A Recombinant Human Stromelysin Catalytic Domain Identifying Tryptophan Derivatives as Human Stromelysin Inhibitors

Qi-Zhuang Ye,*,† Linda L. Johnson,† Ian Nordan,† Donald Hupe,† and Lynn Hupe‡

Departments of Biochemistry and Biotechnology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

Received May 24, 1993•

The human stromelysin catalytic domain (SCD) has been expressed in *Escherichia coli* and purified to homogeneity (Ye et al. *Biochemistry* **1992**, *31*, 11231). We have used this recombinant SCD for inhibitor screening and identified tryptophan derivatives as competitive inhibitors of SCD. Both Cbz-L-Trp-OH (1, IC_{50} 2.5 μM, K_i 2.1 μM) and Boc-L-Trp-OH (3, IC_{50} 10 μM, K_i 8 μM) showed good inhibitory activity. Modification at the indole nitrogen with formyl or mesitylene-2-sulfonyl group (16, IC_{50} 34 μM, K_i 28 μM; 17, IC_{50} 63 μM, K_i 52 μM) showed reduced activity. The amide Cbz-L-Trp-NH_2 (13) was not active, but esters Cbz-L-Trp-OSu (14, IC_{50} 13 μM, K_i 11 μM) and Boc-L-Trp-OSu (15, IC_{50} 102 μM, K_i 84 μM) showed activity. Aromatic amino acid derivatives Cbz-L-Tyr-OH (18, IC_{50} 24 μM, K_i 20 μM) and Cbz-L-Phe-OH (26, IC_{50} 40 μM, K_i 33 μM) were also active, but other amino acid derivatives had no activity. Although Cbz-D-Trp-OH (2, IC_{50} 86 μM, K_i 71 μM) was active, the L-configuration is consistently preferred for inhibitory activity. Some of the SCD inhibitors were tested on full-length human stromelysin purified from cultured human cells, and they showed the same potency rank order. These results demonstrate the usefulness of recombinant DNA technology in generating the authentic human protein with improved properties for drug discovery.

Matrix metalloproteinases are a family of zinc-containing enzymes involved in connective tissue degradation.[1,2] They include interstitial collagenase, neutrophil collagenase, 72-kDa gelatinase, 92-kDa gelatinase, stromelysin, stromelysin-2, stromelysin-3, and matrilysin. Collectively, they degrade extracellular matrix components including interstitial collagens (types I-III), basement membrane type IV collagen, fibronectin, laminin, and various proteoglycans. Matrix metalloproteinases have been implicated in several pathological processes. For example, stromelysin not only degrades proteoglycans but also activates collagenase[3] and has been implicated in cartilage degradation in arthritis.[4,5] The cDNAs of some matrix metalloproteinases such as matrilysin (formerly called pump-1),[6] stromelysin-2,[6] and stromelysin-3[7] were isolated in tumor cells, and their expressions are often associated with the metastatic potential of the cancerous cells. Clearly, potent and selective inhibitors for matrix metalloproteinases are potential therapeutic agents for controlling pathological processes in arthritis, cancer metastasis, and other diseases involving connective tissue degradation.

Matrix metalloproteinases share high protein sequence homology, and their sequences are comprised of signal peptide, propeptide, catalytic domain, and C-terminal domain[1,6] (Figure 1). They are all secreted as a proenzyme and activated in vivo by removal of the propeptide by unknown mechanisms. In vitro, they can be activated with organomercurial compounds, proteolytic enzymes, chaotropic agents, or heat.[8] The activated enzyme undergoes autolytic cleavage at sites close to the C-terminus, producing heterogeneous protein fragments.[9,10] Matrilysin

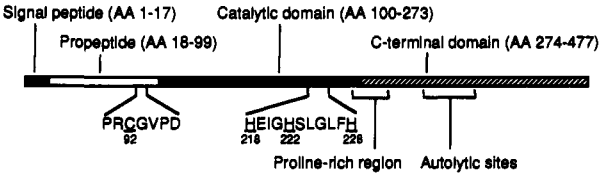

Figure 1. Schematic representation of human stromelysin polypeptide structure. The catalytic domain was expressed and used for inhibition studies. The underlined residues in the partial sequences are believed to be ligands for the catalytic zinc.

is an active matrix metalloproteinase although it lacks the C-terminal domain.[11] C-Terminal truncated matrix metalloproteinases have also been generated by expression and shown to exhibit the proteinase activity.[12-16] Therefore, the catalytic domain of matrix metalloproteinases is responsible and sufficient for the proteinase activity.

Using recombinant DNA technology, we have expressed human stromelysin catalytic domain (SCD) in *Escherichia coli* and purified it to homogeneity.[17] The SCD, which starts at Phe-100 and terminates at Pro-273 (Figure 1), was expressed as an active enzyme without the propeptide and the C-terminal domain. Because there is no propeptide, activation was not required before proteinase assay, and the SCD is stable due to the removal of autolytic sites in the C-terminal domain. The SCD cleaves the natural substrate proteoglycan and synthetic substrates such as the thiopeptolide Ac-Pro-Leu-Gly-S-Leu-Leu-Gly-OEt originally developed for collagenase.[18] We report here the results from the screening for matrix metalloproteinase inhibitors using the recombinant SCD.

Inhibition of the Recombinant SCD by Tryptophan Derivatives. The proteinase activity of SCD was assayed as the hydrolysis of the thiopeptolide substrate Ac-Pro-Leu-Gly-S-Leu-Leu-Gly-OEt by monitoring the change of UV absorbance in the presence of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). The availability of large quantities of recombinant SCD and the convenient assay procedure prompted us to screen randomly for inhibitors

† Department of Biochemistry.
‡ Department of Biotechnology.
* Send correspondence to: Dr. Qi-Zhuang Ye, Department of Biochemistry, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105. Phone: (313) 998-5909. Fax: (313) 996-1355. E-mail: YEQ@AA.WL.COM.
• Abstract published in *Advance ACS Abstracts*, December 1, 1993.

0022-2623/94/1837-0206$04.50/0

Table 1. Inhibitory Activities of the Amino Acid Derivatives on the Recombinant SCD and the Full-Length Stromelysin (FLS)

		SCD		FLS	
no.	compd[a]	IC_{50} (μM)[b]	K_i (μM)[c]	IC_{50} (μM)	K_i (μM)
1	Cbz-L-Trp-OH	2.5 ± 0.9	2.1	7.5 ± 1.8	6.3
2	Cbz-D-Trp-OH	86 ± 34	71	287 ± 31	241
3	Boc-L-Trp-OH	10 ± 4	8		
4	Boc-D-Trp-OH	100–500			
5	H-L-Trp-OH	>500			
6	H-D-Trp-OH	>500			
7	Ac-L-Trp-OH	100–500			
8	Ac-D-Trp-OH	100–500			
9	Fmoc-L-Trp-OH	100–500			
10	Fmoc-D-Trp-OH	100–500			
11	Bz-L-Trp-OH	100–500			
12	FA-L-Trp-OH	145 ± 58	120		
13	Cbz-L-Trp-NH_2	100–500			
14	Cbz-L-Trp-OSu	13 ± 8	11		
15	Boc-L-Trp-OSu	102 ± 39	84		
16	Boc-L-Trp(For)-OH	34 ± 6	28		
17	Boc-L-Trp(Mts)-OH	63 ± 6	52		
18	Cbz-L-Tyr-OH	24 ± 10	20	22 ± 2	18
19	Cbz-D-Tyr-OH	432 ± 215	356	450 ± 69	377
20	Boc-L-Tyr-OH	100–500			
21	Boc-D-Tyr-OH	>500			
22	Ac-L-Tyr-OH	>500			
23	Ac-D-Tyr-OH	>500			
24	Cbz-L-Tyr-NH_2	>500			
25	Cbz-L-Tyr-$NHNH_2$	>500			
26	Cbz-L-Phe-OH	40 ± 8	33	99 ± 52	83
27	Cbz-D-Phe-OH	>500		>500	
28[d]		0.018 ± 0.004	0.015	0.016 ± 0.004	0.013

[a] Abbreviations: Cbz, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Ac, acetyl; Fmoc, 9-fluorenylmethyloxycarbonyl; Bz, benzoyl; FA, 3-(2-furylacryloyl); OSu, *N*-hydroxysuccinimide ester; For, formyl; and Mts, mesitylene-2-sulfonyl. [b] Inhibition for SCD was initially tested at 100 and 500 μM. IC_{50} values were determined when the compound showed more than 50% inhibition at 100 μM. [c] K_i values were derived from IC_{50} values by using the equation $K_i = IC_{50}/(1 + [\text{substrate}]/K_m)$, where substrate concentration was 100 μM, K_m for SCD was 469 μM, and K_m for FLS was 518 μM. [d] 28, $HONHCOCH_2CH(i\text{-}Bu)COLeu\text{-}Phe\text{-}NH_2$.

of matrix metalloproteinases. Tryptophan derivative Cbz-L-Trp-OH (1, IC_{50} 2.5 μM, K_i 2.1 μM) emerged in the screening as a good SCD inhibitor. Most matrix metalloproteinase inhibitors previously described are peptides with a chelating group, e.g., the hydroxamide 28 (U24522, $HONHCOCH_2CH(i\text{-}Bu)COLeu\text{-}Phe\text{-}NH_2$).[19] Although a tryptophan residue can be found in some of the known inhibitors, the good inhibitory activity from a simple modified tryptophan was unexpected. To understand the structure–function relationship for SCD inhibition, we tested structurally related compounds with the recombinant SCD, and the results are summarized in Table 1.

L-Tryptophan (5) itself does not inhibit SCD. Among tryptophans with different amino-protecting groups, Boc-L-Trp-OH (3, IC_{50} 10 μM, K_i 8 μM) showed good activity. Tryptophan with a 3-(2-furylacryloyl) group (12, IC_{50} 145 μM, K_i 120 μM) showed weak activity, and those with 9-fluorenylmethyloxycarbonyl, acetyl, or benzyl groups (7-11) were not active.

Attaching formyl or mesitylene-2-sulfonyl group to the indole nitrogen reduced the activity by only a few fold (16, IC_{50} 34 μM, K_i 28 μM; 17, IC_{50} 63 μM, K_i 52 μM) compared to 3. The amide 13 was not active, but the *N*-hydroxysuccinimide esters 14 (IC_{50} 13 μM, K_i 11 μM) and 15 (IC_{50} 102 μM, K_i 84 μM) showed reduced activity compared to the parents 1 and 3.

The interaction of the inhibitors with SCD is apparently specific as suggested by the different inhibitory activities by L- and D-isomers. Consistently the L-configuration is preferred for inhibition (compare 1 vs 2, 3 vs 4, 18 vs 19, and 26 vs 27). The inhibition of SCD by Cbz-L-Trp-OH (1) is competitive (Figure 2), indicating that the inhibitor binds to SCD at the active site.

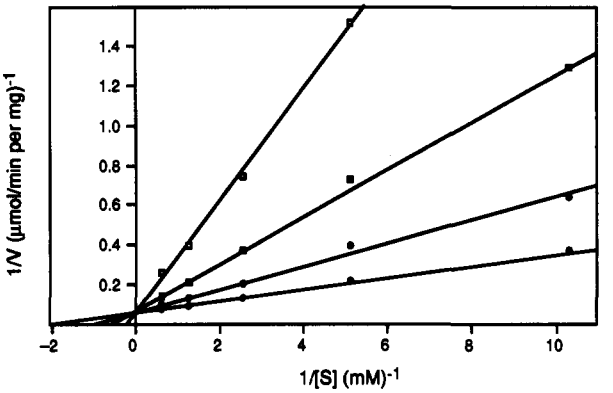

Figure 2. Competitive inhibition of SCD by Cbz-L-Trp-OH (1). Closed circle, no inhibitor; open circle, 1 μM inhibitor; closed square, 3 μM inhibitor; and open square, 10 μM inhibitor.

Besides tryptophan derivatives, other amino acids with the Cbz-protecting group were also evaluated. Two aromatic amino acid derivatives Cbz-L-Tyr-OH (18, IC_{50} 24 μM, K_i 20 μM) and Cbz-L-Phe-OH (26, IC_{50} 40 μM, K_i 33 μM) showed inhibitory activity. All other amino acid derivatives tested showed IC_{50} values higher than 100 μM, which include Cbz-L-Ala-OH, Cbz-D-Ala-OH, Cbz-D-Arg-OH, Cbz-L-Asn-OH, Cbz-D-Asn-OH, Cbz-L-Asp-OH, Cbz-D-Asp-OH, Cbz-L-Gln-OH, Cbz-D-Gln-OH, Cbz-L-Glu-OH, Cbz-D-Glu-OH, Cbz-Gly-OH, Cbz-L-His-OH, Cbz-D-His-OH, Cbz-L-Ile-OH, Cbz-L-Leu-OH, Cbz-D-Leu-OH, Cbz-L-Lys-OH, Cbz-D-Lys-OH, Cbz-L-Met-OH, Cbz-D-Met-OH, Cbz-L-Pro-OH, Cbz-D-Pro-OH, Cbz-L-Ser-OH, Cbz-D-Ser-OH, Cbz-L-Thr-OH, Cbz-D-Thr-OH, Cbz-L-Val-OH, and Cbz-D-Val-OH, in addition to those listed in Table 1.

Comparison of the Inhibitory Activity with SCD and the Full-Length Stromelysin. The SCD was made

by genetic removal of the C-terminal domain, and it is important to know whether this truncation modifies the activity and specificity of the native full-length stromelysin. Marcy et al.[12] have reported that their short form stromelysin (K_m 1.4 mM, k_{cat} 58 min^{-1}), a protein similar to SCD, showed kinetic properties almost identical to the full-length human stromelysin (K_m 0.9 mM, k_{cat} 60 min^{-1}) using nor-Leu[11]-substance P as the substrate.[12] Using our SCD with the thiopeptolide as the substrate, we obtained similar kinetic parameters for SCD (K_m 469 μM, k_{cat} 4.7 s^{-1}) and for full-length human stromelysin (K_m 518 μM, k_{cat} 5.0 s^{-1}). Our results are in good agreement with those reported by Marcy et al.,[12] and both studies confirm that the catalytic domain of stromelysin has the same activity as the full-length stromelysin.

Without structural information, the spatial relationship between the catalytic domain and the C-terminal domain is not clear. If the C-terminal domain interacts with the catalytic domain at or near the active site, then full-length stromelysin may have different substrate and inhibitor specificity than the truncated version, SCD. In order to verify the therapeutic value of the inhibitors discovered from screening using the recombinant SCD, we also tested them on the human stromelysin isolated from cultured human cells, and the results are also summarized in Table 1. The inhibitors for SCD also inhibit the full-length human stromelysin with the same potency rank order. Clearly, SCD retains not only the full proteinase activity but also the specificity for inhibitors. Some of the inhibitors appear less potent against full-length stromelysin compared with SCD, and they may reflect the influence of the C-terminal domain on the conformation of the catalytic domain or steric interference of the C-terminal domain on inhibitor binding.

Discussion

We have demonstrated the utility of the recombinant SCD in inhibitor discovery for human stromelysin. Tryptophan derivatives showed good inhibition for the recombinant SCD, and SCD inhibitors also inhibit full-length human stromelysin with the same potency rank order. This example has shown several advantages of using recombinant DNA technology in drug discovery.

It is difficult to obtain authentic human proteins for drug screening. Similar proteins purified from animal sources or heterogeneous animal tissue homogenates are often used instead for evaluating enzyme inhibitors.[20] Although some matrix metalloproteinases could be purified from mammalian cell lines, expression of SCD in *E. coli* has several advantages. Using recombinant DNA technology, we obtained large quantities of homogenous human protein from *E. coli* for inhibitor screening. It also makes it possible to study enzyme mechanism through mutagenesis of the cloned gene and elucidate protein structure by NMR spectrometry and X-ray analysis. With control over modifications of both the enzyme and inhibitors, we can better understand the mechanism of catalysis and inhibition of SCD and develop potent and selective inhibitors.

Using recombinant DNA technology, we also generated an enzyme with improved properties for inhibitor screening and structural and mechanistic studies. Activated full-length stromelysin is not stable, and autolytic cleavage generates heterogeneous smaller fragments upon incubation.[10,21] The SCD showed remarkable stability due to the removal of the autolytic sites in the C-terminal domain.[17] In addition, no activation is required before the proteinase assay because SCD has no propeptide.

The tryptophan moiety exists in many matrix metalloproteinase substrates and inhibitors. Niedzwiecki et al.[22] studied the substrate specificity of human stromelysin and found that tryptophan is the preferred residue at the P_2' site. The tryptophan residue is also preferred in substrates of both human fibroblast and neutrophil collagenases at the P_2' site.[23] Considering the high protein sequence homology among matrix metalloproteinases, it is not surprising to see that the tryptophan is the preferred P_2' residue in both stromelysin and collagenase substrates. Darlak et al.[24] developed a series of thio-based inhibitors for collagenase and found that the inhibitors were more potent when an aromatic L-amino acid, especially tryptophan, is present at the P_2' site. Galardy and co-workers developed peptide hydroxamic acids[25] and peptide phosphoramidates[26] as human skin fibroblast collagenase inhibitors, and in both cases, tryptophan is favored at the P_2' site. The competitive inhibition pattern shown by 1 (Figure 2) suggests that the tryptophan derivatives identified in this study interact with SCD at the active site and possibly bind to the S_2' site on SCD. Since tryptophan derivatives are a unique class of inhibitors lacking strong chelating groups, the mechanism of inhibition needs further study. With the assignments of main-chain nuclear magnetic resonances and secondary structure for SCD,[27] we will be able to study the interactions between SCD and the tryptophan derivatives by NMR techniques.

The SCD showed the same activity and the same substrate and inhibitor specificity as the full-length stromelysin. Our results are supported by the studies of chimeric proteins of collagenase and stromelysin-2 by Sanchez-Lopez et al.,[15] in which they found that the short peptide sequence coded by exon 5 was the determinant in the substrate gelatin cleavage. The SCD contains the corresponding exon 5 sequence (Gly-210 to Gly-265 in stromelysin, Figure 1). Although some differences between SCD and the full-length stromelysin may appear as more substrates and inhibitors are tested, the recombinant SCD is certainly useful in inhibitor discovery, as well as in enzyme mechanistic and structural studies for stromelysin and other matrix metalloproteinases.

Experimental Section

Sources of Materials. The expression and purification of the recombinant SCD has been described.[17] All the compounds tested on SCD were purchased from Bachem Bioscience Inc. (Philadelphia, PA), except compound **28**,[19] which was synthesized at Parke-Davis. Thiopeptolide substrate Ac-Pro-Leu-Gly-S-Leu-Leu-Gly-OEt was obtained from Bachem. Full-length human stromelysin was generously provided by Dr. Howard Welgus at Washington University in Saint Louis, MO.

Proteinase Assay. The proteinase activity for both SCD and full-length stromelysin was monitored by the hydrolysis of the thiopeptolide substrate as described.[17,18] The 100-μL assay mixture consisted of 50 mM Mes (pH 6.0), 10 mM $CaCl_2$, 0.1% Brij 35, 1 mM DTNB, 100 μM thiopeptolide, the inhibitor, and the enzyme SCD or trypsin-activated full-length stromelysin. For the IC_{50} determinations, 100 nM of SCD or 21 nM full-length stromelysin was used for all the compounds tested except for **28**, in which 10 nM SCD or 10 nM full-length stromelysin was used. The UV absorbance changes were monitored at 405 nm at room temperature for 3 min on a 96-well microplate reader. Inhibition constants (K_i) were derived from IC_{50} values[28] by using the equation $K_i = IC_{50}/(1 + [substrate]/K_m)$.

The human prostromelysin was activated by trypsin digestion at 22 °C for 10 min. The digestion was terminated by the addition of soybean trypsin inhibitor. The progress of the propeptide

removal was monitored by SDS-polyacrylamide gel electrophoresis, and the conversion from prostomelysin to stromelysin was completed the 10-min incubation period. No significant amount of further degradation was detectable by the SDS-polyacrylamide gel electrophoresis analysis.

Acknowledgment. We thank Dr. Howard Welgus for generously providing the human stromelysin and Dr. Tod Holler for his critical reading of the manuscript.

References

(1) Woessner, J. F. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J.* **1991**, *5*, 2145–2154.
(2) McDonnell, S.; Matrisian, L. Stromelysin in tumor progression and metastasis. *Cancer Metastasis Rev.* **1990**, *9*, 305–319.
(3) He, C.; Wilhelm, S. M.; Pentland, A. P.; Marmer, B. L.; Grant, G. A.; Eisen, A. Z.; Goldberg, G. I. Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2632–2636.
(4) Sirum, K. L.; Brinckerhoff, C. E. Cloning of the genes for human stromelysin and stromelysin 2: differential expression in rheumatoid synovial fibroblasts. *Biochemistry* **1989**, *28*, 8691–8698.
(5) Gunja-Smith, Z.; Nagase, H.; Woessner, J. F. Purification of the neutral proteoglycan-degrading metalloproteinase from human articular cartilage tissue and its identification as stromelysin matrix metalloproteinase-3. *Biochem. J.* **1989**, *258*, 115–119.
(6) Muller, D.; Quantin, B.; Gesnel, M.-C.; Millon-Collard, R.; Abecassis, J.; Breathnach, R. The collagenase gene family in humans consists of at least four members. *Biochem. J.* **1988**, *253*, 187–192.
(7) Basset, P.; Bellocq, J. P.; Wolf, C.; Stoll, I.; Hutin, P.; Limacher, J. M.; Podhajcer, O. L.; Chenard, M. P.; Rio, M. C.; Chambon, P. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. *Nature (London)* **1990**, *348*, 699–704.
(8) Nagase, H.; Enghild, J. J.; Suzuki, K.; Salvesen, G. Stepwise activation mechanisms of the precursor of matrix metalloproteinase 3 (stromelysin) by proteinases and (4-aminophenyl)mercuric acetate. *Biochemistry* **1990**, *29*, 5783–5789.
(9) Housley, T. J.; Baumann, A. P.; Braun, I. D.; Davis, G.; Seperack, P. K.; Wilhelm, S. M. Recombinant Chinese hamster ovary cell matrix metalloprotease-3, role of calcium in promatrix metalloprotease-3 activation and thermostability of the low mass catalytic domain of MMP-3. *J. Biol. Chem.* **1993**, *268*, 4481–4487.
(10) Koklitis, P. A.; Murphy, G.; Sutton, C.; Angal, S. Purification of recombinant human prostomelysin. *Biochem. J.* **1991**, *276*, 217–221.
(11) Quantin, B.; Murphy, G.; Breathnach, R. Pump-1 cDNA codes for a protein with characteristics similar to those of classic collagenase family members. *Biochemistry* **1989**, *28*, 5327–5334.
(12) Marcy, A. I.; Eiberger, L. L.; Harrison, R.; Chan, H. K.; Hutchinson, N. I.; Hagmann, W. K.; Cameron, P. M.; Boulton, D. A.; Hermes, J. D. Human fibroblast stromelysin catalytic domain: expression, purification, and characterization of a C-terminally truncated form. *Biochemistry* **1991**, *30*, 6476–6483.
(13) Lowry, C. L.; McGeehan, G.; LeVine, H. I. Metal ion stabilization of the conformation of a recombinant 19-kDa catalytic fragment of human fibroblast collagenase. *Proteins: Struct., Funct., Genet.* **1992**, *12*, 42–48.
(14) Murphy, G.; Allan, J. A.; Willenbrock, F.; Cockett, M. I.; O'Connell, J. P.; Docherty, A. P. The role of the C-terminal domain in collagenase and stromelysin specificity. *J. Biol. Chem.* **1992**, *267*, 9612–9618.
(15) Sanchez-Lopez, R.; Alexander, C. M.; Behrendtsen, O.; Breathnach, R.; Werb, Z. Role of zinc-binding- and hemopexin domain-encoded sequences in the substrate specificity of collagenase and stromelysin-2 as revealed by chimeric proteins. *J. Biol. Chem.* **1993**, *268*, 7238–7247.
(16) Hirose, T.; Patterson, C.; Pourmotabbed, T.; Mainardi, C. L.; Hasty, K. A. Structure-function relationship of human neutrophil collagenase: identification of regions responsible for substrate specificity and general proteinase activity. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2569–2573.
(17) Ye, Q.-Z.; Johnson, L. L.; Hupe, D. J.; Baragi, V. Purification and characterization of the human stromelysin catalytic domain expressed in *Escherichia coli*. *Biochemistry* **1992**, *31*, 11231–11235.
(18) Weingarten, H.; Feder, J. Spectrophotometric assay for vertebrate collagenase. *Anal. Biochem.* **1985**, *147*, 437–440.
(19) Caputo, C. B.; Wolanin, D. J.; Roberts, R. A.; Sygowski, L. A.; Patton, S. P.; Caccese, R. G.; Shaw, A.; DiPasquale, G. Proteoglycan degradation by a chondrocyte metalloprotease: effects of synthetic protease inhibitors. *Biochem. Pharmacol.* **1987**, *36*, 995–1002.
(20) Ye, Q.-Z.; Grunewald, G. L. Conformationally restricted and conformationally defined tyromine analogues as inhibitors of phenylethanolamine N-methyl transferase. *J. Med. Chem.* **1989**, *32*, 478–486.
(21) Okada, Y.; Harris, E. D.; Nagase, H. The precursor of a metalloendopeptidase from human rheumatoid synovial fibroblasts. *Biochem. J.* **1988**, *254*, 731–741.
(22) Niedzwiecki, L.; Teahan, J.; Harrison, R. K.; Stein, R. L. Substrate specificity of the human matrix melloproteinase stromelysin and the development of continuous fluorometric assays. *Biochemistry* **1992**, *31*, 12618–12623.
(23) Netzel-Arnett, S.; Fields, G.; Birkedal-Hansen, H.; Van Wart, H. E. Sequence specificities of human fibroblast and neutrophil collagenases. *J. Biol. Chem.* **1991**, *266*, 6747–6755.
(24) Darlak, K.; Miller, R. B.; Stack, M. S.; Spatola, A. F.; Gray, R. D. Thio-based inhibitors of mammalian collagenase. *J. Biol. Chem.* **1990**, *265*, 5199–5205.
(25) Grobelny, D.; Poncz, L.; Galardy, R. E. Inhibition of human skin fibroblast collagenase, thermolysin, and *Pseudomonas aeruginosa* elastase by peptide hydroxamic acids. *Biochemistry* **1992**, *31*, 7152–7154.
(26) Kortylewicz, Z. P.; Galardy, R. E. Phosphoramidate peptide inhibitors of human skin fibroblast collagenase. *J. Med. Chem.* **1990**, *33*, 263–273.
(27) Van Doren, S. R.; Kurochkin, A. V.; Ye, Q.-Z.; Johnson, L. L.; Hupe, D. J.; Zuiderweg, E. R. P. Assignments for the main chain nuclear magnetic resonances and delineation of the secondary structure of the catalytic domain of human stromelysin-1 as obtained from triple resonance 3D NMR experiments. *Biochemistry* **1993**, *32*, 13109–13122.
(28) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.