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# Purification and Characterization of Two Collagenase Inhibitors From Mouse Sarcoma 180 Conditioned Medium

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**Abstract** We have previously shown that mouse sarcoma 180 cells produce vascular endothelial growth factor [VEGF; Rosenthal et al., 1990, Growth Factors, 4: 53–59], an endothelial mitogen that stimulates angiogenesis. Recent reports have implicated metalloproteinases and their inhibitors in the regulation of vascular morphogenesis, tumor invasion, and metastasis. We report here that mouse sarcoma 180 cells produce two collagenase inhibitors. These inhibitors were purified by heparin-Sepharose affinity chromatography, gel filtration, and C4 reverse phase h.p.l.c. Analytical gel electrophoresis of the purified inhibitors (MS-22 and MS-31) revealed molecular masses of 22,000 and 31,000 Da under reducing conditions, and 20,000 and 30,000 Da under nonreducing conditions, respectively. The $NH_2$-terminal amino acid sequence of MS-22 was identical to that of tissue inhibitor of metalloproteinases type 2 (TIMP-2) produced by human melanoma cells [Stetler-Stevenson et al., 1989, J. Biol. Chem. 264: 17374–17378) over the first 30 amino acids. The $NH_2$-terminal amino acid sequence of MS-31 was identical to that of murine TIMP-1 [Gewert et al., 1989, EMBO J 6: 651–657]. Statistical analysis of the amino acid composition data of these two mouse sarcoma 180-derived collagenase inhibitors confirms the identification of MS-22 as TIMP-2 and MS-31 as TIMP-1. 

**Key words:** mouse sarcoma 180 cells, tissue inhibitor of metalloproteinases, angiogenesis, endothelial cells, metalloproteinases

When injected into nude mice, mouse sarcoma 180 cells produce highly vascularized tumors that metastasize to the lung and axilla (Brem, H. and Folkman, J., unpublished data). We have recently found that mouse sarcoma 180 cells produce and secrete vascular endothelial growth factor [VEGF; Rosenthal et al., 1990], an endothelial cell mitogen that stimulates angiogenesis [Leung et al., 1989; Connolly et al., 1989]. It is becoming apparent that angiogenesis is not regulated solely by an increase in the production of endothelial growth factors, but also by other events, such as a decrease in the production of inhibitors of endothelial cell growth [Rastinejad et al., 1989] and/or an increase in matrix metalloproteinase activity [Rifkin et al., 1982; Montesano and Orci, 1985; Herron et al., 1986; Mignatti et al., 1989; Pepper et al., 1990].

Tumor cells, including mouse sarcoma 180, degrade the extracellular matrix in order to invade tissues and metastasize. Matrix metalloproteinases are believed to function in degradation and turnover of connective tissue under normal and pathological conditions. Imbalances between metalloproteinases and their inhibitors are involved in diseases such as scleroderma, fibrosis, and atherosclerosis (excess deposition), and arthritis and tumor invasion (excess destruction). Inhibitors of collagenase include:

Abbreviations used: TIMP, tissue inhibitor of metalloproteinases; CDI, cartilage-derived collagenase inhibitor; BCDI, bovine cartilage-derived collagenase inhibitor; VEGF, vascular endothelial growth factor; MS-22, mouse sarcoma 180-derived collagenase inhibitor, $M_r$ 22,000; MS-31, mouse sarcoma 180-derived collagenase inhibitor, $M_r$ 31,000.

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low molecular weight cationic proteins (11 kDa) found in cartilage and small blood vessels [Kuettner et al., 1976], $\alpha_2$-macroglobulin [Werb et al., 1974], and a family of proteins referred to as tissue inhibitors of metalloproteinases (TIMPs). The first reported member of this family is TIMP-1, a glycoprotein of approximately 30,000 Da, which is produced by normal cells such as fibroblasts [Stricklin and Welgus, 1983; Carmichael et al., 1986] and endothelial cells [DeClerck et al., 1989], and by tumor cells such as mammary carcinoma [Korczak et al., 1991], glioma cells [Apodaca et al., 1990], and other intracranial tumor cells [Halaka et al., 1983]. Moses et al. [1990] have described a TIMP-1-like cartilage-derived collagenase inhibitor (CDI) which is anti-angiogenic. A second member of the TIMP family, termed TIMP-2, has also been described. TIMP-2 has a molecular mass of 22,000 Da, and it is not glycosylated. It is produced by endothelial cells [DeClerck et al., 1989], human alveolar macrophages [Shapiro et al., 1992], and bovine scapular cartilage [Murray et al., 1986] and also by tumor cells such as human melanoma [Stetler-Stevenson et al., 1989], mouse colon 26 tumor cells [Kishi et al., 1991], and neoplastic epithelial cells [Whitelock et al., 1991].

Since metalloproteinases and their inhibitors are involved in the process of angiogenesis [Moses et al., 1990; Liotta et al., 1991], we tested the conditioned medium of mouse sarcoma 180 cells for its effect on collagenase activity. We detected considerable collagenase inhibitory activity in the conditioned medium from mouse sarcoma 180 cells, and we proceeded to purify, identify, and characterize the factor(s).

## METHODS

### Materials

Nutridoma-SP was purchased from Boehringer Mannheim (Indianapolis, IN). Armour Pharmaceuticals (Tarrytown, NY) supplied BSA. Electrophoresis chemicals were obtained from Bio-Rad (Hercules, CA). Gibco (Gaithersburg, MD) supplied Dulbecco's modified Eagle's medium (DMEM), OptiMEM, RPMI 1640, glutamine, and antibiotics. Tissue culture serum was from Hyclone (Logan, VT), except as noted. Heparin-Sepharose and Sephadex G-100 were purchased from Pharmacia (Piscataway, NJ). NEN/Dupont (Wilmington, DE) supplied $^3$H-thymidine. A C4 reverse phase h.p.l.c. column was obtained from Rainin (Woburn, MA). Sigma (St. Louis, MO) supplied immunological and other reagents.

### Cell Culture

Mouse sarcoma 180 cells were cultured and conditioned medium was collected as described by Rosenthal et al. [1990].

### Collagenase Inhibitor Assay

This assay was performed as described by Murray et al. [1986]. One unit of collagenase inhibitory activity was defined as the amount of inhibitor required to produce 50% inhibition in this assay.

### Protein Determination

Protein was determined by the method of Bradford [1976], and BSA was used as standard.

### Purification of Collagenase Inhibitors From Mouse Sarcoma 180 Conditioned Medium

Conditioned medium (2.0 l) was applied directly to a heparin-Sepharose column (8 × 2.5 cm) which had been equilibrated with 20 mM Tris-HCl (pH 7.4) and 50 mM NaCl. The column was washed with 5 column volumes of the same buffer. Bound proteins were eluted from the column with a linear gradient of 0.05–3.0 M NaCl over a total volume of 200 ml.

The fractions containing collagenase inhibitory activity were pooled and concentrated by vacuum dialysis. The concentrated sample (0.5 ml) was applied to a Sephadex G-100 column (50 × 1.5 cm) which had been equilibrated with 10 mM Tris-HCl (pH 7.4) and 0.2 M NaCl. The column was run with the same buffer, and fractions (2.7 ml) were collected, and collagenase inhibitory activity was found to elute at an approximate molecular mass of 30,000 Da.

The fractions containing peak inhibitory activity were pooled and concentrated by vacuum dialysis. The sample was diluted in 0.05% trifluoroacetic acid (v/v) in water and applied to a C4 reverse phase h.p.l.c. column (0.46 × 25 cm) equilibrated in the same buffer. The column was washed with a linear gradient of 0–30% acetonitrile (v/v) and 0.05% trifluoroacetic acid (v/v) over 15 min, followed by a gradient of 30–60% acetonitrile (v/v) and 0.05% trifluoroacetic acid (v/v) over 60 min. The flow rate was 1.0 ml/min, and fractions were collected by peaks and assayed for their inhibitory effect on collagenase activity. The collagenase inhibitory activity

eluted in a single peak at approximately 35% acetonitrile (v/v).

### Amino Acid Analysis and Sequencing

The purified MS-22 protein from the C4 h.p.l.c. column was dried by centrifugation in a Speed Vac (Savant, Farmingdale, NY). In addition, samples from the C4 h.p.l.c. column containing MS-22 and MS-31 were separated on an analytical SDS-PAGE gel and blotted to polyvinylidene difluoride (PVDF) membranes by the method of Matsudaira [1987]. The bands were stained by incubation in 0.2% Ponceau S (w/v) in 1% acetic acid (v/v) and then destained by extensive washing with water. Bands corresponding to MS-22 and MS-31 were excised with a razor blade and analyzed as described below. Analyses of MS-22 obtained directly from the h.p.l.c. fraction or obtained after subsequent SDS-PAGE and blotting to PVDF membrane yielded the same results.

A portion of the purified sample(s) was analyzed for amino acid composition on an Applied Biosystems 420A derivatizer/analyzer [Atherton, 1989]. The results were analyzed using the SΔn index of Cornish-Bowden: $S\Delta n = \frac{1}{2} \Sigma (n_{iA} - n_{iB})^2 - 0.035 (N_A - N_B)^2 + 0.535 |N_A - N_B|$, where $n_{iA}$ is the number of residues of the ith type of amino acid in protein A, and $N_A = \Sigma n_{iA}$ [Cornish-Bowden, 1979]. If the value of SΔn calculated for two proteins is $< 0.4$ N, then the proteins are likely ($> 95\%$) to be related [Cornish-Bowden, 1977]. The lower the value of SΔn, the more related the proteins. This method has been previously used to compare the amino acid compositions of different collagenase inhibitors [Murray et al., 1986; Staskus et al., 1991]. Only residues determined for both proteins were used in the calculation.

The remaining sample was reduced, and cysteine residues were alkylated. The desalted sample was subjected to automated Edman degradation on an ABI Model 477A protein sequencer with modifications for a faster cycle time (36 min). The resultant phenylthiohydantoin amino acid fractions were subsequently identified manually using an on-line ABI Model 120A h.p.l.c. Further details are available upon request.

### Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed as described by Laemmli and Favre [1973]. Polyacrylamide gels (12%) were run at 25 mA constant current at room temperature in an electrode buffer composed of 25 mM Tris (pH 8.3), 192 mM glycine, and 0.1% SDS. Silver staining [Oakley et al., 1980] was used to visualize protein bands.

In order to separate MS-22 and MS-31, preparative SDS-PAGE on a Bio-Rad Model 491 Prep Cell was performed according to the manufacturer's instructions. The separating gel consisted of 12% acrylamide (w/v), and the resolving gel was 4% acrylamide (w/v). The gel sample buffer was prepared without β-mercaptoethanol. The gel was run at 40 mA constant current for 6 h, and flow rate of the elution buffer (10 mM Tris and 10 mM NaCl, pH 8.3) was 45 ml/h. Fractions were collected and analyzed by analytical SDS-PAGE and tested for collagenase inhibitory activity following dialysis against 10 mM Tris-HCl, pH 7.4, and 10 mM NaCl.

## RESULTS

We have previously reported that conditioned medium from mouse sarcoma 180 cells stimulated DNA synthesis in bovine capillary endothelial cells, due in part to the presence of vascular endothelial growth factor [Rosenthal et al., 1990]. Because metalloproteinases and their inhibitors are also involved in the processes of angiogenesis and metastasis, we tested this conditioned medium for its effect on collagenase activity and found considerable collagenase inhibitory activity (Table I). To ensure the separation of VEGF from this collagenase inhibitory activity, the conditioned medium was applied to a heparin-Sepharose column, and the column was subsequently washed with a linear gradient

**TABLE I. Purification of Collagenase Inhibitors From Mouse Sarcoma 180 Conditioned Medium**

| Step | Protein (mg) | Collagenase inhibition, total activity (units) | Collagenease inhibition, specific activity (units/mg) | *Recovery* (%) |
|---|---|---|---|---|
| Conditioned medium | 260 | 13,423 | 52 | 100 |
| Heparin-Sepharose | 2.28 | 93 | 41 | 0.71 |
| Sephadex G-100 | 0.768 | 760 | 990 | 5.7 |
| C4 h.p.l.c. | 0.045 | 2,024 | 44,978 | 15.1 |

of NaCl. The peak of collagenase inhibitory activity was eluted at approximately 0.4 M NaCl. This activity was well separated from VEGF, which elutes at about 0.9 M NaCl [Rosenthal et al., 1990].

The collagenase inhibitory activity was further purified by gel filtration and C4 reverse phase h.p.l.c. (Table I). It is unclear why the total collagenase inhibitory activity increased during the course of the purification, but these results were reproducible over a number of batches analyzed. One possible explanation is that an unknown protein or factor present in the conditioned medium was interfering with the assay, and this factor was gradually removed during the purification.

Silver staining of an SDS-PAGE gel of the h.p.l.c.-purified fractions revealed two protein bands: one corresponding to 22,000 Da (MS-22); and another at 31,000 Da (MS-31; Fig. 1), which was present in smaller amounts. MS-22 was present in some fractions in the absence of MS-31, but MS-31-containing fractions also contained MS-22. Pure MS-22 and MS-31 were obtained by fractionation of these latter fractions on preparative SDS-PAGE (Fig. 1).

Like other similar collagenase inhibitors, the mouse sarcoma 180-derived collagenase inhibitors exhibited higher apparent molecular masses under reducing than under nonreducing conditions [Stetler-Stevenson et al., 1989; Stricklin and Welgus, 1983; Murray et al., 1986]. MS-22 exhibited an apparent molecular mass of 22,000 Da under reducing conditions and 20,000 Da under nonreducing conditions (Fig. 1). Similarly, MS-31 ran as 31,000 Da under reducing conditions and 30,000 Da under non-reducing conditions (Fig. 1).

The $NH_2$-terminal amino acid sequence of the mouse sarcoma 180-derived MS-22 was determined to be: Cys-Ser-Cys-Ser-Pro-Val-His-Pro-Gln-Gln-Ala-Phe-Cys-Asn-Ala-Asp-Val-Val-Ile-Arg-Ala-Lys-Ala-Val-Ser-Glu-Lys-Glu-Val-Asp. Comparison with $NH_2$-terminal amino acid sequences of TIMPs and related molecules (Table II) reveals that MS-22 was identical to TIMP-2

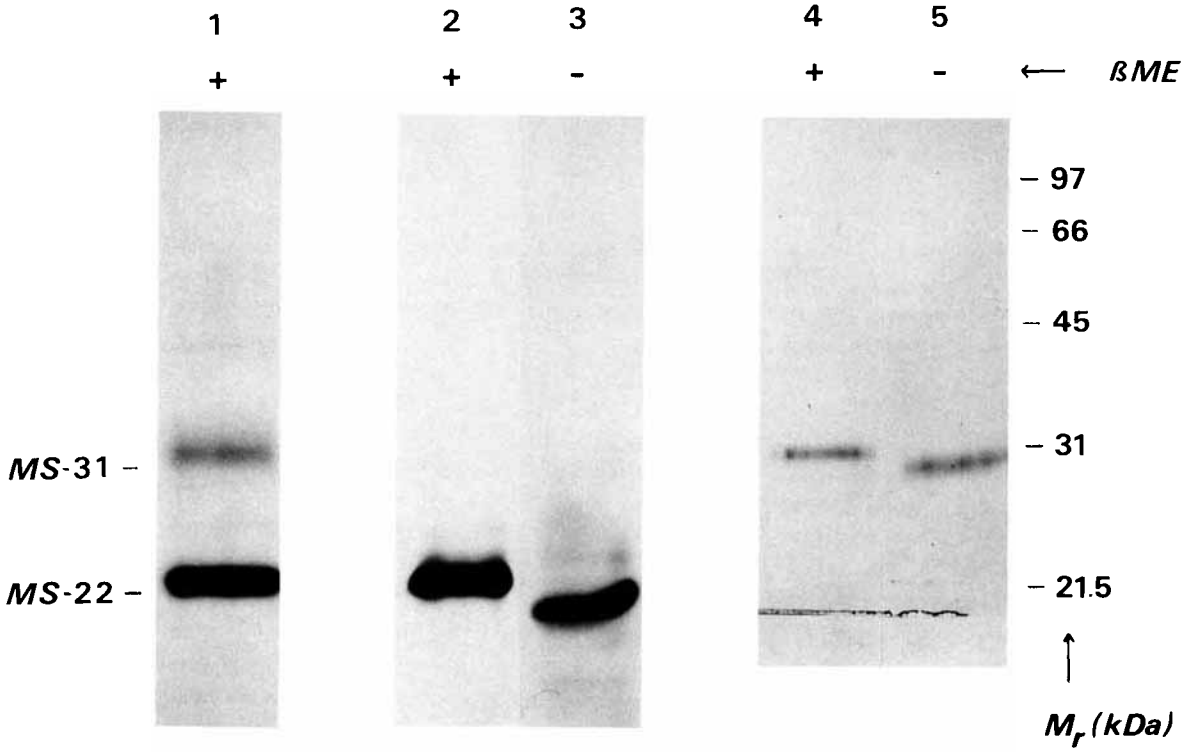


**Fig. 1.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). C4 column fractions containing both MS-22 and MS-31 (lane 1) were analyzed by SDS-PAGE in the presence of β-mercaptoethanol (βME) as described by Laemmli and Favre [1973]. The C4 column fraction (50 ng) containing MS-22 was analyzed by SDS-PAGE in the presence (lane 2) or absence (lane 3) of 0.18 mM β-mercaptoethanol. A fraction from preparative SDS-PAGE containing pure MS-31 was also run in the presence (lane 4) or absence (lane 5) of 0.18 mM β-mercaptoethanol. The protein bands were visualized by silver staining [Oakley et al., 1980]. The positions of the reduced molecular weight standards are indicated in the margin.

[Stetler-Stevenson et al., 1989] and 96% identical to bovine cartilage-derived collagenase inhibitor [BCDI; Murray et al., 1986] over the first 30 amino acids. It shared 60% sequence identity to murine TIMP-1 [Gewert et al., 1987] over the same region (Table II). The $NH_2$-terminal amino acid sequence of MS-31 was determined to be: X-Ser-Cys-Ala-Pro-Pro-His-Pro-Gln-Thr-Ala-Phe-Cys-Asn-Ser-Asp-Leu-Val-Ile-Arg- Ala-Lys-Phe-Met-Gly-Ser. This sequence was identical to the $NH_2$-terminal sequence of murine TIMP-1 [Gewert et al., 1987].

Because of the highly conserved nature of the $NH_2$-terminal amino acid sequence within the TIMP family [Staskus et al., 1991], MS-22 and MS-31 were subjected to amino acid analysis. The composition data is reported in Tables III and IV. The data were analyzed by the method of Cornish-Bowden, using the index SΔn, where SΔn is a conservative estimate of the number of differences between two protein sequences [Cornish-Bowden, 1977, 1979]. According to this method, the lower the value of SΔn, the more related the proteins. The Cornish-Bowden method has been previously used to compare the amino acid composition data of various members of the TIMP family [Murray et al., 1986; Staskus et al., 1991]. When applied to our data, this analysis predicted that MS-22 was closely related to TIMP-2 (SΔn = 21; Table III) and MS-31 was closely related to TIMP-1 (SΔn = 48; Table IV). These predictions are consistent with the $NH_2$-terminal amino acid sequence data.

## DISCUSSION

We report here that mouse sarcoma 180 cells produce at least two different but related metalloproteinase inhibitors, MS-22 and MS-31. Given the low recovery of collagenase inhibitory activity in the first step of the purification, it is possible that another metalloproteinase inhibitor(s) is also present in the mouse sarcoma 180 conditioned medium. Like members of the TIMP family, MS-22 and MS-31 exhibit molecular masses of 22,000 and 31,000 Da under reducing conditions, and slightly lower molecular masses under non-reducing conditions. In addition, we have found the $NH_2$-terminal amino acid sequence of MS-22 to be identical to TIMP-2 over the first 30 amino acids [Stetler-Stevenson et al., 1989] and almost identical to BCDI [Murray et al., 1986]. The $NH_2$-terminal sequence of MS-31 is identical to that of murine TIMP-1 (Table II). Analysis of amino acid composition data (see Table III) of MS-22 and MS-31 is consistent with their identification as TIMP-2 and TIMP-1, respectively.

Because of the high degree of sequence identity in the $NH_2$-terminus among members of the TIMP family [Murray et al., 1986; Boone et al., 1990; Staskus et al., 1991], $NH_2$-terminal amino acid sequence similarities may not necessarily predict that two collagenase inhibitors are the same. In addition to $NH_2$-terminal sequence data, amino acid composition analysis has been used to compare potentially related proteins, including metalloproteinase inhibitors [Murray et al., 1986; Staskus et al., 1991]. The TIMP family of inhibitors has been subdivided into two groups based on significant amino-terminal sequence similarities and amino acid composition analysis [Staskus et al., 1991; Moses et al., 1991a]. One group consists of TIMP-1 (tissue inhibitor of metalloproteinase type 1) from human skin fibroblasts [Stricklin and Welgus, 1983], CDI, a cartilage-derived collagenase inhibitor [Moses et al., 1990], and an avian 21 kDa protein [Staskus et al., 1991]. The second group consists of BCDI, a second cartilage-derived inhibitor [Murray et al., 1986], TIMP-2 from human melanoma cells [Stetler-Stevenson et al., 1989], and metalloproteinase inhibitor from bovine aortic endothelial cells [DeClerck et al., 1989]. Table II compares the $NH_2$-terminal se-

**TABLE II. $NH_2$-Terminal Amino Acid Sequences of MS-22 and MS-31***

| | 10 | 20 | 30 |
|---|---|---|---|
| TIMP-2[a] | **CSCSPVHPQQAFCNADVVIRAKA V SEKEVD** | | |
| MS-22 | **CSCSPVHPQQAFCNADVVIRAKA V SEKEVD** | | |
| mTIMP-1[b] | **CSCAPPHPQTAFCNSDLVIRAKFMGSPRIN** | | |
| MS-31 | **—SCAPPHPQTAFCNSD**LVIR**AKFM**GS | | |

*The $NH_2$-terminal amino acid sequences were determined as described in Methods. —, no determination. **Bold** residues indicate determination with high confidence. Non-bold residues indicate determination with less than full confidence.
[a]Human TIMP-2, Stetler-Stevenson et al., 1989.
[b]Murine TIMP-1, Gewert et al., 1987.

**TABLE III. Amino Acid Composition of MS-22 and Other TIMP-2-Like Proteins***

| | MS-22 (pmol) | MS-22 (residues/molecule) | TIMP-2[a] (residues/molecule) | Bovine MI[b] (residues/molecule) | BCDI[c] (residues/molecule) |
|---|---|---|---|---|---|
| Ala | 851 | 14 | 14 | 16 | 18 |
| Asx | 1,215.9 | 20 | 19 | 22 | 27 |
| Glx | 1,306.4 | 21 | 22 | 19 | 28 |
| Phe | 437.7 | 7 | 7 | 7 | 9 |
| Gly | 907 | 15 | 12 | 13 | 22 |
| His | 262.6 | 4 | 4 | 4 | 4 |
| Ile | 907.4 | 15 | 18 | 19 | 21 |
| Lys | 1,066.9 | 18 | 19 | 17 | 21 |
| Leu | 566.5 | 9 | 7 | 7 | 12 |
| Met | 192.8 | 3 | 1 | 5 | 5 |
| Pro | 737 | 12 | 10 | 12 | 15 |
| Arg | 465.7 | 8 | 7 | 6 | 11 |
| Ser | 750.5 | 12 | 12 | 10 | 17 |
| Thr | 458.9 | 8 | 8 | 6 | 10 |
| Val | 572.4 | 9 | 9 | 8 | 11 |
| Tyr | 229.9 | 4 | 6 | 7 | 9 |
| Cys | — | — | 12 | 12 | 10 |
| Trp | — | — | 4 | 4 | — |
| SΔn *vs.* MS-22 | | 0 | 21 | 32 | 53 |
| SΔn *vs.* MS-31 | | 118 | 175 | 216 | 222 |

*Amino acid analysis was performed as described in Methods. Residues/molecule were calculated from pmol data to obtain a total number of amino acids consistent with that reported for other members of the TIMP family,[a,b,c] excluding Cys and Trp. SΔn is an unbiased estimator of the number of differences between the two sequences [Cornish-Bowden, 1977]. The amino acid composition of MS-31 is given in Table IV. —, not determined.

[a]Stetler-Stevenson et al., 1989, from amino acid sequence analysis.

[b]Metalloproteinase inhibitor [Boone et al., 1990] from cDNA sequence analysis.

[c]Bovine cartilage-derived collagenase inhibitor [Murray et al., 1986] from amino acid composition analysis.

quences of TIMP-1 and TIMP-2 with MS-22 and MS-31. As previously reported, TIMP-1 and TIMP-2 do not appear to be related to each other as judged by statistical analysis of the amino acid composition despite the similarities in $NH_2$-terminal sequences [Murray et al., 1986; Staskus et al., 1991]. The $NH_2$-terminal sequence of MS-22 is identical to TIMP-2 (see above); statistical analysis of the amino acid composition data confirms that MS-22 is closely related to TIMP-2, bovine metalloproteinase inhibitor (MI), and BCDI (see Results and Table III). Similarly, both the $NH_2$-terminal sequence (Table II) and the amino acid composition analysis (Table IV) of MS-31 and TIMP-1 support the conclusion that they are closely related.

Mouse sarcoma 180, like other solid tumors, requires angiogenesis for growth [Folkman, 1971, 1990]. Tumors produce endothelial cell growth factors [Folkman and Klagsbrun, 1987] and inhibitors [Rastinejad et al., 1989]. We have observed that mouse sarcoma 180 produces VEGF [Rosenthal et al., 1990], an endothelial cell mitogen which stimulates angiogenesis in vivo [Leung et al., 1989; Connolly et al., 1989]. VEGF has also been shown to stimulate the production of urokinase-type and tissue-type plasminogen activators by microvascular endothelial cells [Pepper et al., 1991]. Another endothelial cell mitogen, basic fibroblast growth factor, has also been shown to enhance production of proteases by endothelial cells [Rifkin, 1982; Pepper et al., 1990]. Degradation of the extracellular matrix is one step in the angiogenic process; correspondingly, at least one endogenous protease inhibitor can block neovascularization. The TIMP-like cartilage-derived collagenase inhibitor [CDI; Moses et al., 1990; Moses and Langer, 1991b] and a related chondrocyte-derived collagenase inhibitor [ChDI; Moses et al., 1992] have been shown to inhibit angiogenesis in vivo and in vitro.

In addition to regulation of vascular morphology, the balance between proteinases and their inhibitors helps to regulate tumor invasion and metastasis [Herron et al., 1986; Mignatti et al.,

**TABLE IV. Amino Acid Composition of MS-31 and Other TIMP-1-Like Proteins***

| | MS-31 (pmol) | MS-31 (residues/molecule) | Murine TIMP-1[a] (residues/molecule) | Human TIMP-1[b] (residues/molecule) | Chicken 21-kDa[c] (residues/molecule) |
|---|---|---|---|---|---|
| Ala | 134.4 | 16 | 13 | 11 | 9–10 |
| Asx | 126.9 | 15 | 13 | 9 | 14–15 |
| Glx | 158.9 | 19 | 13 | 21 | 17 |
| Phe | 59.3 | 7 | 9 | 10 | 5 |
| Gly | 124.8 | 15 | 11 | 12 | 23–24 |
| His | 48.4 | 6 | 5 | 6 | 3 |
| Ile | 60.7 | 7 | 7 | 7 | 8–9 |
| Lys | 101.2 | 12 | 11 | 8 | 8–9 |
| Leu | 121.9 | 15 | 16 | 18 | 12 |
| Met | 14.6 | 2 | 5 | 3 | 4 |
| Pro | 94.1 | 11 | 10 | 10 | 8–9 |
| Arg | 100.2 | 12 | 13 | 10 | 10 |
| Ser | 127.5 | 16 | 15 | 13 | 14 |
| Thr | 103.7 | 13 | 11 | 16 | 15 |
| Val | 85.4 | 10 | 9 | 9 | 11 |
| Tyr | 38.4 | 5 | 7 | 6 | 9–10 |
| Cys | — | — | 12 | 12 | 8–9 |
| Trp | — | — | 3 | 3 | — |
| SΔn vs. MS-22 | | 118 | 180 | 239 | 197 |
| SΔn vs. MS-31 | | 0 | 48 | 68 | 101 |

*Amino acid composition and statistical analysis was performed as described in the legend to Table III. The amino acid composition of MS-22 is given in Table III.
[a]Gewert et al., 1987, from cDNA sequence analysis.
[b]Carmichael et al., 1986, from cDNA sequence analysis.
[c]Staskus et al., 1991, from amino acid composition analysis.

1989; Pepper et al., 1990; Liotta et al., 1991], and collagenase inhibitors produced by mouse sarcoma 180 cells may also be involved in these processes. Tumor cells produce both proteinases and proteinase inhibitors [for review see Goldberg and Eisen, 1991; Khokha and Denhardt, 1989]. Accumulating evidence suggests that increased production of proteinases or decreased production of proteinase inhibitors could enhance tumorigenesis and metastasis. Several studies have demonstrated that the production of metalloproteinase activity by tumor cells is correlated with tumor invasion and metastatic potential [Liotta et al., 1980; Turpeenniemi-Hujanen et al., 1985; Nakajima et al., 1987; Korczak et al., 1991]. Murine fibrosarcoma cells which secrete decreased TIMP levels were found to have a higher invasive potential than normal cells or fibrosarcoma cells which secrete normal levels of TIMP [Hicks et al., 1984]. In addition, transfection of TIMP antisense RNA into Swiss 3T3 cells has been reported to result in the ability of the transfected cells to form tumors and metastases in nude mice [Khokha et al., 1989]. In contrast to these reports, Stetler-Stevenson et al. [1990] observed that expression of TIMP-1 mRNA in adenocarcinoma was elevated compared to normal colonic mucosa. Shifting the proteolytic balance by the addition of exogenous proteinase inhibitors has also been demonstrated to block metastasis. For example, recombinant TIMP-1 [Schultz et al., 1988; Alvarez et al., 1990] and recombinant TIMP-2 [DeClerck et al., 1991] inhibit tumor metastasis in mice. These findings suggest that one strategy for treating a number of tumors and other "angiogenic diseases" characterized by pathological neovascularization [Folkman and Klagsbrun, 1987] might be the control of metalloproteinases [Hicks et al., 1984; Moses and Langer, 1991a].

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