIMP-2) related to tissue inhibitor of metalloproteinases (TIMP) has been recently described by our laboratory (DeClerck, Y. A., Yean, T. D., Ratzkin, B. J., Lu, H. S., and Langley, K. E. (1989) J. Biol. Chem. 264, 17445–17453; Boone, T. C., Johnson, M. J., DeClerck, Y. A., and Langley, K. E. (1989) Proc. Natl. Acad. Sci. U. S. A. 87, 2800–2804). We have transfected Chinese hamster ovary cells with a vector containing human MI/TIMP-2 cDNA and purified recombinant-derived MI/TIMP-2 (rMI/rTIMP-2) from the conditioned medium of such cells. We have investigated the inhibitory activity of rMI/rTIMP-2 toward rabbit fibroblast interstitial collagenase. The inhibition of activated collagenase by rMI/rTIMP-2 is stoichiometric and consistent with the formation of a 1:1 molar ratio complex. In addition to blocking the activated enzyme, rMI/rTIMP-2 inhibits the conversion of 52-kDa procollagenase to the 42-kDa active enzyme initiated by organomercurials. When plasmin is used as activator, rMI/rTIMP-2 does not inhibit the plasmin-mediated conversion of the 52-kDa proenzyme to the 46-kDa inactive intermediate but blocks further conversion of the 46-kDa intermediate to the 42-kDa active enzyme. The data indicate that rMI/rTIMP-2 blocks the autoproteolytic activation of procollagenase. Also, rMI/rTIMP-2 forms complexes with the 52-kDa procollagenase, the 46-kDa intermediate, and with the 42-kDa activated enzyme which are stable to sodium dodecyl sulfate (SDS), such that the complexes can be visualized by SDS-polyacrylamide gel electrophoresis. It appears that the formation of a SDS-stable complex with procollagenase requires an initial conformational change of the procollagenase brought about by organomercurials or by plasmin cleavage. The data suggest that MI/TIMP-2 may be able to control the extracellular action of certain metalloproteinases not only at the level of the activated enzyme but also at the level of proenzyme activation.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases with proteolytic activities toward several components of the extracellular matrix (1). Major members of this family are interstitial collagenase (MMP-1), 72-kDa type IV collagenase (MMP-2, 72-kDa gelatinase), stromelysin (MMP-3, rat transin, proteoglycanase), and 92-kDa type IV collagenase (92-kDa gelatinase). These proteases have activities specific for various components of the extracellular matrix. Interstitial collagenase degrades types I, II, and III collagens, 72-kDa gelatinase degrades type IV collagen, stromelysin degrades laminin, fibronectin, and proteoglycans, and 92-kDa gelatinase degrades types IV and V collagens. These enzymes, whose cDNA sequences have been recently described (2–5), have several structural and functional properties in common. They share a similar amino-terminal region, a common zinc binding domain, and a hemopexin-like domain near the carboxy terminus. In comparison with interstitial collagenase and stromelysin, 72- and 92-kDa gelatinases have an additional fibronectin-like collagen binding domain, and 92-kDa gelatinase has a unique α3(V) collagen-like domain. Secretion in an inactive precursor form is also an important feature of the MMPs that regulates their activity in the extracellular milieu.

Activation of interstitial procollagenase can be accomplished by several processes, which all lead to the conversion of a 52-kDa precursor enzyme to a 42-kDa active enzyme (6). Organomercurials activate the proenzyme in vitro by inducing a conformational change involving disruption of the cysteine-zinc interaction and freeing the zinc to participate in an intramolecular autoproteolytic reaction. This reaction removes the amino-terminal "pro" segment, permanently converting the enzyme to the active form. Stromelysin can then "superactivate" interstitial collagenase by proteolytic cleavage near the carboxy terminus resulting in a 49-kDa enzyme with 5–8-fold higher activity (7). Trypsin and plasmin can also activate the proenzyme. These serine proteases cleave the peptide bond between Arg6 and Asn6, generating a 46-kDa inactive intermediate. This intermediate is further converted to the 42-kDa active enzyme by an intramolecular autoproteolytic reaction that cleaves one of three peptide bonds around residues 99–102 (6). Therefore, a cascade mechanism involving plasminogen activators and plasmin has been proposed for the activation of procollagenase in vivo, and inhibitors of plasminogen activator have been suggested to play a role in the control of the activation process (8, 9).

Further regulation of the activity of metalloproteinases in the extracellular milieu is achieved by specific inhibitors*.

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§ To whom correspondence should be addressed: Division of Hematology/Oncology, Children’s Hospital of Los Angeles, 4650 Sunset Blvd., Los Angeles, CA 90027. Tel.: 213-669-2207; Fax: 213-664-9455.

¶ The abbreviations used are: MMP, matrix metalloproteinase; MI, metalloproteinase inhibitor; TIMP, tissue inhibitor of metalloproteinases; pAPA, p-aminophenylmercuric acetate; SDS, sodium dodecyl sulfate; DMEM, Dulbecco’s modified Eagle’s medium; r, recombinant; CHO, Chinese hamster ovary.

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Recombinant Expression of MI/TIMP-2 by Chinese Hamster Ovary Cells—The construction of the expression plasmid is illustrated in Fig. 1. Briefly, the EcoRI/NcoI fragment of pUCHMI (16) containing the intact coding sequence of human MI was first subcloned into the multiple cloning site of pCFM1156 (20) to give plasmid pDSRa2MI. The MI cDNA was retrieved from plasmid pCFM1156 (20) to give plasmid pDSRa2MI.

In our system the NcoI to EcoRI fragment of pUCHMI (16) containing the terminal repeat region of human T-cell leukemia/lymphoma virus 1 (17) was picked by using cloning rings and expanded separately for testing of collagenase. Of particular note is the blocking of autolysis from conditioned medium for in vitro collagenase inhibition activity (below).

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Recently it has become clear that there is an inhibitor related to but distinct from TIMP. We have reported the isolation and characterization of this inhibitor, which we designated metalloproteinase inhibitor or MI (14). We have also described bovine and human cDNA clones, and expression of recombinant MI from the human clone in Escherichia coli (16). The inhibitor has about 40% homology with TIMP, with conservation of all 12 cysteine residues. Simultaneously Stetler-Stevenson et al. (17) and subsequently Goldberg et al. (18) have reported on an inhibitor designated TIMP-2. Comparison between the amino acid sequence of MI deduced from nucleotide sequence (16) and that for TIMP-2 determined by amino acid sequencing of the protein (17) indicated differences at 10 positions. More recently Stetler-Stevenson et al. (19) have reported the cDNA cloning of TIMP-2. Comparison of cDNA sequences shows that MI and TIMP-2 are the same molecule (the reported cDNA sequences for the coding region are identical). The existence of this inhibitor has raised the intriguing possibility that there is a family of TIMP-like inhibitors whose members could interact differentially with members of the MMP family that they inhibit.

In order to explore such possibilities, we now describe the purification of MI/TIMP-2 recombinantly expressed and secreted by Chinese hamster ovary cells. Using the recombinant MI/TIMP-2, we characterize interactions with interstitial collagenase. Of particular note is the blocking of autolysolytic activation of procollagenase.

MATERIALS AND METHODS

Recombinant Expression of MI/TIMP-2 by Chinese Hamster Ovary Cells—The construction of the expression plasmid is illustrated in Fig. 1. Briefly, the NcoI to EcoRI fragment of pUCHMI (16) containing the intact coding sequence of human MI was first subcloned into the multiple cloning site of pCFM1156 (20) to give plasmid p1156HMINR. The MI cDNA was retrieved from plasmid p1156HMINR as a 0.65-kilobase pair HindIII to SstI fragment and subsequently inserted into the expression vector pDSRα2 to form plasmid pDSRα2MI. The vector pDSRα2 is a derivative of the pCD1 plasmid (21) with several modifications. First, the “R” element derived from the long terminal repeat region of human T-cell leukemia/lymphoma virus 1 (22) was added between the Simian virus 40 early promoter and the splicing donor sequences (“S”). Second, the simian virus 40 polyadenylation sequence was replaced by the signal from the α-subunit of bovine follicular stimulating hormone (23). Also a mouse dihydrofolate reductase minigene (24) was inserted downstream from the expression cassette to allow selection and amplification of integrated cDNA.

Dihydrofolate reductase-deficient Chinese hamster ovary cells (25) were maintained routinely in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% (v/v) fetal bovine serum, nonessential amino acids (100 μg/ml), hypoxanthine (100 μg/ml), thymidine (16 μg/ml), penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml). Approximately 1 × 10⁶ cells were transfected with 20 μg of pDSRα2MI plasmid DNA by a modified calcium phosphate precipitation method (26). Three days post-transfection, the cells were split into eight 100-mm dishes containing selective medium (DMEM supplemented with 10% (v/v) fetal bovine serum, nonessential amino acids, and penicillin/streptomycin). Stable individual transfectants were picked by using cloning rings and expanded separately for testing of conditioned medium for in vitro collagenase inhibition activity (below).

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Fig. 1. Construction of the expression plasmid pDSRα2MI.

The plasmid pDSRα2MI was constructed as described in the text. Restriction sites were utilized as indicated. The 3′- and 5′-untranslated regions (UTR) and the human MI/TIMP-2 coding region (HMI) are indicated. The nature of the R and S elements is indicated in the text. SVE is the simian virus 40 early promoter/enhancer element. Stuffer refers to a 600-base pair spacer fragment.

MI/TIMP-2 (rMI/rTIMP-2), the selected recombinant clone was grown in spinner flasks in DMEM containing 5% (v/v) dialyzed fetal bovine serum, glutamine, and nonessential amino acids. Cells were then transferred to 850-cm² roller bottles (2 × 10⁶ cells/bottle) containing the same medium. After 3-4 days at 37 °C, the cell monolayers were washed with phosphate-buffered saline, and fresh medium (150-200 ml/bottle; as above, but lacking serum) was added. Conditioned medium was harvested 6-7 days later. Sodium azide (final concentration 0.02%, w/v) and the protease inhibitors pepstatin A (final concentration 1 μg/ml) and phenylmethanesulfonyl fluoride (final concentration 0.6 mM) were added.

Purification of rMI/rTIMP-2—Conditioned medium from Chinese hamster ovary cells transfected with pDSRα2MI plasmid was obtained as described in the preceding section. When purified by SDS-polyacrylamide gel electrophoresis (below) the medium contained a prominent 25-kDa band (reduced) or 21.5-kDa band (unreduced) not evident for medium derived from untransfected Chinese hamster ovary cells. These bands represent rMI/rTIMP-2 with the mobility shift after reduction observed for natural bovine MI/TIMP-2 (14) and characteristic of proteins with intrachain disulfide bonds. Since
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RESULTS

Purification of rMI/rTIMP-2—Purification of rMI/rTIMP-2 is summarized in Table I. The recovery of in vitro inhibitory activity was approximately 50%, and 5.6-fold purification was required. The final preparation had a specific activity of 1015 units/mg, which is similar to that observed for purified natural bovine MI/TIMP-2 (14). We do not have an explanation for the drop in specific activity between the Q-Sepharose and Sephacryl S-200 HR steps. By SDS-polyacrylamide gel electrophoresis, the expected protein band with apparent molecular weight of 21,500 (unreduced) and 25,000 (reduced) was present (Fig. 2); purity was judged as greater than 95%. As mentioned above, the mobility shift observed upon SDS-polyacrylamide gel electrophoresis after reduction corresponds to that observed for natural bovine MI/TIMP-2 (14) and is characteristic of proteins with intrachain disulfide bonds. This result suggests that the disulfide structure of the recombinant-derived material probably matches that of the natural. Using Ellman’s reagent (36) in the presence of denaturing agents, we find no reactivity with the rMI/rTIMP-2 (data not shown), suggesting that all 12 cysteine residues are in disulfide linkage. The 12 cysteine residues of TIMP align precisely with those of MI/TIMP-2 (see Ref. 16), and assignments for the 6 disulfide bonds in TIMP have recently been reported (37). It is likely that MI/TIMP-2 has 6 disulfide bonds homologous to those of TIMP. By automated aminoterminal amino acid sequencing carried out through 20 cycles (see Ref. 14 for methodology), the purified material showed

<table>
<thead>
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<th>Step</th>
<th>Volume</th>
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<td>Chelating Sepharose</td>
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<td>630,000</td>
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<td>Sephacryl S-200 HR</td>
<td>180</td>
<td>260</td>
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* Determined by the method of Bradford (30) using bovine serum albumin as standard, except where indicated otherwise.

** Activity was determined by the collagen filter assay (31).

* Determined by A_{420} cm, using a value of 1.82 for the absorbance at 280 nm of a 1 mg/ml solution.

FIG. 2. SDS-polyacrylamide gel electrophoresis of rMI/rTIMP-2 purified from conditioned medium of CHO cell. Purified material was electrophoresed on a 12.5% (w/v) acrylamide gel. After electrophoresis, the gel was silver-stained. Lane 1, reduced sample (1 μg); lane 2, unreduced sample (1 μg). The lane at left shows reduced molecular weight markers, with molecular weight values (× 10^3) indicated.
the sequence expected for the mature protein (16). Thus the 26-amino acid leader sequence encoded by the plasmid pDSRoz2MI is efficiently removed during synthesis and secretion of the rMI/rTIMP-2 by CHO cells. From absorbance at 280 nm and quantitative amino acid composition analysis, a value of 1.82 for $\varepsilon_{0,280}$ was obtained and used to determine concentrations of working rMI/rTIMP-2 solutions.

Like natural bovine MI (14), the human rMI/rTIMP-2 appears to be unglycosylated (note that the bovine and human MI/TIMP-2 cDNAs both encode polypeptides lacking N-linked glycosylation sites (16)) and elutes from gel filtration columns with apparent molecular weight of about 20,000-25,000 relative to proteins used as molecular weight markers. Taken together, the similarities between natural bovine MI/TIMP-2 and human rMI/rTIMP-2 with regard to specific activity and other biochemical properties indicate that the rMI/rTIMP-2 has an active conformation equivalent to that of the natural molecule.

**Inhibition of Interstitial Collagenase by rMI/rTIMP-2 Is Stoichiometric with a 1:1 Molar Inhibitor:Enzyme Ratio**—Inhibition of the degradation of $^{14}$C-labeled type I collagen by pAPMA-activated rabbit fibroblast interstitial collagenase incubated with increasing concentrations of purified rMI/rTIMP-2 is shown in Fig. 3. The inhibition is dose-dependent with a near complete suppression of enzyme activity achieved as the molar inhibitor/enzyme ratio approaches 1. This observation is consistent with a stoichiometric inhibition and the formation of a 1:1 molar complex between the enzyme and the inhibitor.

**rMI/rTIMP-2 Inhibits the Specific Single Peptide Bond Cleavage of Type I Collagen by Collagenase**—The specificity of the inhibition of type I collagen degradation by fibroblast collagenase in the presence of rMI/rTIMP-2 was verified by SDS-polyacrylamide gel electrophoresis of the collagen degradation products (Fig. 4). Interstitial collagenase typically generates $\frac{3}{4}$ and $\frac{1}{4}$ length fragments ($T_{C}^{a}$ and $T_{C}^{b}$) of collagen (lanes 2). These were not seen when rMI/rTIMP-2 (lanes 3) or EDTA (lanes 4) was added to the incubation mixtures. At 37°C, further degradation of the collagen chains was observed, possibly due to minor contamination of the preparation with gelatinase. The initial cleavage of the collagen as well as its further degradation were completely blocked by rMI/rTIMP-2.

**rMI/rTIMP-2 Inhibits the Activation of Procollagenase by pAPMA**—We investigated whether rMI/rTIMP-2 could interfere with the activation of procollagenase by organomercurials. Treatment of procollagenase with pAPMA resulted in the conversion of the 52-kDa proenzyme to the 42-kDa activated enzyme, as visualized by SDS-polyacrylamide gel electrophoresis (Fig. 5). This conversion was completed over 30 min (Fig. 5A). Its rate was unaffected by the addition of trypsin-activated collagenase (Fig. 5B). This observation is consistent with the initiation by organomercurials of an intramolecular autoproteolytic process rather than an intermolecular process that would have been accelerated by the activation of collagenase (6). In the presence of rMI/rTIMP-2 at a molar inhibitor/enzyme ratio of 1.7:1 conversion of the 52-kDa procollagenase to the 42-kDa enzyme was completely inhibited (Fig. 5C). From these experiments we conclude that rMI/rTIMP-2 blocks the activation of procollagenase initiated by organomercurials.

**rMI/rTIMP-2 Inhibits the Autoproteolytic Activation of Procollagenase Induced by Plasmin**—In the presence of plasmin at 4°C, procollagenase was converted from the 52-kDa proenzyme to a 46-kDa intermediate clearly identified on SDS-polyacrylamide gels (Fig. 6, lanes 1 and 2). Activation of this intermediate by autocalytic reaction was initiated by incubating the sample at 37°C (lane 3) (6). The addition of rMI/rTIMP-2 had no effect on the conversion of the 52-kDa proenzyme to the 46-kDa intermediate (lane 4), as expected since we have previously demonstrated that MI/TIMP-2 has no antiplasmin activity (14). In contrast, the conversion of
fig. 5. rMI/rTIMP-2 inhibits pAPMA-induced activation of fibroblast interstitial procollagenase. Interstitial fibroblast procollagenase (0.84 μg = 16 pmol) in 5 μl of 50 mM Tris-HCl, 200 mM NaCl, 10 mM CaCl₂, pH 7.5, was incubated at 37 °C in the presence of pAPMA (5 mM). At the indicated times, the reactions were terminated by the addition of EDTA to 20 mM. Samples were then heated at 68 °C for 10 min in the presence of 50 mM dithiothreitol and run on 12.5% (w/v) acrylamide SDS gels. Gels were stained with Coomassie Blue. Gel A, fibroblast interstitial procollagenase activated with pAPMA; gel B, as A, but trypsin-activated fibroblast interstitial collagenase (0.84 μg) was added prior to pAPMA addition; gel C, as A, but rMI/rTIMP-2 (0.57 μg = 27 pmol) was added prior to pAPMA addition. The time at which the reaction was terminated with EDTA is indicated across the top of the figure (in minutes). The arrows at the right indicate migration positions of procollagenase (PRO), collagenase (COLL), and rMI/rTIMP-2 (rMI).

fig. 6. rMI/rTIMP-2 inhibits plasmin-induced activation of fibroblast interstitial procollagenase. Interstitial fibroblast procollagenase (0.84 μg = 16 pmol) in 5 μl of 50 mM Tris-HCl, 200 mM NaCl, 10 mM CaCl₂, pH 7.5, was incubated as indicated below. After incubation, samples were reduced in the presence of 50 mM dithiothreitol, heated at 68 °C for 10 min, and subjected to SDS-polyacrylamide gel electrophoresis as described for Fig. 5. Lane 1, procollagenase; lane 2, procollagenase incubated with plasmin (0.6 μg) at 4 °C for 30 min followed by the addition of soybean trypsin inhibitor (3 μg) to inactivate plasmin; note the presence of plasmin (70-kDa band) and soybean trypsin inhibitor (21.5-kDa band) on the gel; lane 3, as lane 2 but the sample was further incubated at 37 °C for 30 min after the addition of soybean trypsin inhibitor; note the conversion of the 46-kDa intermediate to 42-kDa activated collagenase; lane 4, as lane 2, but rMI/rTIMP-2 (0.57 μg = 27 pmol) was added along with the plasmin; note the presence of 25-kDa rMI/rTIMP-2 (reduced); lane 5, as lane 3, but rMI/rTIMP-2 was added prior to incubation at 37 °C; lane 6, as lane 3, but EDTA was added to 20 mM prior to incubation at 37 °C; lane 7, rMI/rTIMP-2 (27 pmol) alone; lane 8, reduced molecular weight markers with molecular mass values indicated on the right (in kDa). Positions of the 52-kDa procollagenase, 46-kDa intermediate, and 42-kDa activated collagenase are indicated on the left.

Fig. 7. rMI/rTIMP-2 forms complexes with procollagenase and collagenase that are stable to SDS. Fibroblast interstitial procollagenase (0.84 μg = 16 pmol) in 5 μl of 50 mM Tris-HCl, 200 mM NaCl, 10 mM CaCl₂, pH 7.5, was incubated as indicated below, in duplicate for each case. After incubations, samples were prepared for SDS-polyacrylamide gel electrophoresis as usual with dithiothreitol. Samples in lanes 6-8 were not heat-treated prior to electrophoresis. After electrophoresis (12.5%, w/v, acrylamide), gel A was stained with Coomassie Blue and gel B was immunoblotted using a rabbit anti-rMI/rTIMP-2 antiserum (see "Materials and Methods"). Migration positions of reduced molecular weight markers are indicated on the right (gel A, as in Fig. 6; gel B, prestained markers); values represent molecular mass in kDa. Lanes 1, interstitial procollagenase; lanes 2, interstitial procollagenase activated with pAPMA (5 mM) at 37 °C for 30 min; lanes 3 and 6, interstitial procollagenase incubated with rMI/rTIMP-2 (0.57 μg = 27 pmol) for 15 min at 22 °C; lanes 4 and 7, as lanes 2 followed by incubation with rMI/rTIMP-2 for 15 min at 22 °C; lanes 5 and 8, as lanes 3 and 6 followed by incubation with pAPMA for 30 min at 37 °C; lanes 9, rMI/rTIMP-2 alone.
Confirmation that the bands representing putative complexes truly contain rMI/rTIMP-2 was obtained by immunoblot analysis using antiserum against rMI/rTIMP-2 (Fig. 7B) and by the use of $^{125}$I-labeled rMI/rTIMP-2 and autoradiography (Fig. 8).

**DISCUSSION**

The characterization and cloning of MI/TIMP-2 by ourselves (14, 16) and others (17-19) has made clear the relatedness between this molecule and TIMP. Despite the amino acid sequence divergence and the fact that TIMP is glycosylated whereas MI/TIMP-2 is not, the inhibitors have a similar spectrum of activities toward different metalloproteinases (14, 17, 18), at least in a general way. It is of interest to know whether there are specific differences in the mechanism of inhibition that could reflect different functional roles for the inhibitors in vivo.

Several recent reports (4, 17, 18) have included data on the interactions between TIMP and TIMP-2 on the one hand and 72-kDa type IV collagenase (MMP-2) and 92-kDa type IV collagenase on the other. Complexes of TIMP-2 with 72-kDa type IV procollagenase were isolated from medium conditioned by human A2058 melanoma cells (17) and from medium conditioned by simian virus 40-transformed human lung fibroblasts or by several other cell types (18). The simian virus 40-transformed human lung fibroblasts also secreted a complex of TIMP with 92-kDa type IV procollagenase (4, 18), and since there was no apparent crossover in components of the complexes, it was concluded that TIMP interacts preferentially with the 92-kDa type IV procollagenase and TIMP-2 interacts preferentially with the 72-kDa type IV procollagenase (18). The isolated complexes were stable to 1 M NaCl or 2 M urea (4, 18) but unstable to 0.1% (w/v) SDS or 0.1% (v/v) trifluoroacetic acid/acetonitrile (4, 17, 18). The effects of binding of the inhibitors to the proenzymes have remained unexplored because of the unavailability of native, inhibitor-free proenzymes (4, 18).

In the present paper we have utilized recombinant-derived MI/TIMP-2 and an inhibitor-free preparation of rabbit fibroblast interstitial procollagenase (MMP-1) to study the inhibitor-enzyme interactions. It appears that rMI/rTIMP-2 inhibits activated interstitial collagenase by formation of a 1:1 molar stoichiometric complex. This finding is comparable to findings for TIMP interacting with interstitial collagenase (15), stromelysin (38), 92-kDa type IV collagenase (4), and a 97-kDa gelatinase from pig polymorphonuclear leukocytes (39); and for TIMP-2 interacting with 72-kDa type IV collagenase (17, 18).

It is of particular interest that rMI/rTIMP-2 also completely inhibits the pAPMA-induced autoproteolytic activation of interstitial procollagenase. Our data suggest that the inhibition of autoproteolysis, like the inhibition of the activated metalloproteinase, occurs by formation of a SDS-stable noncovalent complex between inhibitor and proenzyme/enzyme. The SDS-stable rMI/rTIMP-2-collagenase complexes and rMI/rTIMP-2-procollagenase complexes are visualized after SDS-polyacrylamide gel electrophoresis, and, as noted, they are also stable to dithiothreitol treatment. Thus the complexes do not appear to involve intermolecular disulfide bonds. Comparison between the apparent molecular mass values for the rMI/rTIMP-2-collagenase complex (55 kDa) and the rMI/rTIMP-2-procollagenase complex (81 kDa) relative to the corresponding values for the uncomplexed enzymes (42 and 52 kDa, respectively) indicates a much greater mobility shift in the latter case. Conceivably, the rMI/rTIMP-2-procollagenase complex includes two molecules of MI/TIMP-2 per molecule of procollagenase. Alternatively, the complexes may have differences in structure or SDS binding that lead to anomalous electrophoretic mobilities.

In vitro, the autoproteolytic activation of interstitial procollagenase can be initiated by pAPMA treatment or by plasmin-catalyzed cleavage to the 46-kDa intermediate. rMI/rTIMP-2 does not prevent pAPMA or plasmin from having an effect on procollagenase; in fact, formation of the SDS-stable complexes only occurs if pAPMA or plasmin is added to the rMI/rTIMP-2-procollagenase mixtures. Therefore it can be postulated that pAPMA or plasmin treatment leads to a conformational change in procollagenase (the 46-kDa intermediate in the case of plasmin treatment) such that rMI/rTIMP-2 is then able to bind and inhibit the autoproteolysis. It is possible that rMI/rTIMP-2 also forms a complex with interstitial procollagenase in the absence of pAPMA that is not SDS-stable, especially since this is the case with TIMP-2 and 72-kDa type IV procollagenase (18).

With regard to mechanistic or functional differences in the interactions of TIMP and TIMP-2 with MMPs, it is worthwhile to discuss our data with MI/TIMP-2 in the context of other reported data. TIMP was found to form a complex with activated interstitial collagenase but not with interstitial procollagenase (15). By analogy to our data with TIMP-2, it remains possible that TIMP could form a complex with interstitial procollagenase in the presence of pAPMA. In contrast, TIMP clearly forms a complex with the 92-kDa type IV procollagenase, and the presence of TIMP in the complex did not prevent phenylmercuric acid-induced activation of the proenzyme (4). Murphy et al. (39) have also reported that TIMP did not prevent PAPMA-induced autoproteolytic activation of the 97-kDa metalloproteinase proenzyme from pig polymorphonuclear leukocytes. In the case of TIMP-2, our data show that it can bind to interstitial procollagenase (at least in the presence of pAPMA) and can completely inhibit the PAPMA-induced autoproteolytic activation. The reports of Stetler-Stevenson et al. (17) and Goldberg et al. (18) show that TIMP-2 can bind to the 72-kDa type IV procollagenase; furthermore, TIMP-2 inhibits PAPMA-induced autoproteo-
lytic activation of this proenzyme (18). Again, the preference of TIMP-2 for binding to 72-kDa type IV procollagenase versus 92-kDa type IV procollagenase (18) has been noted above. Thus TIMP and TIMP-2 appear to defer from each other in their interactions with MMPs with regard to 1) the proenzymes that they can bind to and 2) whether or not they inhibit autoprotoeltyc activation of the proenzymes to which they do bind. Additional studies will be required to characterize such interactions further. For example, to our knowledge no studies on the interaction of TIMP or TIMP-2 with prostromelysin (MMP-3) have been reported.

It appears that both TIMP and TIMP-2 can bind to and inhibit the activated forms of most MMPs. In the case of TIMP, this has been shown for interstitial collagenase (39), stromelysin (38, 40), 72-kDa type IV collagenase (18), and 97-kDa type IV collagenase from pig polymorphonuclear leukocytes (39). TIMP-2 seems to have the same broad spectrum of inhibitory activity toward the activated MMPs (this paper and Refs. 14 and 18). It remains to be determined whether there might be differences in the relative affinities of TIMP and MI/TIMP-2 for activated MMPs.

In conclusion, available data suggest that MI/TIMP-2 may be able to control the extracellular action of some MMPs at two levels, the activation of the proenzyme and the activity of the activated enzyme.

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REFERENCES