Peng, R. X., Tu, Y. Y., & Yang, C. S. (1982) *Carcinogenesis (London) 3*, 1457–1461.
Preussmann, R., & Eisenbrand, G. (1984) *ACS Monogr. 182*, 829–868.
Preussmann, R., & Stewart, B. W. (1984) *ACS Monogr. 182*, 643–828.
Reik, L. M., Levin, W., Ryan, D. E., & Thomas, P. E. (1982) *J. Biol. Chem. 257*, 3950–3957.
Reik, L. M., Levin, W., Maines, S. L., & Thomas, P. E. (1985) *Arch. Biochem. Biophys. 242*, 365–382.
Reinke, L. A., Sexter, S. H., & Rikans, L. E. (1985) *Res. Commun. Chem. Pathol. Pharmacol. 47*, 97–106.
Ryan, D. E., Thomas, P. E., Korzeniowski, D., & Levin, W. (1979) *J. Biol. Chem. 254*, 1365–1374.
Ryan, D. E., Thomas, P. E. & Levin, W. (1980) *J. Biol. Chem. 255*, 7941–7955.
Ryan, D. E., Thomas, P. E., & Levin, W. (1982) *Arch. Biochem. Biophys. 216*, 272–288.
Ryan, D. E., Iida, S., Wood, A. W., Thomas, P. E., Lieber, C. S., & Levin, W. (1984) *J. Biol. Chem. 259*, 1239–1250.
Ryan, D. E., Ramanathan, L., Iida, S., Thomas, P. E., Haniu, M., Shively, J. E., Lieber, C. S., & Levin, W. (1985) *J. Biol. Chem. 260*, 6385–6393.
Ryan, D. E., Koop, D. R., Thomas, P. E., Coon, M. J., & Levin, W. (1986) *Arch. Biochem. Biophys. 246*, 633–644.
Sipes, I. G., Stripp, B., Krishna, G., Malling, H. M., & Gillette, J. (1973) *Proc. Soc. Exp. Biol. Med. 142*, 237–240.
Sipes, I. G., Slocumb, M. L., & Holtzman, G. (1978) *Chem.-Biol. Interact. 21*, 155–166.
Stock, B. H., Hansen, A. R., & Fouts, J. R. (1970) *Toxicol. Appl. Pharmacol. 16*, 728–739.
Thomas, P. E., Lu, A. Y. H., Ryan, D. E., West, S. B., Kawalek, J., & Levin, W. (1976) *J. Biol. Chem. 251*, 1385–1391.
Thomas, P. E., Reik, L. M., Ryan, D. E., & Levin, W. (1981) *J. Biol. Chem. 256*, 1044–1052.
Thomas, P. E., Reik, L. M., Ryan, D. E., & Levin, W. (1983) *J. Biol. Chem. 258*, 4590–4598.
Thomas, P. E., Reik, L. M., Ryan, D. E., & Levin, W. (1984) *J. Biol. Chem. 259*, 3890–3899.
Tu, Y. Y., & Yang, C. S. (1985) *Arch. Biochem. Biophys. 242*, 32–40.
Tu, Y. Y., Sonnenberg, J., Lewis, K. F., & Yang, C. S. (1981) *Biochem. Biophys. Res. Commun. 103*, 905–912.
Tu, Y. Y., Peng, R. X., Cheng, Z. F., & Yang, C. S. (1983) *Chem.-Biol. Interact. 44*, 247–260.
Wrighton, S. A., Thomas, P. E., Molowa, D. T., Haniu, M., Shively, J. E., Maines, S. L., Watkins, P. B., Parker, G., Mendoz-Picon, G., Levin, W., & Guzelian, P. S. (1986) *Biochemistry 25*, 6731–6735.
Yang, C. S., Tu, Y. Y., Koop, D. R., & Coon, M. J. (1985) *Cancer Res. 45*, 1140–1145.
Yasukochi, Y., & Masters, B. S. S. (1976) *J. Biol. Chem. 251*, 5337–5344.

Molecular Cloning of Human Cathepsin G: Structural Similarity to Mast Cell and Cytotoxic T Lymphocyte Proteinases†

Guy Salvesen,* David Farley, Jon Shuman, Alan Przybyla, Christopher Reilly,‡ and James Travis*
Department of Biochemistry, University of Georgia, Athens, Georgia 30602
Received September 29, 1986; Revised Manuscript Received November 21, 1986

ABSTRACT: Human cathepsin G is a serine proteinase with chymotrypsin-like specificity found in both polymorphonuclear leukocytes (neutrophils) and the U937 leukemic cell line. Utilizing RNA from the latter, we have constructed a cDNA library in λgt11 and isolated a clone which apparently codes for the complete amino acid sequence of this enzyme. Analysis of the sequence reveals homology with rat mast cell proteinase II (47%) but a greater degree of identity (56%) with a product of activated mouse cytotoxic T lymphocytes. The close relationship between the three proteins indicates similarities in substrate specificity and in biosynthesis which we predict involves removal of a two amino acid activation peptide during or just before packaging into their respective storage granules.

In humans, the polymorphonuclear leukocyte (neutrophil) is the most abundant circulating white blood cell. Among the several properties of these cells, perhaps their most important functions are the ability to control the growth of microbial pathogens and to partake in the generation and regulation of inflammation (Lisiewicz, 1980). To accomplish these tasks, neutrophils contain specialized granules whose contents, including the serine proteinases cathepsin G (cat G)[1] and elastase, may participate in the killing and digestion of engulfed pathogens, and in connective tissue remodeling at sites of inflammation (Starkey, 1977). In the absence of controlling inhibitors, elastase appears to cause the abnormal degradation of elastin and proteoglycan, resulting in the development of pulmonary emphysema (Janoff, 1985). Cathepsin G, on the

† This work was supported by Grant HL 26887 from the U.S. Public Health Service.
* Correspondence should be addressed to this author.
‡ Present address: Whitaker College of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139.

[1] Abbreviations: cat G, cathepsin G; RMCP II, rat mast cell proteinase II; CCP I, putative protein from mouse cytotoxic T lymphocytes; SDS, sodium dodecyl sulfate; pfu, plaque-forming unit; bp, nucleotide base pairs; SSC, 0.15 M NaCl and 0.015 M sodium citrate, pH 7; ds, double stranded; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PTH, phenylthiohydantoin; HPLC, high-performance liquid chromatography.

0006-2960/87/0426-2289$01.50/0

other hand, is not known to be directly involved in disease development. The biological role of this enzyme is far from clear in spite of the fact that it can degrade connective tissue proteins, albeit somewhat more slowly than elastase (Roughley & Barrett, 1977).

Senior and Campbell (1984) have shown that the human leukemic cell line U937 synthesizes cat G, and we have used RNA from this line to investigate its structure by recombinant DNA technology. This report describes both the isolation and sequence analysis of a cDNA clone encoding cat G, as well as a comparison of its derived protein sequence with similar mammalian serine proteinases, including rat mast cell proteinase II (Woodbury et al., 1978) and a putative mouse cytotoxic T lymphocyte proteinase (Lobe et al., 1986).

Materials and Methods

DNA polymerase I, T4 DNA polymerase, and *Eco*RI methylase were from New England Biolabs. T4 polynucleotide kinase and *Escherichia coli* ligase were from Pharmacia. *Eco*RI (120 and 5 units/μL), T4 DNA ligase, *Hin*dIII, and calf alkaline phosphatase were purchased from Boehringer Mannheim. RNase H was obtained from Bethesda Research Laboratories. AMV reverse transcriptase was from Life Sciences.

Alkaline phosphatase conjugated anti-goat IgG, nitroblue tetrazolium, bromochloroindolyl phosphate, bromochloroindolyl galactoside, and isopropyl thiogalactoside were from Sigma. *S*-Adenosylmethionine and *Eco*RI linkers d-(CGGAATTCCG) were obtained from New England Biolabs. M13 mp18 RF, M13 mp19 RF, $(dT)_{12-18}$, oligo(dT)–cellulose, and deoxy- and dideoxynucleotide triphosphates were products of Pharmacia. A λ packaging system (Packagene) was purchased from Promega Biotech. [^{32}P]dCTP (3000 Ci/mmol) and [^{35}S]thio-dATP (400 Ci/mmol) were from Amersham. RPMI 1640 medium and fetal calf serum were from Flow Laboratories.

The U937 cell line (Sundstrom & Nilsson, 1976) was a gift of Dr. Robert Senior, Department of Medicine, Washington University, St. Louis, MO. *E. coli* strain TG1 (Gay & Walker, 1985) was a gift of Dr. Nick Gay, MRC Laboratory of Molecular Biology, Cambridge, England. λgt11 was a gift of Dr. Ron Makula, and *E. coli* strains Y1088 and Y1090 were provided by Dr. Claiborn Glover, both of this department.

Human neutrophil cathepsin G was prepared by the method of Travis et al. (1978). A monospecific antiserum was raised in goats, and the IgG fraction obtained by (NH_4) SO_4 precipitation was used to detect enzyme produced by in vitro translation of mRNA and to screen the λgt11 library.

Isolation of RNA. U937 cells were grown at 37° C/5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum (Senior et al., 1982) and were passaged every 4 days. RNA was isolated from 1.2 g of cells by using guanidine thiocyanate, as described by Chirgwin et al. (1979) Poly(A) RNA was selected (Maniatis et al., 1982) and used as a template for the synthesis of cDNA.

Cell-Free Translation. Preparation of rabbit reticulocyte lysate and translation of exogenous RNA were as described by Merrick (1983). Translation mixtures contained 70 μCi of [^{3}H]leucine and 30 μg of total U937 RNA. Translates were reacted with goat anti-human cat G for 3 h at room temperature followed by precipitation with staphylococcal cell walls (Kessler, 1981). Washed immunoprecipitates were fractionated in 10% SDS–polyacrylamide gels (Laemmli, 1970), and the gels were stained and impregnated with 1 M sodium salicylate (Chamberlain, 1979) as fluorophore. This procedure allows direct alignment of the fluorogram with stained protein bands, thus optimizing molecular weight estimations.

Synthesis of cDNA. cDNA synthesis employed ribonuclease H and DNA polymerase I as previously described (Gubler & Hoffman, 1983) except that the precipitation step following first-strand synthesis was omitted and the mixture simply diluted 2-fold with second-strand buffer. This modification increased the yield of ds-cDNA.

One microgram of cDNA was methylated with *Eco*RI methylase and blunt-ended as described (Huynh et al., 1985). Phosphorylated *Eco*RI linkers were ligated with cDNA (Huynh et al., 1985) in a 5 -μL reaction containing 0.5 unit of T4 ligase. Following ligation, the mixture was made 10 mM in NaCl, heated to inactivate the ligase, and restricted for 5 h at 37 °C with 120 units of *Eco*RI in a total volume of 11 μL. The mixture was applied to a 1.5% low gelling temperature agarose, and molecules of 0.5–7 kbp were selected (Sanger et al., 1980). The gel slice was melted at 65 °C, and the aqueous phase was extracted twice with 0.5 volume of phenol and once with an equal volume of chloroform/phenol. DNA was precipitated with ethanol/sodium acetate, washed with 70% ethanol, and dried (Maniatis et al., 1982).

Construction of Library. Twenty-two micrograms of λgt11 was restricted with *Eco*RI and dephosphorylated as described (Huynh et al., 1985). The vector was then ligated with 0.5 μg of cDNA in a final volume of 5 μL (Huynh et al., 1985) followed by packaging of 1-μL portions with one tube of particles as described by the manufacturer. A small portion of the library was plated by using *E. coli* Y1088 in the presence of isopropyl thiogalactoside and bromochloroindolyl galactoside to determine the titer and proportion of recombinants. The packaged particles were used, without amplification, to infect *E. coli* Y1090 (Huynh et al., 1985).

Library Screening. The library was plated at a density of 50 000 pfu per 132-mm plate and incubated for 4 h at 42 °C followed by blotting in duplicate with nitrocellulose filters as described (Reinach & Fischman, 1985). The filters were processed separately in petri dishes (Reinach & Fischman, 1985) with the following modifications: filter blocking with 1% albumin was decreased to 15 min with no increase in background; alkaline phosphatase conjugated rabbit anti-goat IgG was used to visualize positive signals, by diluting 1000-fold in 10 mM Tris, pH 8, 0.15 M NaCl, and 0.05% Tween 20 and incubating for 30 min with a volume of 10 mL per filter. The filters were rinsed 3 times in the same buffer, followed by incubation in 15 mL of 0.1 M Tris, pH 9.5, 0.1 M NaCl, and 5 mM $MgCl_2$ containing 5 mg each of bromochloroindolyl phosphate and nitroblue tetrazolium. Color was allowed to develop for 15 min in the dark on a rotating table before the filters were rinsed with 10 mM Tris, pH 8.5, and 5 mM EDTA, dried on filter paper, and inspected for positives. Duplicate positive signals were aligned with the respective agar plate, and the corresponding areas were picked with the large end of a Pasteur pipet.

Isolation and Analysis of Recombinants. Plaques containing phage that reacted with the antiserum to cat G were purified by replating at lower density followed by rescreening. The plaque giving the strongest signal was plate amplified and the DNA purified essentially as descirbed by Maniatis et al. (1982). The recombinant DNA was released by digestion with *Eco*RI and purified after electrophoresis through a 1% low gelling temperature agarose gel (Sanger et al., 1980). The recombinant DNA was inserted into the *Eco*RI site of M13 mp19 (Yanish-Perron et al., 1985) and sequenced by a modification (Biggin et al., 1983) of the dideoxy chain termination

method (Sanger et al., 1977) using *E. coli* strain TG1 as host. The *Sma*I and *Hin*dIII sites (Figure 2) proved useful in allowing the insert to be sequenced in the reverse orientation following ligation into *Eco*RI/*Hin*dIII or *Eco*RI/*Sma*I-cut M13 mp19 (Bankier et al., 1983). Preliminary sequence data enabled the construction of three synthetic primers that were used to complete the sequence. The primers had the following sequence and were gifts of Dr. Phil Barr, Chiron Corp., Emeryville, CA.

P1	3′TCGTTGTGTAGTGACGC5′
P2	3′GCAGAGACCTATAACAC5′
P3	3′CATCCTTGGCTTCTACG5′

The DNA sequences generated were compiled and analyzed with the help of the "Microgenie" program of Beckman Corp. The derived protein sequences were screened against the National Biomedical Research Foundation protein databank, version 8, using the FASTP search program of Lipman and Pearson (1985).

Analysis of RNA. Samples of total and poly(A) RNA for "Northern" blotting were denatured with formaldehyde, subjected to electrophoresis in a 1.4% agarose/formaldehyde gel, and transferred to a nitrocellulose filter (Maniatis et al., 1982). The blot was hybridized with a single-stranded "prime cut probe" (Farrell et al., 1983) containing the sequence complementary to the RNA from the 5′ *Eco*RI site to the *Hin*dIII site; 10^7 cpm of probe was hybridized overnight at 42 °C in 50% formamide containing 5 × SSC, 50 mM sodium phosphate, pH 6.5, 5 × Denhardt's solution, and 100 μg/mL denatured salmon DNA, followed by washing for 4 h at room temperature in 2 × SSC and 0.1% SDS.

Protein Sequence Analysis. Amino-terminal sequence analysis of reduced, carboxamidomethylated cat G, through 40 residues, was performed by using a Beckman Model 890C protein sequencer and the 0.1 M Quadrol program designed by the manufacturer. PTH–amino acids were analyzed in a Waters HPLC system.

Results

The specificity of the antiserum used in the following experiments was tested by double immunodiffusion against purified neutrophil cat G and a crude extract of azurophil granules. The antiserum reacted only with cat G and was considered to be a suitable probe for cloning, and investigating the biosynthesis of the enzyme.

In vitro translation of 30 μg of total U937 RNA, followed by precipitation in the presence of the cat G antiserum, revealed a radioactive band of $\sim M_r$ 28K–30K (Figure 1a). This indicated that RNA capable of driving the synthesis of this protein is present in U937 cells, confirming the results of Senior and Campbell (1984), who showed that this cell line synthesizes cat G.

The cDNA library constructed from 0.1 μg of U937 poly(A) RNA contained 3×10^5 pfu of which 30% were recombinants. The whole library was screened with antiserum, and 16 positives were verified through 2 rounds of rescreening. Differences in the intensity of color of the 16 positives were noticed and were consistent through each round of screening. These qualitative differences may be attributable to recombinant proteins containing different numbers of sites reacting with the polyvalent antiserum, resulting from the expression of different length transcripts. On the assumption that the most intense positive would contain the longest transcript, the recombinant giving the strongest signal was purified, and the sequence of its insert is shown in Figure 2. The *Eco*RI sites

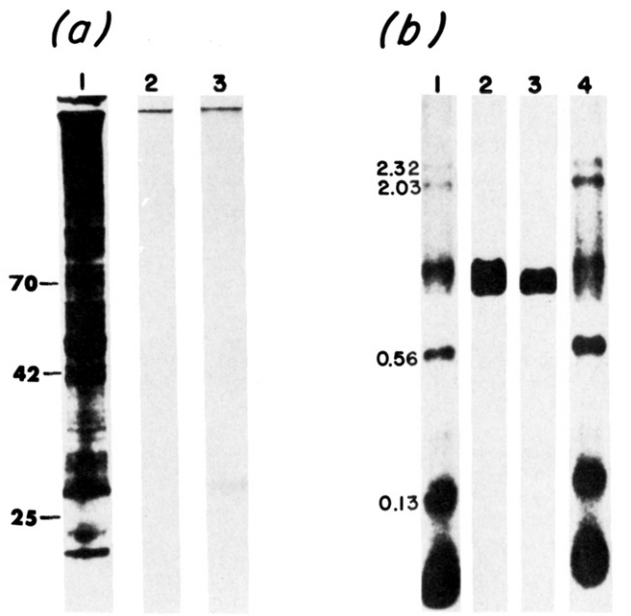

FIGURE 1: Analysis of cat G mRNA. (a) In vitro translation of U937 RNA was carried out as described under Materials and Methods; samples were run in a 10% SDS–polyacrylamide gel which was fluorographed for 5 days. Lane 1, total translate; 2, translate reacted with goat nonimmune serum; 3, translate reacted with goat anti-cat G serum. The band visible in lane 3 appears as a doublet of M_r 28K–30K. The numbers on the left represent molecular weight marker proteins $\times 10^{-3}$. (b) "Northern" blot of U937 RNA probed with a ^{32}P-labeled cat G cDNA probe as described under Materials and Methods. Lanes 1 and 4, *Hin*dIII-cut λDNA used as markers with sizes in kilobase pairs; 2, 20 μg of total RNA; 3, 5 μg of poly(A) RNA.

at each end of the insert are omitted as they are derived from the linkers, and not internal sites in the cDNA.

Northern blotting (Figure 1b) indicated that the mRNA is approximately 900 bp in length, inferring that the 870 bp of cDNA represents an almost full-length transcript. Present in the cDNA is a characteristic polyadenylation signal (AATAAA) followed 28 nucleotides later by an A_{13} tract which we assume is part of the poly(A) tail of the mRNA.

The derived protein sequence of the cDNA insert was found to contain the amino-terminal sequence (residues 1–40) of the reduced, carboxamidomethylated protein obtained from either normal or leukemic neutrophils, and we take this as evidence that the cDNA encodes neutrophil cat G. Indeed, it would appear that the mature protein contains 235 residues, has 3 disulfide bonds, and has a potential glycosylation site 6 residues downstream from the active-site histidine (see Figure 2). Protein data-base screening revealed a good match between cat G and rat mast cell proteinase II (RMCP II); however, we found an even better match between cat G and the putative product of activated mouse cytotoxic T lymphocytes (CCP I; Lobe et al., 1986). These three proteins are aligned with bovine chymotrypsin A in Figure 3 so as to show the extent of homology, with residue numbering according to chymotrypsinogen. Cat G, RMCP II, and CCP I are probably not species variants of the same protein for, although they are closely related, their degree of identity is in the region of 50–60%, wheras identities in excess of 75% characterize species variants of mammalian proteins in the serine proteinase superfamily.

Discussion

The N-terminal 18 residues of the inferred protein sequence (Figure 2), containing the characteristic hydrophobic region preceded by a hydrophilic residue (Carne & Scheele, 1984), almost certainly represent the signal peptide of pre-cat G. note

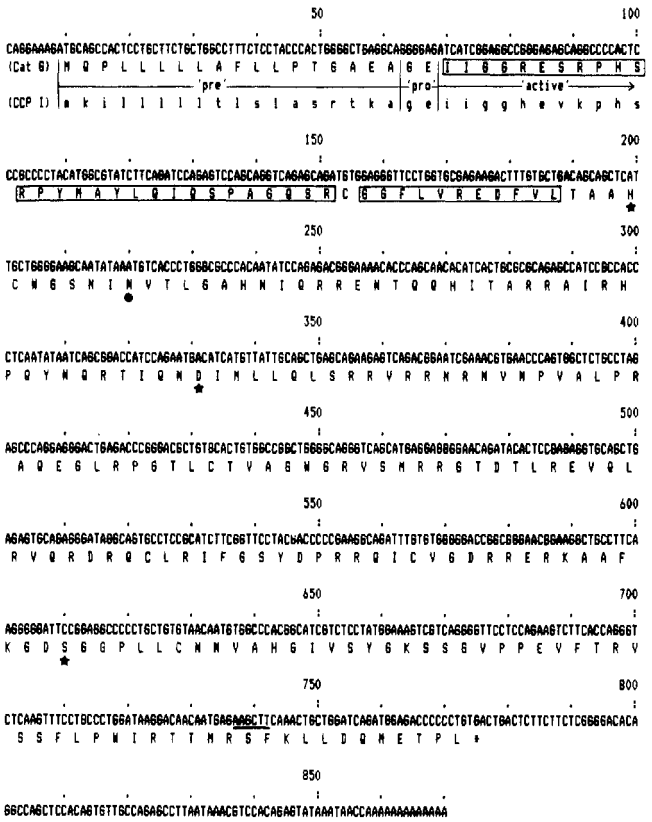

FIGURE 2: Nucleotide and inferred amino acid sequence of cDNA. The sequence was determined on both strands, with the exception of 80 bp of 3′-untranslated region, by using the primers described under Materials and Methods and the *Sma*I and *Hin*dIII sites (underlined). The nucleotide sequence is numbered starting from the first base after the *Eco*RI linker site. The limits of the proposed transient regions of pre-pro-cat G and CCP I (lower case) are indicated by vertical bars. The catalytic residues characteristic of serine proteinases (Kraut, 1977) are starred, and a potential N-linked glycosylation site is indicated by a solid circle. The boxed region of the amino acid sequence depicts the residues determined by automated protein sequence analysis of cat G from neutrophils.

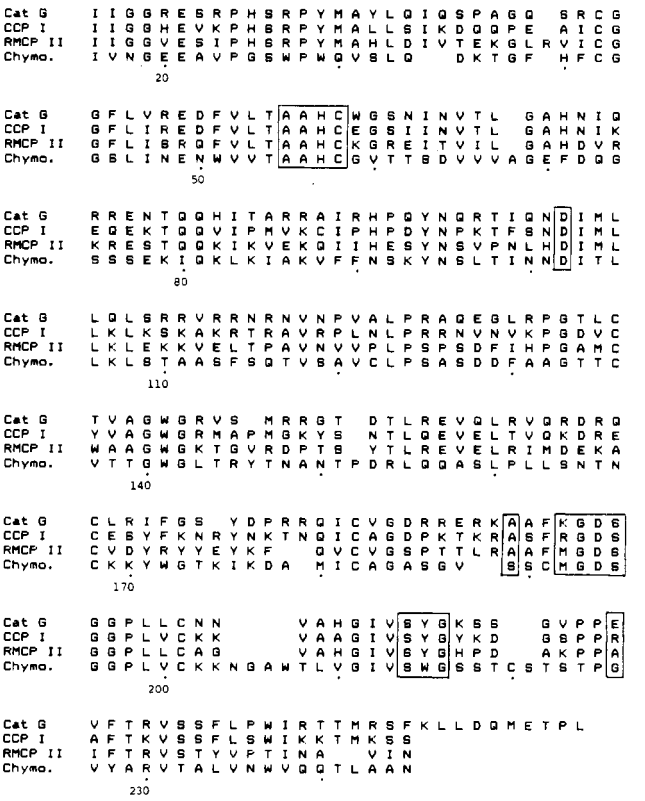

FIGURE 3: Composite alignment of the amino acid sequences of cat G, CCP I, RMCP II, and bovine chymotrypsin A. Gaps are included to maximize homology, and the sequences are numbered following the standard chymotrypsinogen notation. Residues have been boxed to emphasize regions of homology surrounding the catalytic His-57, Asp-102, and Ser-195 residues, and residues surrounding the primary specificity pocket of chymotrypsin. In this alignment, the extent of identity between cat G and the other proteins is as follows: 56%, CCPI; 47%, RMCP II; 31%, chymotrypsin; CCP I and RMCP II are 49% identical.

that this region is very similar in CCP I, indicating that the first ATG codon in the sequence is the initiator codon. The sequence Ala-Xaa-Ala is the most frequent one preceding the signal peptidase cleavage site (Carne & Scheele, 1984); therefore, we predict that cleavage would be between Ala-18 and Gly-19 in both cat G and CCP I to yield a zymogen containing a dipeptide N-terminal extension (Gly-Glu), especially since the mature protein, as isolated from neutrophils, contains a typical hydrophobic sequence (Ile-Ile) at its amino terminus. It seems likely, therefore, that the proposed activation peptide of pro-cat G enables it to be transported and sorted without posing the danger of proteolysis and that activation takes place during or just after packaging as has been proposed for lysosomal enzymes (Portnoy et al., 1986). Activation of serine proteinases usually follows cleavage after Lys or Arg (Isackson et al., 1984); therefore, the enzyme responsible for the removal of the cat G pro piece must have an unusual specificity. Clearly, these predictions for pre-pro-cat G must be tested by biosynthetic labeling, but they are consistent with what we know of the activation of serine proteinases (Kraut, 1977).

Alignment of the mature (active) sequences of cat G, CCP I, and RMCP II with chymotrypsin (Figure 3) reveals some interesting observations, many of which were considered by Woodbury et al. (1978) in their description of the protein structure of RMCP II. Clearly, cat G, CCP I, and RMCP II are similar in primary structure, with the former two being most closely related. The relationship of all three proteinases to chymotrypsin is somewhat more distant. Nevertheless, we have included this enzyme because (a) cat G and RMCP I have chymotrypsin-like specificity (Powers et al., 1985) and (b) the three-dimensional structure of chymotrypsin is known to high resolution (Blow, 1971). Sequence homology also implies that the putative protease CCP I should have the same general specificity as cat G and RMCP II, particularly as the residues lining the primary specificity pocket in chymotrypsin (Ser-189, Cys-190–Gly-192, and Ser-214–Gly-216; Kraut, 1977) are well conserved between the three other proteinases. Despite their evident chymotrypsin-like specificity, the geometry of this pocket in cat G and RMCP II may be significantly different from that of chymotrypsin as Gly-226, conserved in trypsins and chymotrypsins and forming part of the bottom of the pocket (Kraut, 1977), has undergone a radical change in the other enzymes shown in Figure 3. Cat G, like CCP I and RMCP II, also lacks the disulfide Cys-191–Cys-220, the only serine proteinases known to lack this feature (Woodbury et al., 1981). As previously pointed out (Woodbury et al., 1978), this omission may affect the substrate binding sites of the enzymes and, in the case of cat G at least, may explain the unusually low reactivity against a range of substrates (Tanaka et al., 1985).

Both cat G and CCP I contain a potential N-linked carbohydrate attachment site at Asn-64. We have preliminary evidence for glycosylation of this residue in cat G from protein sequence analysis (W. Watorek, unpublished observations) which is surprising as the equivalent position in chymotrypsin

lies close to the substrate binding sites of the enzyme (Park, 1985; M. Laskowsky, Jr., personal communication). Differences in the extent of glycosylation of this residue may at least partly explain the characteristic charge heterogeneity of the enzyme (Travis et al., 1980), although this may also be due to C-terminal processing, a phenomenon seen in lysosomal proteinases such as cathepsin D (Erickson & Blobel, 1983).

Because it is restricted to neutrophils and transformed cells of the neutrophil lineage such as U937 (Senior & Campbell, 1984) and HL60 (Olsson & Olofsson, 1981), one assumes that the role of cat G is related to the defensive function of the neutrophil; however, the aspects of neutrophil function that cat G is involved in are far from clear. Although the chymotrypsin-like primary specificity of cat G is well established (Tanaka et al., 1985), little is known of its natural substrates. Cat G is reasonably efficient in the degradation of proteoglycans (Roughley & Barrett, 1977), but it acts only slowly on other substrates (Roughley & Barrett, 1977; Tanaka et al., 1985); indeed, it has been speculated that the enzyme may have a very limited substrate specificity (Reilly et al., 1982). However, it should also be possible to use the cat G cDNA clone described here to investigate the biology of the enzyme in both differentiating hematopoietic and transformed cells by RNA mapping and gene inactivation experiments. Such studies are currently under way in this laboratory.

ACKNOWLEDGMENTS

We thank Sandy Cummings for raising the antiserum, M. Banda, A. Erickson, and H. Nagase for valuable discussions, and M. Laskowsky, Jr., for communicating results before publication.

REFERENCES

Bankier, A. T., & Barrell, B. G. (1983) in *Techniques in Nucleic Acid Biochemistry, B508* (Flavell, R. A., Ed.) pp 1–34, Elsevier Scientific Publishers, Ireland.

Biggin, M. D., Gibson, T. J., & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1212–1215.

Blow, D. M. (1971) *Enzymes (3rd Ed.) 3*, 185–212.

Carne, T., & Scheele, G. (1985) in *Cell Biology of the Secretory Process* (Cantin, M., Ed.) pp 73–101, S. Karger, Basel.

Chamberlain, J. P. (1979) *Anal. Biochim. 98*, 132–135.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.

Erickson, A. H., & Blobel, G. (1983) *J. Biol. Chem. 261*, 1760–1765.

Farrell, P. J., Deininger, P. L., Bankier, A. T., & Barrell, B. G. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1565–1569.

Gay, N. J., & Walker, J. E. (1985) *Biochem. J. 225*, 707–713.

Gubler, U., & Hoffman, B. J. (1983) *Gene 25*, 263–273.

Huynh, T. V., Young, R. A., & Davis, R. W. (1985) in *DNA Cloning* (Glover, D. D., Ed.) pp 49–78, IRL Press, Oxford.

Isackson, P. J., Ullrich, A., & Bradshaw, R. A. (1984) *Biochemistry 23*, 5997–6002.

Janoff, A. (1985) *Am. Rev. Respir. Dis. 132*, 417–433.

Kessler, S. W. (1981) *Methods Enzymol. 73*, 606–615.

Kraut, J. (1977) *Annu. Rev. Biochem. 46*, 331–358.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lippman, D. J., & Pearson, W. R. (1985) *Science (Washington, D.C.) 227*, 1435–1441.

Lisiewicz, J. (1980) *Human Neutrophils*, Charles Press, Bowie, MD.

Lobe, C. G., Finlay, B. B., Paranchych, W., Paetkau, V. H., & Bleakley, R. C. (1986) *Science (Washington, D.C.) 232*, 858–861.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Merrick, W. C. (1983) *Methods Enzymol. 101*, 606–615.

Olsson, I., & Olofsson, T. (1981) *Exp. Cell Res. 131*, 225–230.

Park, S. J. (1985) Ph.D. Thesis, Purdue University.

Portnoy, D. A., Erickson, A. H., Kochan, J., Ravetch, J. W., & Unckeless, J. C. (1986) *J. Biol. Chem. 261*, 14697–14703.

Powers, J. C., Tanaka, T., Harper, J. W., Mimematsu, Y., Barker, L., Lincoln, D., Crumley, K. V., Fraki, J. E., Schechter, N. M., Lazarus, G. G., Nakajima, K., Nakashino, K., Neurath, H., & Woodbury, R. G. (1985) *Biochemistry 24*, 2048–2058.

Reilly, C. F., Tewkesbury, D., Schechter, N., & Travis, J. (1982) *J. Biol. Chem. 257*, 8619–8623.

Reinach, F. C., & Fischman, D. A. (1985) *J. Mol. Biol. 181*, 411–422.

Roughley, P. J., & Barrett, A. J. (1977) *Biochem. J. 167*, 629–637.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5476.

Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H., & Roe, B. A. (1980) *J. Mol. Biol. 143*, 161–178.

Senior, R. M., & Campbell, E. J. (1984) *J. Immunol. 132*, 2547–2551.

Senior, R. M., Campbell, E. J., Landis, J. A., Cox, F. R., Kuhn, C., & Koren, H. S. (1982) *J. Clin. Invest. 69*, 384–393.

Starkey, P. M. (1977) in *Proteinases in Mammalian Cells and Tissues* (Barrett, A. J., Ed.) pp 57–89, North-Holland, Amsterdam.

Sundstrom, C., & Nilsson, K. (1976) *Int. J. Cancer 17*, 565–577.

Tanaka, T., Mimematsu, Y., Reilly, C. F., Travis, J., & Powers, J. C. (1985) *Biochemistry 24*, 2040–2047.

Travis, J., Bowen, J., & Baugh, R. (1978) *Biochemistry 17*, 5651–5657.

Travis, J., Porcelli, L., Reilly, C. F., Baugh, R., & Powers, J. (1980) *Ciba Found. Symp. 75*, 51–68.

Woodbury, R. G., Katanuma, N., Kobayashi, K., Titani, K., & Neurath, H. (1978) *Biochemistry 17*, 811–819.

Woodbury, R. G., Everitt, M. T., & Neurath, H. (1981) *Methods Enzymol. 80*, 588–609.

Yanisch-Perron, C., Vieira, J., & Messing, J. (1985) *Gene 33*, 103–119.