SV40-transformed Human Lung Fibroblasts Secrete a 92-kDa Type IV Collagenase Which Is Identical to That Secreted by Normal Human Macrophages*

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We have reported that SV40-transformed human lung fibroblasts secrete a 92-kDa metalloprotease which is not detectable in the parental cell line IMR-90. We now present the complete structure of this enzyme along with the evidence that it is identical to the 92-kDa metalloprotease secreted by normal human alveolar macrophages, phorbol ester-differentiated monocyctic leukemia U937 cells, fibrosarcoma HT1080 cells, and cultured human keratinocytes. A similar, perhaps identical, enzyme can be released by polymorphonuclear cells. The preproenzyme is synthesized as a polypeptide of predicted M, 78,426 containing a 19 amino-acid-long signal peptide and secreted as a single 92,000 glycosylated proenzyme. The purified proenzyme complexes noncovalently with the tissue inhibitor of metalloprotease (TIMP) and can be activated by organomercurials. Activation with phenylmercuric chloride results in removal of 73 amino acids from the NH2 terminus of the proenzyme, yielding an active form capable of digesting native types IV and V collagen. The in vitro substrate specificity of the enzyme using these substrates was indistinguishable from that of the 72-kDa type IV collagenase. The 92-kDa type IV collagenase consists of five domains: the amino-terminal and zinc-binding domains shared by all members of the secreted metalloprotease gene family, the collagen-binding fibronectin-like domain also present in the 72-kDa type IV collagenase, a carboxyl-terminal hemopexin-like domain shared by all known enzymes of this family with the exception of PUMP-1, and a unique 54-amino-acid-long proline-rich domain homologous to the a2 chain of type V collagen.

Resident cells of tissues are capable of secreting an array of enzymes initiating the degradation of the surrounding macromolecules of the extracellular matrix (ECM)1 (1–13), which presumably contributes to the initial steps of tissue remodeling during morphogenesis, wound healing, angiogenesis, and tumor invasion (11–22). Several of the metalloproteases of this class have been shown to be structurally related and compromise a novel secreted protease gene family, which includes interstitial collagenase (1–5), stromelysin (6, 7), the rat equivalent of stromelysin, transin (8, 9), a 72-kDa type IV collagenase (10), and two enzymes of unknown function, transin 2 and PUMP-1 (11). The latter may be identical to the small molecular weight metalloprotease recently isolated from rat uterus (23). The expression of genes coding for these enzymes is cell-type specific and is regulated by growth factors (8, 24), oncogenes (18, 25), mediators of inflammation (26–29), and tumor promoters (2, 6, 8, 30). The extracellular activity of these enzymes is modulated by proenzyme activation, interaction with the specific tissue inhibitor of metalloprotease (TIMP) (31, 32), and microenvironmental factors such as tissue localization.

The ECM metalloproteases are secreted as zymogens which undergo extracellular activation. One possible pathway of activation of interstitial collagenase involves a cascade of proteolytic events in which processing of the amino-terminal portion of the enzyme by plasmin results in removal of 84 amino acid residues (5). A second step requires the presence of plasmin-activated stromelysin, which removes approximately 15 residues from the carboxyl end of the molecule, resulting in the fully activated enzyme (5). Interstitial collagenase once activated can form an enzyme inhibitor complex with TIMP. However, no complex formation between the latent procollagenase and TIMP occurs (33, 34).

We have reported (10) that human bronchial epithelial cells secrete a 72-kDa type IV collagenase in response to transformation with the H-ras oncogene (TBE-1). The same enzyme is secreted by a variety of tumor cells as well as by normal human skin fibroblasts. SV40-transformed human lung fibroblasts secrete an additional 92-kDa gelatinolytic enzyme which was not detected in the parental cells (10). This enzyme has a molecular weight similar to that of the enzyme secreted by normal alveolar macrophages (35, 36), phorbol ester-treated U937 monocyctic leukemia cells, and granulocytes (37). The secretion of this enzyme was not detected in any normal cell strain of fibroblast origin. We now report the primary structure of this enzyme, its substrate specificity, and evidence for a proenzyme-inhibitor complex.

MATERIALS AND METHODS

Cell Culture—SV40-transformed fetal lung fibroblasts and the parental line IMR-90 (from the National Institutes of Health Insti-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) M70370.

1 The abbreviations used are: ECM, extracellular matrix; TIMP, tissue inhibitor of metalloprotease; TPA, 12-O-tetradecanoylphorbol acetate; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; PMC, phenylmercuric acid; bp, base pairs.

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Enzyme Purification—The 92-kDa metalloproteinase was purified from conditioned medium of SV40-transformed fibroblasts or TPA-differentiated U937 cells using a three-step procedure. Serum-free medium was adjusted to 0.01 M Tris-HCl, pH 7.5, and applied to a 2.5 × 10-cm column of reactive Red Agarose (Sigma) equilibrated in 0.02 M Tris-HCl, pH 7.5, containing 0.005 M CaCl₂ (Tris-CaCl₂ buffer) and 0.15 M NaCl. The enzyme was eluted from the column using a 0.15-2.0 M NaCl linear gradient in Tris/CaCl₂ buffer and fractions were assayed by gelatin zymography (38). Fractions containing 92-kDa gelatinolytic activity were pooled, adjusted to 0.5 M NaCl and 0.01% Brij-35 and chromatographed on a 1.0 × 10-cm column of gelatin-Sepharose (Sigma) as previously described (10, 37) except that the enzyme was eluted using a 0-10 M NaCl linear gradient in Tris/CaCl₂ buffer and fractions were assayed by gelatin zymography (38). Gel filtration was also performed in 0.02 M Tris-HCl, pH 7.5, containing either 2 M urea or 0.1% SDS in an effort to separate the proenzyme and TIMP complex. Hydrophobic interaction chromatography was carried out by adjusting this preparation to 4.0 M NaCl, and applying the sample to a 0.5 × 2.0-cm column of phenyl-Sepharose (Pharmacia LKB Biotechnology Inc.). The column was eluted stepwise with 1.0, 0.5, 0.2, and 0.1 M NaCl and 0.5 M NaCl containing 50% ethylene glycol in Tris-CaCl₂ buffer. Antibody Preparation—Monospecific antiserum was prepared using SDS gel slices containing 50-75 μg of protein emulsified in Freund’s complete adjuvant and injected into multiple sites at bi-weekly intervals for up to 2 months. Electrophoretic (Western) blotting was performed as described (2) except that the nitrocellulose blots were developed using a 1:250 dilution of primary antiserum and applying the sample to a PVDF membrane by electroblotting (39) were sequenced using an Applied Biosystems 470A gas-phase sequenator (40). Enzyme Assays—Samples of purified 92-kDa U937 proenzyme in 0.05 M Tris-HCl, pH 7.5, 0.005 M CaCl₂, 0.05 M NaCl, and 0.01% SDS were applied to the assay. U937 or human promonocytes were used for the assays as previously described (41). The amino acid sequence of three internal peptides from the 92-kDa enzyme purified from the conditioned medium of TPA-differentiated U937 cells. To determine the primary structure and confirm the relationship of the 92-kDa proenzyme are shown in Fig. 1. The NH₂-terminal sequence of the 92-kDa enzyme purified from the conditioned medium of SV40-transformed human lung fibroblasts was determined in a similar fashion and found to be identical to that of the enzyme secreted by U937 cells (shown in Fig. 1). In addition, immunoblot analysis (Fig. 2) demonstrated that the rabbit antibody raised against the enzyme purified from U937 cells recognized the 92-kDa enzyme secreted by TPA-stimulated SV40-transformed lung fibroblasts, HT1080 cells, alveolar macrophages, polymorphonuclear leukocytes, and keratinocytes (data not shown), but not the 72-kDa type IV collagenase purified from human fibroblasts or human bronchial epithelial cells or human skin fibroblasts.
92-kDa Peptide and Oligonucleotide Probe Sequence Data

| Peptide | Probe  |
|---------|--------|
| TP12    | CAGGTGCCGAGGGGAGATGGCAGGGCTG/CA/TCTGICG/T~GIC/GGGGGGC |
| TP16    | CACGTCGCCACGGMCAGCACCAGGGTIG/CA/TCTGICG/T~GIC/GGGGGGC |
| V7      | CAGGTGCCGAGGGGAGATGGCAGGGCTG/CA/TCTGICG/T~GIC/GGGGGGC |
| NA1     | CAGGTGCCGAGGGGAGATGGCAGGGCTG/CA/TCTGICG/T~GIC/GGGGGGC |
| NA2     | CAGGTGCCGAGGGGAGATGGCAGGGCTG/CA/TCTGICG/T~GIC/GGGGGGC |

**Fig. 1.** Amino acid sequence of peptides derived from 92-kDa human type IV collagenase. Tryptic (TP) and V8 (V) peptides were fractioned and sequenced as described under “Materials and Methods.” Purified type IV procollagenase (NP) or FMC-activated enzyme (NA1 and NA2) were electroblotted onto a PVDF membrane and subjected to NH2-terminal sequence analysis. The ON 2209 oligonucleotide probe was synthesized (58) using the NH2-terminal sequence of purified type III procollagenase. The numbers represent the positions of the amino acid residues and the nucleotides in the sequence presented in Fig. 3.

**Fig. 2.** Immunoblot analysis of 92-kDa type IV collagenase secreted by normal and transformed cells. Samples of purified type IV collagenase (100 ng) and serum-free conditioned medium (200 μl) were subjected to immunoblot analysis (unreduced). Lanes 1 and 2, purified 72-kDa (TBE-1) and human skin fibroblast type IV collagenase (HSF). Lanes 3 and 9, purified and crude U937 92-kDa type IV collagenase. Lanes 4 and 6, purified and crude SV-40 92-kDa type IV collagenase. Lanes 5, 7, 8, and 10 represent samples of conditioned medium from HT-1080 cells, human skin keratinocytes (HSK), alveolar macrophages (AO), and polymorphonuclear leukocytes (PMN). The PMN enzyme is unreduced and therefore shows the 92-kDa in addition to several other immunoreactive bands (see Ref. 39). All bands displayed gelatinolytic activity on zymography (data not shown).

Insert in the clone p92174.1 is presented in Fig. 3. The insert contains 2334 bp, excluding the poly(AT) and the poly(GC) tails and represents the mRNA encoding the 92-kDa proenzyme. The sequence contains a 19-bp 5'-untranslated region followed by an AUG initiating methionine codon and an open reading frame with coding capacity for a 707 amino-acid-long proenzyme of predicted Mr, 78,426. The 193-nucleotide long 3'-untranslated region contains the putative poly(A) addition site 19 bases upstream from the 3' end of the RNA. The first 19 amino acids of the predicted sequence constitute a hydrophobic signal peptide followed by the start of the mature secreted protein at APRQR, as determined by amino acid sequence analysis (see Fig. 1).

The sequences of peptides TP12, TP16, and V7 obtained from the enzyme secreted by the U937 cells are present in the deduced amino acid sequence of the clone p92174.1 isolated from the HT1080 cell cDNA library. The cDNA clone hybridizes to a 2.8-kilobase message in TPA-treated U937 cells, HT1080, and SV40-transformed human lung fibroblasts (Fig. 4). These data indicate that the 92-kDa metalloprotease secreted from TPA-differentiated U937 cells is identical to the enzyme secreted by HT1080 fibrosarcoma cells and SV40-transformed human lung fibroblasts and in all likelihood is identical to the 92-kDa enzyme secreted by alveolar macrophages, polymorphonuclear leukocytes, and keratinocytes. Based on the similarity of the enzymatic properties and structural homology of this enzyme and the 72-kDa type IV collagenase described previously (10, see below) this enzyme will be referred to as 92-kDa type IV collagenase.

The apparent discrepancy between the predicted molecular weight and the molecular weight of the secreted protein determined by SDS-PAGE is due to a post-translational modification of the enzyme through addition of several oligosaccharide side chains. The results presented in Fig. 5 demonstrate that treatment of the U937 metalloprotease with N- or O-glycanase but not endoglycosidase H leads to a reduction in molecular weight, indicating that the mature secreted 92-kDa proenzyme contains both O-linked and complex N-linked carbohydrates. The reduced molecular weight of the enzyme after removal of carbohydrates is consistent with the M, 81,000 of the cell-free translation product synthesized using either the U937 or HT1080 mRNA (data not shown). In agreement with this result, three potential N-linked glycosylation sites are found in the predicted amino acid sequence of the 92-kDa metalloprotease at positions 38, 120, and 127. Only one of these sites at Asn-120 is conserved in both interstitial collagenase (3) and stromelysin (6).

**92-kDa Type IV Collagenase Consists of Five Distinct Domains and Is a Member of the Secreted ECM Metalloprotease Gene Family**—A comparison of the primary structure of the 92- and 72-kDa type IV collagenases presented in Fig. 6 demonstrates that the 92-kDa type IV collagenase consists of five domains. The amino-terminus and fibronectin-like collagen-binding domain adjacent to the zinc-binding domain (10), and the hemopexin-like carboxyl end proximal domain (11) are homologous to those found in the 72-kDa type IV collagenase. In addition, the 92-kDa type collagenase contains a 54-amino-acid-long proline-rich collagen-like domain of unknown function which is not found in other members of the secreted ECM metalloprotease gene family (Fig. 7). The homology of this domain with a portion of the helical region of α2(V) collagen (42) is shown in Fig. 6. The size of this domain corresponds to a 3-fold multiple of the basic exon unit of 54 bp typically found in the collagen genes (43). Both the sequence homology and the length of the insertion suggest that the appearance of this domain in the 92-kDa type IV collagenase is the result of a recombinational event between the enzyme precursor gene and a collagen gene.

**The Expression of 92-kDa Type IV Collagenase Can Be Induced by Protein C Kinase Activators and Growth Factors**—As shown in Fig. 4, phorbol esters (TPA), and Indolactam V (−), both potent protein C kinase activators (44, 45) induce expression of the 92-kDa type IV collagenase in cultures of U937 cells, SV40-transformed fetal lung fibroblasts, and...
The nucleotide sequence of the human 92-kDa type IV collagenase cDNA. The sequence was determined on both strands as described under "Materials and Methods." The predicted amino acid sequence of the enzyme is shown under the DNA sequence. The amino terminus of the mature and PMC-activated enzyme is indicated by an asterisk. The potential glycosylation sites and the putative poly(A) addition signal are underlined.

For reference, the peptides shown in Fig. 1 are located at the following positions within the nucleotide sequence. TP12, begins at nucleotide 1784 (aa 589-599); TP16, begins at nucleotide 1667 (aa 550-564); V7, begins at nucleotide 1967 (aa 650-656); NP, begins at nucleotide 77 (aa 20-37); NAI, begins at nucleotide 299 (aa 94-104); NAZ, begins at nucleotide 236 (aa 73-82).

HT1080 cells, but not in the parental fetal lung fibroblast cell line IMR-90. Those agents do not induce expression of the 72-kDa type IV collagenase. The inactive diastereoisomer indolactam V (+) had little if any effect on expression of the 92-kDa enzyme in these cell lines. The epidermal growth factor and interleukin-1 induced a steady-state level of the 92-kDa enzyme specific mRNA (Fig. 4) in SV40-transformed cells.

The 92-kDa Type IV Collagenase Proenzyme Exists in a Nonequivalent Complex with TIMP, Which Is Activatable by p-

FIG. 3. Nucleotide sequence of the human 92-kDa type IV collagenase cDNA. The sequence was determined on both strands as described under "Materials and Methods." The predicted amino acid sequence of the enzyme is shown under the DNA sequence. The amino terminus of the mature and PMC-activated enzyme is indicated by an asterisk. The potential glycosylation sites and the putative poly(A) addition signal are underlined.
Aminophenylmercuric Acetate, Yielding an Enzyme with a Protein was identified as TIMP by Western blot analysis (Fig. 4). Hydrophobic interaction chromatography on phenyl-Sepharose (Fig. 9B) was also unsuccessful. However, gel filtration in the presence of 0.1% SDS did resolve the proenzyme and TIMP proteins (Fig. 9C).

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**Fig. 5.** N- and O-linked glycosylation of the 92-kDa type IV collagenase. Samples of purified (U937 cells) type IV procollagen (2 µg) were incubated with either endoglycosidase H (lane 2), N-glycanase (lane 3), O-glycanase (lane 4), or in buffer only (lanes 1 and 5) for 16 h at 37°C and subjected to immunoblot analysis. Alternatively TPA-differentiated U937 cells were biosynthetically labeled in the presence of 0.5 µg/ml of tunicamycin for 16 h, and samples of conditioned medium were immunoprecipitated using 92-kDa monospecific antiserum. These data indicate that the enzyme isolated from any cell type that secretes significant amounts of TIMP may be found in the conditioned medium.

**Fig. 6.** Structural relationship between human 72- and 92-kDa type IV collagenases. **Top line**, amino acid sequence of the 92-kDa type IV collagenase as in Fig. 3. **Second line**, human 72-kDa type IV collagenase. The sequence of the signal peptide of this protein was reconstructed from the DNA sequence of the genomic clone of the enzyme. The lines designated with + represent the partial sequence of the α2 chain of type V collagen (positions 961–1011 (44)), homologous to the collagen like domain of the 92-kDa type IV collagenase. Deletions are represented by –. Only the amino acid residues that differ from the top line are indicated.

These data indicate that the 92-kDa purified zymogen and TIMP exist in a very stable noncovalent complex. These data are in agreement with our previous results demonstrating that TIMP is coimmunoprecipitated with the 92-kDa proenzyme using a monospecific antibody directed against the 92-kDa protein which does not recognize TIMP. These findings demonstrate that the enzyme isolated from any cell type that secretes significant amounts of TIMP may be found in the conditioned medium.
The specific activity of the PMC-activated enzyme against 

\[^{14}C\]gelatin was between 900 and 1200 units/mg of enzyme protein. The activated enzyme has no activity on type I collagen, proteoglycan, laminin, or fibronectin, but is capable of degrading native types IV and V collagen (Fig. 10) at 32 °C. The cleavage products appeared to be identical to those generated by the 72-kDa type IV collagenase (10), as well as purified rabbit bone gelatinase (46) and polymorphonuclear leukocytes 92-kDa gelatinase (37). Analysis of substrate specificity of the 72-kDa type IV collagenase and the 92-kDa enzyme will need to be reassessed when the enzymes free of the inhibitor complex become available. Recombinant enzyme isolated from a cell line which does not express TIMP is the most likely candidate as a source of such a preparation because all the cells secreting the enzyme constitutively also secrete TIMP.

**DISCUSSION**

We have recently reported the substrate specificity and the primary structure of a 72-kDa type IV collagenase secreted by H-ras-transformed TBE-1 cells and normal skin fibroblasts which can degrade gelatin, basement membrane type IV collagen, and type V collagen (10). This metalloprotease is identical to the human tumor cell enzyme secreted by A2058 melanoma cells (47) and most probably is the human homolog of the 68-kDa murine type IV collagenase (48). In this report we describe the primary structure, purification, and substrate specificity of a new member of the secreted ECM metalloprotease gene family: 92-kDa type IV collagenase. Although this enzyme is very similar to the 72-kDa type IV collagenase in structure, substrate specificity, and properties, a striking different appears in tissue specificity and regulation of its expression. The 72-kDa type IV collagenase is expressed in vitro by normal human skin fibroblasts and a variety of tumor cells, but the 92-kDa enzyme is secreted only by some transformed cell lines of fibroblast origin and several other tumor cells. Under normal circumstances it is expressed by macrophages and epidermal keratinocytes in preference to the 72-kDa enzyme. Regulation of 92-kDa type IV collagenase expression resembles more closely that of interstitial collagenase (2). Unlike the 72-kDa type IV collagenase, expression of the 92-kDa enzyme is induced by epidermal growth factor, interleukin 1, and the tumor promoter TPA. Enzyme secretion is also increased with the differentiation of blood monocytes into alveolar macrophages.

Unlike the 72-kDa enzyme, the 92-kDa type IV collagenase contains three potential N-glycosylation sites and is glycosylated. The role of glycosylation in the metabolism of the secreted metalloproteases is not understood. Both interstitial collagenase and stromelysin are partially glycosylated (2, 6) without any noticeable effect of glycosylation on the behavior of the purified enzyme in vitro. In contrast, the bulk and most
were digested with the 92-kDa enzyme at 1:10 ratio for 6 h at 32 °C and the digestion products analyzed by SDS-PAGE and silver stained. Interaction with TIMP in vitro has been studied primarily using interstitial collagenase as a model (31-34). Our results indicate that the 72-kDa proenzyme complex become readily available. Likely all of the 92-kDa type IV collagenase protein is glycosylated (Fig. 5).

Interaction of the ECM metalloproteases with the specific inhibitor TIMP is essential for regulation of their activity in the extracellular space. Interaction with TIMP in vitro has been studied primarily using interstitial collagenase as a model (31-34).

Interaction of the 92-kDa Proenzyme with TIMP and Activation by Organomercurials—TIMP has been reported to inhibit interstitial collagenase (31-34), stromelysin (49, 50), and rabbit bone gelatinase (46, 51), by forming an enzyme-inhibitor complex only with activated enzymes. The formation of a complex between the inhibitor and the latent form of the enzyme has not been reported. Our results indicate that the purified 92-kDa type IV procollagenase and TIMP exist in a noncovalent complex that is stable in the presence of 1.0 M NaCl or 2.0 M urea. The 92-kDa enzyme isolated from PMNs was found in a TIMP-free form which is consistent with the fact that these cells do not produce TIMP (52). The absence of TIMP from that enzyme preparation most likely accounts for the 10-fold higher specific activity it exhibited against both gelatin and type IV collagen. The presence of TIMP in the U937 enzyme preparation did not prevent an activation initiated by treatment with an organomercurial resulting in the generation of an 84-kDa enzyme species. This is in agreement with the results of Murphy et al. (53) who reported that the presence of TIMP slowed but did not prevent organomercurial-initiated autoactivation of a 97-kDa metalloprotease from pig PMNs. Our data demonstrate that the 72-kDa type IV procollagenase also forms a noncovalent complex with the inhibitor. The physiological significance of the formation of a TIMP-proenzyme complex in the case of 92-kDa type IV collagenase is unclear while both interstitial collagenase and stromelysin require enzyme activation for binding to TIMP. We have not been able to distinguish the substrate specificity of organomercurial-activated 72-kDa type IV collagenase from that of the 92-kDa enzyme using types IV, or V collagen, or gelatin substrates. This issue will have to be addressed again when the proteases free of the inhibitor complex become readily available.

Mosaic Structure of the Secreted ECM Metalloproteases—Comparison of the amino acid sequences of the 92- and 72-kDa type collagenases (Fig. 6) shows that the former acquired an additional 54-amino-acid-long proline-rich domain not found in any of the previously described gene family members. The sequence of the inserted domain is homologous to the sequence of the helical region of the collagen proteins. The alignment presented in Fig. 6 was obtained with a fragment of the α2 chain of type V collagen. The size of the inserted domain corresponds to a 3-fold multiple of a typical 54-bp long exon found in the collagen genes (43). The homology and size of the insert suggest that the presence of the domain in the 92-kDa type IV collagenase is the result of the shuffling of the functional domains between this ECM degrading enzyme and structural ECM macromolecules. This is similar to the recruitment of the collagen-binding domain from fibronectin (10) present in both type IV collagenases.

Secreted ECM metalloproteases are constructed of five distinct protein domains. The simplest member of the gene family, PUMP-1 (putative metalloprotease) (11), most likely identical to the low molecular weight protease isolated from rat uterus (23, 54), consists of the amino-terminal domain...
adjacent to the zinc-binding domain. The amino-terminal domain participates in maintenance of the latent state of the enzymes and the activation of the proteases is achieved by the proteolytic processing of this domain (5, 40). The conserved amino acid sequence PRCGVPDV found within the amino-terminal domains of procollagenase, prostromelysin, transin (55), and the 72-kDa type IV procollagenase, immediately upstream from the amino terminus of the active enzymes plays an important role in the function of this domain. In transin (55) mutations within this sequence lead to variants with an increased tendency to undergo spontaneous activation. Interestingly, in the 92-kDa type IV collagenase this sequence is present immediately downstream from the amino terminus of the active enzyme. The zinc-binding domain is involved in the catalytic center of each enzyme and is encoded by a separate exon in each metalloprotease gene (4, 7, 10, 11, 56, 57). All known members of the gene family contain these two domains.

Fibroblast interstitial collagenase and stromelysin contain an additional hemopexin-like domain (11), the function of which remains speculative. We have demonstrated recently that a short, carboxyl-terminal portion of this domain is cleaved in the second step of the proteolytic activation of interstitial collagenase (5).

Both 92- and 72-kDa type IV collagenases contain an additional fibronectin-like collagen-binding domain (10). This domain consists of three 58-residue head-to-tail repeats and is responsible for the ability of both enzymes to bind to a gelatin affinity column (10).

Finally, the 92-kDa type IV collagenase contains yet an additional collagen-like domain described above. The function of this domain is not known, although it is tempting to speculate that it is responsible for the formation of the multimeric forms of the enzyme often seen on the zymogram gels. However, these forms have not been demonstrated in solution or sufficiently characterized.

It is of interest that the fibronectin-like and collagen-like domains are inserted into the protein (see Figs. 6 and 7) immediately adjacent to the zinc-binding domain. Introduction of the new sequences into the vicinity of the catalytic domain may contribute to the change in substrate specificity of the enzyme. The divergence of the fibronectin-like repeats of the 72-kDa type IV collagenase is evidently directed in a way that leads to the conservation of the secondary structural features present in interstitial collagenase immediately adjacent to the zinc-binding site (10), although no sequence homology in this portion of the two enzymes can be found. A comparison of the sequence of this domain between the 72- and 92-kDa type IV collagenases suggests a strong sequence conservation in this region (Fig. 6). Similar considerations may explain the sequence conservation pattern in the collagen-like domain of the 92-kDa type IV collagenase. The half of this domain that is proximal to the zinc-binding site is 30% identical to the α2 (V) collagen chain. In the distal half of the domain, 55% identity between the two sequences is found (Fig. 6) suggesting that the proximity to the active center, as in the case of the fibronectin-like domain, imposes additional structural constraint on the inserted protein sequence.

An understanding of the relationship between the function of the protein domains found in secreted metalloproteases and the tissue-specific pattern of their expression is indispensable for understanding the physiologic role of these enzymes.

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