Human Neutrophil Collagenase

A DISTINCT GENE PRODUCT WITH HOMOLOGY TO OTHER MATRIX METALLOPROTEINASES*

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We have identified and sequenced a cDNA encoding human neutrophil collagenase from a λgt11 cDNA library constructed from mRNA extracted from the peripheral leukocytes of a patient with chronic granulocytic leukemia. The library was screened with an oligonucleotide probe constructed from the putative zinc-binding region of fibroblast collagenase. Eleven positive clones were identified, of which the one bearing the largest insert (2.2 kilobases (kb)) was sequenced. From the nucleotide sequence of the 2.2-kb cDNA clone we have deduced a 467-amino acid sequence representing the entire coding sequence of the enzyme. The deduced protein was confirmed as neutrophil collagenase by conformity with the amino-terminal sequence analyses of three tryptic peptides of purified neutrophil collagenase. The cDNA clone hybridizes to a 3.3-kb mRNA present in RNA extracted from human bone marrow but did not hybridize with RNA isolated from U937 cells induced to differentiate with phorbol myristate acetate. Neutrophil collagenase was found to possess 57% identity with the deduced protein sequence for fibroblast collagenase with 72% chemical similarity. Certain regions of the molecule, including the putative zinc-binding region, are highly conserved. When compared with the published sequence for fibroblast collagenase, neutrophil collagenase contains four additional sites for glycosylation. Medium from COS-7 cells transfected with a pcDNA1 eucaryotic expression vector containing cDNA for neutrophil collagenase degraded type I collagen into the three-quarter, one-quarter fragments characteristic of mammalian interstitial collagenase activity. Thus, definitive evidence based on the cDNA sequence confirms the neutrophil collagenase is a distinct gene product and a member of the family of matrix metalloproteinases.

Mammalian interstitial collagensases are metalloproteinases that can degrade native interstitial collagen types I, II, and III and are members of the family of matrix metalloproteinases. These enzymes cleave the triple helical collagen molecule three-fourths of the distance from the NH₂ terminus between residues 771 and 772 into C-terminal and HC² degradation products that further denature at 37 °C and become susceptible to degradation by a variety of other proteases (1). Skin fibroblast and synovial cell interstitial collagenases are well characterized enzymes that are identical (2, 3) while neutrophil collagenase has been reported to differ in substrate specificity (4) and immunological cross-reactivity (5). We have investigated the substrate specificity of neutrophil collagenase for the interstitial collagens and have shown the preference of the neutrophil enzyme for type I collagen in contrast with the greater susceptibility of type III collagen to digestion by fibroblast collagenase (6). Our immunological data suggest that while there are epitopes present in neutrophil collagenase which are distinct to this proteinase, other epitopes are shared with fibroblast collagenases (7). Although we have shown that polyclonal antibodies prepared to neutrophil collagenase cross-react significantly with fibroblast collagenase, we have identified a specific inhibitory monoclonal antibody to neutrophil collagenase which did not recognize synovial cell or fibroblast collagenase (8).

In contrast to human skin or synovial cells which constitutively synthesize and release collagenase, in the neutrophil the enzyme is synthesized and stored intracellularly in specific granules during the maturation of the neutrophil in the bone marrow (9). We have purified the secreted neutrophil collagenase and have shown the enzyme to have a molecular mass of 75 kDa (10). In addition, a smaller 57-kDa form of the enzyme, presumably the result of proteolytic processing, is often present.

In this communication, we present definitive evidence that neutrophil collagenase is a unique member of the family of matrix metalloproteinases. Analysis of the cDNA sequence and the derived amino acid sequence confirm the previous reports from ours and other laboratories that neutrophil collagenase is a distinct enzyme from fibroblast collagenase.

MATERIALS AND METHODS

Isolation and Sequence Analysis of cDNA for Neutrophil Collagenase—A λgt11 cDNA library constructed from peripheral leukocytes from a patient with chronic granulocytic leukemia (the kind gift of Dr. Fred Hagen, ZymoGenetics, Inc., Seattle, WA) was screened using a 24-mer oligonucleotide GTTGCGGCTCATGAACTCGGCCAT radiolabeled (11) at the 5' end with [32P]ATP (5' DNA terminus labeling system, Bethesda Research Laboratories) representing the presumptive zinc-binding region of fibroblast collagenase. Replicate transfers were done using GeneScreen filters, and the blots were prehybridized in 6 × SSC (sodium chloride, sodium citrate), 5 × Denhardt's solution, 0.05% sodium pyrophosphate, 100 μg/ml salmon sperm DNA, and 0.05% SDS for 4 h at 37 °C. Hybridizations were performed in 6 × SSC, 1 × Denhardt's solution, 0.1 μg/ml yeast tRNA, and 0.05% sodium pyrophosphate at 48 °C overnight. The blots were washed three times at room temperature for 15 min in 6 × SSC with 0.05% sodium pyrophosphate and once for 30 min at 60 °C.

Eleven clones out of 250,000 plaques were identified as positive by

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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) J05556.

The abbreviations used are: SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.
hybridization with the oligonucleotide probe. One of the largest clones, NC 7.2, containing a 2.2-kb insert, was subcloned into the EcoRI site of pUC18 and sequenced by the dideoxynucleotide chain termination method as modified for double-stranded DNA using [35S]dATP (Sequenase kit, United States Biochemical Corp.) and a sequencing kit containing terminal deoxyribonucleotidyl transferase. Both the 2.2-kb cDNA were sequenced. An additional clone containing a 2.0-kb insert was also sequenced and found identical to that of the 2.2-kb clone. 

**Northern Blot Analysis**—RNA was extracted with guanidinium thiocyanate (12) from human bone marrow cells and U937 cells which had been previously incubated at 37 °C for 24 h with phorbol 12-myristate 13-acetate to induce differentiation (13). The extracted RNA was pelleted through a CsCl gradient and 15 µg of total RNA was electrophoresed in a 1% agarose gel containing 2.2 M formaldehyde and transferred to nitrocellulose. The RNA on the blot was cross-linked by exposure to UV light, prehybridized at 42 °C for 4 h in 60% formamide, 5 × SSC, 5 × Denhardt's solution, 100 µg/ml denatured salmon sperm DNA, and 50 mM sodium phosphate, pH 7.4, and hybridized overnight at 42 °C in the same buffer containing 10% dextran sulfate with either 1) cDNA from the NC 7.2 clone after EcoRI digestion and purification of the insert in low melting point agarose; or 2) a 1.7-kb cDNA for human synovial collagenase (3) (the kind gift of Dr. Constance Brinckerhoff, Dartmouth Medical School) similar to the purified inserts was labeled by random primer extension (random primer DNA labeling system, Bethesda Research Laboratories) and [32P]dCTP. Unincorporated nucleotides were removed by chromatography on a nucleic acid chromatography system Prepac column (Bethesda Research Laboratories) using 0.2 M NaCl in 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA (TE buffer), and the radioactive hybrids were eluted with 50 mM NaCl in TE buffer. The blots were hybridized with 500,000 cpm of the radiolabeled probe/ml of hybridization buffer.

**Purification of Polymorphonuclear Interstitial Collagenase**—Neutrophil collagenase was purified from supernatants of neutrophils stimulated to degranulate with 5 ng/ml phorbol 12-myristate 13-acetate to induce differentiation (13). Supernatants were diluted 3-fold to bring the salt concentration to 50 mM NaCl and adjusted to 50 mM Tris-HCl, pH 7.5, 1 mM phenylmethylsulfonyl fluoride, 100 µg/ml pepstatin A. The resulting material was loaded onto a 1 × 6.5-cm zinc-chelating Sepharose 4B column (Pharmacia LKB Biotechnology Inc.) connected in tandem with DEAE-52 and Reactive Red-120 agarose columns. The void through this column was activated with 1 µM p-chloromercuribenzoate and applied onto a Pro-Leu-Gly-NHOH peptide affinity column. Briefly, the column was equilibrated with 50 mM Tris-HCl, pH 9, buffer containing 100 mM CaCl2 and 500 mM NaCl. The 2-ml fractions were collected onto 400 µl of 1 M NaOH and digested with 5% (w/w) L-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin at room temperature for 18 h and separated on a high performance liquid chromatography column as previously described. Individual peaks were collected, and the amino-terminal sequence was then determined using an Applied Biosystems 470A gas-phase sequenator.

**Results and Discussion**

From the nucleotide sequence analysis of the 2.2-kb cDNA NC 7.2 clone we have deduced a 467-amino acid sequence (Fig. 1). This amino acid sequence contains the presumptive zinc-binding sequence VAAHEFGH which has 85% identity to a similar region in the metalloproteinases, fibroblast collagenase and stromelysin (16). Similar to other proteins, a signal peptide of 18 amino acids is present at the amino terminus. In addition to the coding sequences, the clone contains 70 nucleotides at the 5'-end of the cDNA and 751 nucleotides at the 3'-end which are untranslated. The identity of the clone as neutrophil collagenase was confirmed by comparison with the NH2-terminal sequences of isolated tryptic peptides of the purified enzyme.

In order to identify the species of mRNA complementary to this cDNA, we utilized mRNA extracted from human bone marrow cells, since granular constituents are synthesized during the maturation of the neutrophil. As a comparison we
performed parallel analyses using mRNA extracted from differentiated U937 cells which have been shown to express fibroblast collagenase in culture (13). Northern blot analysis of mRNA extracted from human bone marrow and U937 cells showed that the NC 7.2 clone hybridized to a 3.3-kb mRNA from bone marrow but did not bind to mRNA isolated from U937 cells (Fig. 2). By contrast, a 1.7-kb cDNA probe specific for human synovial collagenase did not hybridize to bone marrow mRNA but clearly recognized a 2.2-kb mRNA in U937 mRNA in agreement with previously published data.

To confirm that the cDNA isolated was indeed collagenase, the cDNA was transfected into COS-7 cells using the NC 7.2 cDNA subcloned into a pCDNA1 vector. Medium from the cells transfected with the pCDNA1 vector bearing the insert degraded the collagen substrate into the characteristic a1A, a2A, a1B, and a2B degradation products typical of interstitial collagenase activity (Fig. 3). Cells transfected with the pCDNA1 vector alone did not show significant collagenase activity. Interestingly, the majority of the activity was present in the medium and not in cell lysates (data not shown), suggesting that these cells constitutively secrete the collagenase and do not store it intracellularly.

Although neutrophil collagenase is similar to fibroblast collagenase in initiating degradation of native interstitial collagen molecules at a single site in the triple helix, there is only 57% identity and 72% chemical similarity (Fig. 4) (2). By comparison, neutrophil collagenase has 52% identity to human stromelysin, a metalloproteinase with a broader range of substrates. Many of the residues identical with those of both collagenase and stromelysin are conserved in other members of the family of known metalloproteinases. This conservation may relate to properties shared by all metalloproteinases such as zinc and calcium binding or may govern the conversion of latent enzyme to active enzyme. The sequence PRCGPVD located between residues 89 and 95 in neutrophil collagenase is highly conserved among the characterized matrix metalloproteinases and may play a critical role in activation of these enzymes. Mutations of this sequence in transcin, the rat homolog to stromelysin, enhanced spontaneous activation of the enzyme (17).

Figures 2 and 3 depict the Northern blot and collagenase activity analysis, respectively, used in these experiments. The techniques employed for these analyses are described in detail in the Materials and Methods section of the paper.
targeting this enzyme to intracellular secretory granules. With the isolation of a cDNA probe for this enzyme, such questions can be addressed.

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REFERENCES
1. Gross, J., Harper, E., Harris, E. D., McCroskery, P. A., Highberger, J. H., Corbett, C., and Kang, A. H. (1974) Biochem. Biophys. Res. Commun. 61, 605-612
2. Goldberg, G. I., Wilhelm, S. M., Kronberger, A., Bauer, E. A., Grant, G. A., and Eisen, A. Z. (1986) J. Biol. Chem. 261, 6600-6605
3. Brinckerhoff, C. E., Ruby, P. L., Austin, S. D., Fini, M. E., and White, H. D. (1987) J. Clin. Invest. 79, 542-546
4. Horowitz, A. L., Hance, A. J., and Crystal, R. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 897-901
5. Woolley, D. E., Crossley, M. J., and Evanson, J. M. (1976) Eur. J. Biochem. 69, 421
6. Hasty, K. A., Jeffrey, J. J., Hibbs, M. S., and Welgus, H. G. (1987) J. Biol. Chem. 262, 10048-10052
7. Hasty, K. A., Stricklin, G. P., Hibbs, M. S., Mainardi, C. L., and Kang, A. H. (1987) Arthritis Rheum. 30, 655-669
8. Hasty, K. A., Hibbs, M. S., Mainardi, C. L., and Kang, A. H. (1984) J. Exp. Med. 159, 1455-1463
9. Bainton, D. F., Ullion, J. L., and Farquhar, M. C. (1971) J. Exp. Med. 134, 967-984
10. Hasty, K. A., Hibbs, M. S., Kang, A. H., and Mainardi, C. L. (1986) J. Biol. Chem. 201, 5045-5050
11. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 123, 6-13
12. Chirn, J., Pryzbyla, A. E., MacDonald, R. J., and Rutter, W. (1979) Biochemistry 18, 5294-5299
13. Wilhelm, S. M., Collier, I. E., Marmer, B. L., Eisen, A. Z., Grant, G. A., and Goldberg, G. I. (1989) J. Biol. Chem. 264, 17213-17221
14. Hibbs, M. S., Hasty, K. A., Saper, J. M., Kang, A. H., and Mainardi, C. L. (1985) J. Biol. Chem. 260, 2493-2500
15. Nagai, Y., Lapiere, C. M., and Gross, J. (1966) Biochemistry 5, 3123-3130
16. Whitham, S. E., Murphy, G., Angel, P., Rahmsdorf, H.-J., Smith, B. J., Lyons, A., Harris, J. J., Herrlich, P., and Docherty, A. J. P. (1986) Biochem. J. 240, 313-316
17. Sanchez-Lopez, R., Nicholson, R., Gesnel, M. C., Matrisian, L. M., and Breathnach, R. (1988) J. Biol. Chem. 263, 11892-11899
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