Proteinase 3 is a human polymorphonuclear leukocyte serine proteinase that degrades elastin in vitro and causes emphysema when administered by intratracheal insufflation into hamsters. Proteinase 3, stored in the azurophilic granules, is expressed in progenitor cells of myeloid origin. In the present study, the biosynthesis, processing, and intracellular transport of the enzyme was investigated in the human myelomonocytic cell line U937. Proteinase 3 is initially identified as a 35-kDa precursor and converted into the 29-kDa mature form within 3 h. By using a combination of techniques including amino-terminal sequencing, we identified the 35-kDa form as a zymogen containing an activation dipeptide but lacking the amino-terminal 25 residues, presumably the result of cleavage by a signal peptidase. Tunicamycin treatment and alkalization of acidic cell compartments with NH₄Cl did not prevent the processing of the proteinase 3 zymogen into the mature form, suggesting that the enzyme is targeted to the cytoplasmic granules by a mechanism other than the mannose 6-phosphate receptor. Brefeldin A inhibited the zymogen processing, suggesting that the dipeptide cleavage occurred in a post-Golgi organelle. The enzyme responsible for the removal of the dipeptide is a cysteine proteinase since E-64d, a class-specific inhibitor, prevented processing. However, treatment of cells with a dipeptidyl peptidase I inhibitor, Gly-Phe-diazomethyl ketone and with the lysosomotropic agents, NH₄Cl and chloroquine, did not prevent dipeptide cleavage, indicating that the processing enzyme for proteinase 3 is not dipeptidyl peptidase I. In contrast, Gly-Phe-diazomethyl ketone inhibited cleavage of the dipeptide from cathepsin G. This indicates that processing of proteinase 3 is distinct from that of cathepsin G. Proteinase 3 is also processed at the COOH-terminal extension. Cleavage takes place next to Arg-222, suggesting that a trypsin-like proteinase is involved in the COOH-terminal processing.

Proteinase 3 (PR-3, EC 3.4.21.76), is a third neutral serine proteinase in azurophilic granules of human polymorphonuclear leukocytes (PMNL) (1, 2), distinct from elastase (HLE, EC 3.4.21.37) and cathepsin G (Cat G, EC 3.4.21.20). PR-3 degrades several structural proteins in vitro, including elastin, suggesting that it plays a role in several PMNL-mediated physiologic and pathologic events (1, 3). Physiologically, the proteolytic activity of PR-3 may facilitate the movement of neutrophils from the vasculature through basement membranes at sites of inflammation (4) or may assist in the digestion of phagocytosed microorganisms (5). Pathologically, the elastolytic property of PR-3 suggests a role in the development of emphysema. This possibility is supported by the demonstration of emphysematous lesions in the lungs of hamsters following intratracheal insufflation of PR-3 (1). The prospect that PR-3 also is a mediator of airway injury is strengthened by its resistance to inhibition by secretory leukocyte protease inhibitor, the primary serine proteinase inhibitor in human upper respiratory tract (3, 6).

PR-3, in addition to its proteolytic activity against extracellular matrix proteins, has a variety of other potentially important actions. PR-3 is identical to myeloblastin, which has been ascribed a central role in the control of growth and differentiation of leukemic cells (7). Recently, PR-3 was found to degrade the 28-kDa mammalian heat shock protein (8), previously linked to differentiation of normal and neoplastic cells and to cleave the nuclear factor KB subunit p65 (9). PR-3 also has microbicidal activity that is independent of its serine proteinase activity (10). Perhaps most importantly, PR-3 is the antigen recognized by cytoplasmic-staining anti-neutrophil cytoplasmic autoantibodies in patients with Wegener's granulomatosis (11–13), a disease characterized by a prominent neutrophilic vasculitis. PR-3 could contribute to the pathogenesis of this disease either by inactivating complement pathway inhibitor (C1) (14) or by its presence on the surfaces of PMNL and human endothelial cells, turning these cells into targets for activation by cytoplasm-staining anti-neutrophil cytoplasmic autoantibodies (15, 16). Because of these diverse roles, an in-depth knowledge of factors that influence the expression of PR-3 are of substantial importance. In the present investigation, we report on the biosynthesis, processing, and intracellular transport of this enzyme.

**EXPERIMENTAL PROCEDURES**

Materials—RPMI 1640, RPMI 1640 methionine-deficient medium, RPMI 1640 select-amine kit, and minimum essential medium nonessential amino acids were from Life Technologies, Inc. Defined fetal bovine serum was from Hydnone (Logan, UT). 1-[^35S]Methionine (1000 Ci/mmol), in vivo cell labeling grade and L-[4,5-^3H]leucine (100 Ci/mmol) were from Amersham Corp. Tunicamycin, 3,4-dichloroisocoumarin, and E-64 were from Boehringer Mannheim. Rabbit antisera to Cat G was from Athens Research and Technology Inc. (Athens, GA). Gly-Phe-diazomethyl ketone (GF-CHN2) was from Enzyme Systems Products (Dublin, CA). Electrophoresis chemicals were from Bio-Rad. prolBlt membrane and all reagents for peptide synthesis and protein
Fig. 1. PMNL serine proteinases primary structure, location of peptide sequences used for peptide antisera production, and proposed COOH-terminal cleavage sites. The amino acid sequences are deduced from the cDNA and genomic studies reported for PR-3 (7, 34, 56–58) HLE (26, 59), and Cat G (60). All of the residues are numbered starting with the first amino acid residue of the active proteinase. The residues in the prepro region are assigned negative numbers relative to the first amino acid residue. The peptide sequences used for raising antisera were shown in italics of purified PR-3 and that reported for purified HLE (61) are shown in boldface. The sequence obtained for the COOH-terminal tryptic peptide of purified PR-3 (3) and that for purified HLE (61) are shown in italics. The sequence obtained for the peptide resulting from the cleavage of each proteinase by iodosobenzoic acid is underlined. The arrow next to Arg indicates the probable cleavage site of the COOH-terminal extension peptide.

Antisera—Rabbit polyclonal antisera were raised to purified PR-3 and HLE as described previously (1) and then further purified by HPLC to ensure that the preparations were free from peptides produced by autolysis. The highly purified proteinases (30 μg) were suspended in 30 μl of 4 mM guanidine hydrochloride in 80% acetic acid, and then 30 μl of iodosobenzoic acid (10 mg/ml dissolved in the same solvent) was added (10-fold excess over the protein). Iodosobenzoic acid cleaves peptide bonds on the carboxyl side of Trp (17, 18), which is strategically located near the COOH termini of PR-3, HLE, and Cat G (see Fig. 1). The reaction mixture was incubated for 24 h in the dark at room temperature. The reaction was terminated by diluting with water followed by lyophilization. The peptides in the digest were fractionated by HPLC and sequenced. The sequence data was confirmed by mass spectrometry analysis.

Antisera—Rabbit polyclonal antisera were raised to purified PR-3 and HLE as described previously (1). Rabbit polyclonal antisera also were raised to the synthetic peptides representing the partial NH2-terminal prepro region (Nab) and the COOH-terminal region (Cab) of PR-3 deduced from the cDNA and from sequencing peptides of the mature protein (see Ref. 7 and “Results”). For Nab, the peptide consisted of 33 residues, cAA211LLS220EDNPCPHPRD230 and cAA224FKLLDQMETPL235 were synthesized by McGuire et al. (24). Briefly, the reaction mixture contained 50 μM Gly-Phe-β-naphthylamide buffer, pH 8, containing 10 mM Tris/Tris-HCl, 140 mM NaCl, 1% (v/v) Nonidet P-40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, and a proteinase inhibitor mixture (1 mM phenanTRAN, 50 mM 3,4-dichloroisocoumarin, 10 μM E-64) (20).

Cell lysates and conditioned media were each mixed with 30 μl of a 10% suspension of protein A-Sepharose CL-4B beads in radioimmunoprecipitation assay buffer and incubated for 1 h at 4 °C with gentle agitation. Supernatants were recovered by centrifugation, and then 50 μl of protein A beads, preincubated with 20 μl of the appropriate antisera, was added and incubated overnight at 4 °C with gentle mixing. Protein A-bound immune complexes were then washed, suspended in 40 μl of SDS sample buffer, boiled for 5 min, and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography or radiosequencing.

SDS-PAGE and Fluorography—SDS-PAGE was performed (21) in 1.5-mm thick 12.5% acrylamide minigels. Gels were stained with Coomassie Blue R-250. For fluorography, destained gels were treated with ENLIGHTENING for 30 min according to the manufacturer’s directions. After drying, the gels were exposed to Kodak X-Omat XAR2 film at −80 °C.

Radiosequencing Analysis—For radiosequence analysis, immunoprecipitates were subjected to SDS-PAGE as above and then electrophoretically separated onto a proBlott membrane using a Bio-Rad mini Trans-Blot system (22, 23). Transfer time was 1 h in a buffer, pH 8.3, containing 25 mM Tris, 192 mM glycine, and 10% (v/v) methanol at 200 mA. The membrane was then stained for 1 min in 0.1% Coomassie Blue R-250 in 40% (v/v) methanol and 1% (v/v) acetic acid and destained in 50% (v/v) methanol. The dried membrane surface was sprayed evenly with three light coats of ENHANCE spray. The treated membrane was wrapped in plastic and exposed to Kodak film as above at −80 °C. Using the developed film as a template, appropriate bands were excised from the membrane and sequenced by Edman degradation on an automated pulse liquid sequenator (ABI 477A, Applied Biosystem). The sequenced samples were collected and counted for 10 min in 10 ml of Optifluor scintillation fluid. The radioactivity from each cycle was plotted against the cycle number and aligned with the sequence of the appropriate proteinase.

Dipetidyl Peptidase I (DPP-I, EC 3.4.14.1) Assay—DPP-I activity in U937 cells was assayed using Gly-Phe-β-naphthylamide, as described by McGuire et al. (24). Briefly, the reaction mixture contained 50 μM acetylacetocacid buffer, 5.5 mM EDTA, 1 mM diithiothreitol, 30 mM NaCl, and 0.1 mM Gly-Phe-β-naphthylamide in a final volume of 1
lated from their electrophoretic mobilities relative to standards. The apparent molecular weights of the biosynthetic forms were calculated from their electrophoretic mobilities relative to standards.

The apparent molecular weights of the biosynthetic forms were calculated from their electrophoretic mobilities relative to standards. By the end of a 3-h chase, the 35-kDa biosynthetic form-or that of purified mature PR-3 suggested that the larger form may include a preprosequence on the NH2-terminal side and/or an extension peptide on the COOH-terminal end. This possibility was explored in several ways. A comparison of the amino acid composition of the predicted sequence of PR-3 (the expected residues at position 1-229) and the determined composition of mature PR-3 (25) indicated that PR-3 has a COOH-terminal extension that is removed during processing. In order to gain insight into the processing of the COOH-terminal extension peptide, we initially determined the COOH-terminal amino acid of PR-3 using iodosobenzoic acid to generate peptides. The amino acid sequence of the smallest peptide started with Ile and ended with Arg at positions 6 and 7. Mass spectrum analysis of this peptide confirmed that the amount of arginine recovered at cycle 7 was not a residual Arg from cycle 6 and thus demonstrated that the last two residues of this peptide were Arg. This peptide matches residues 216-222 of the deduced sequence of PR-3 cDNA (see Fig. 1), indicating that the COOH-terminal amino acid of PR-3 is Arg222.

The results establish that PR-3 has a seven-residue COOH-terminal extension that is removed during processing to the mature enzyme. In parallel experiments carried out with HLE and Cat G, we found that these proteinases also have COOH-terminal arginine residues, Arg12 and Arg223, respectively indicating that COOH-terminal extension peptides of HLE (SED-NPCPHPRDPDPASRTLH) (26) and Cat G (SFKLLDQMETPL) (27) were removed during the processing (28). Next, U937 cells were pulse labeled for 30 min with radioactive methionine, and the signal peptide and COOH-terminal extension peptide had been removed. The same results were obtained with HLE and Cat G. Similar results were obtained when the cells were pulse labeled for periods as short as 5 min, suggesting that the signal peptide and the COOH-terminal extension were removed co-translationally. Alternatively, the peptide antibodies may have been unable to precipitate the precursor protein.

To further investigate the nature of the precursor processing, we used radiolabeling to identify the amino terminus of the precursor forms of PR-3. For these studies, cells were labeled with [3H]isoleucine since isoleucine is at the amino terminus of mature PR-3 (see Fig. 1). The results are shown in Fig. 3. The 35-kDa form of PR-3 contained an isoleucine at position 3. Thus, the earliest identifiable biosynthetic form (35 kDa) was pro-PR-3, which consists of a dipeptide preceding the amino-terminal isoleucine of mature PR-3 (see Fig. 1). These results confirmed that the 25-amino acid signal peptide was removed co-translationally. In the later biosynthetic form of PR-3 (29 kDa), the prosegment (Ala2-Glu-1) was removed resulting in isoleucine at position one.

The Role of Glycosylation in the Processing of PR-3—Since removal of the dipeptide from the precursor protein did not account for the apparent molecular weight differences between the early 35-kDa (pro) and the late 29-kDa (mature) forms of PR-3, we next investigated the contribution of glycosylation at consensus sites (Asn-X-{Ser/Thr}) at Asn residues 102 and 147. We determined the effects of tunicamycin, which blocks the Asn-linked core glycosylation by inhibiting the addition of N-acetylglucosamine to dolichol phosphate, the first step in the formation of core oligosaccharides. Cells were preincubated with various concentrations of tunicamycin prior to pulse la-

![Fig. 2. Pulse-chase experiment showing processing of PR-3 in U937 cells.](image-url)
belonging with [35S]methionine in the continued presence of the inhibitor. The results demonstrate that increasing concentrations of tunicamycin resulted in the earliest biosynthetic form of PR-3 having a molecular mass of 29 kDa (Fig. 4). Tunicamycin did not lead to secretion of PR-3 biosynthetic forms into the conditioned medium (data not shown). The results indicate that conversion of the 35-kDa form to the 29-kDa form is due largely to “trimming” of Asn-linked oligosaccharides.

Next we conducted chase experiments with [3H]isoleucine in the presence of tunicamycin to explore the role of glycosylation in PR-3 trafficking and processing. The radiosequence analysis demonstrated that inhibition of glycosylation did not prevent the isoleucine shift from the third to the first position during the chase period (data not shown). Thus, glycosylation is not essential for cleavage of the pro dipeptide and by inference is not critical for transport of the protein through intracellular compartments.

Effect of Brefeldin A (BFA) on Processing of PR-3—To investigate the subcellular location of the dipeptide removal that converts thezymogen to an active form, we examined the effect of BFA on processing of PR-3. BFA, a fungal product, is known to inhibit the transport of proteins out of the Golgi apparatus by inducing resorption of the Golgi into the endoplasmic reticulum. BFA-treated cells were labeled with [3H]isoleucine, chased for 4 h in the presence of the drug, and then subjected to immunoprecipitation, electrophoresis, blotting, and sequenc ing. The radiosequence results of PR-3 immunoprecipitated from BFA-treated cells revealed an isoleucine in the third position. This suggests that excision of the activation dipeptide from PR-3 occurs in a post-Golgi compartment. Similarly, BFA blocked removal of the activation dipeptide from HLE and C a t G (Fig. 5).

A Cysteine Proteinase(s) Is Involved in Cleavage of the Dipeptide from PR-3—Recently, McGuire et al. (29) reported that the granules of U937 cells (analogous to azurophilic granules of PMNL) contain a cysteine exopeptidase, DPP-I, that removes the dipeptide from the Cat Gzymogen to convert it to the active mature form of the enzyme. To examine the possibility that the enzyme responsible for removing the dipeptide from PR-3 might belong to the same catalytic class as DPP-I, we tested the effects of various cysteine proteinase inhibitors. Of the inhibitors tested, N-ethylmaleimide (10 μM), iodoacetic acid (200 μM), and the active site cysteine-specific inhibitor 2, 2'-dithiodipyridine (30) (300 μM), were toxic to the cells when used at effective inhibitory concentrations. The lysosomal cysteine proteinase inhibitors that were not toxic to U937 cells included egg white cystatin (8 μM), leupeptin (0.21 mM), E-64 (1.4 mM), E-64c (0.32 mM), and E-64d (0.36 mM). However, cystatin and leupeptin failed to inhibit DPP-I in cell cultures, suggesting an inability to access the enzyme within cells. E-64 and E-64c inhibited DPP-I in cell cultures but only by 70% after 4 h of treatment. E-64d inhibited more than 96% within 1 h of treatment. This difference may be related to the slower entry of the former two inhibitors into the cells via pinocytosis (31, 32). We examined whether inhibition of cysteine proteinases with E-64d prevented PR-3 processing. We found that not only pro-PR-3 processing but also processing of pro-HLE and pro-Cat G was abrogated by E-64d (Fig. 6). These results indicate that, like Cat G, a cysteine proteinase is involved in processing of PR-3 and HLE.

Since E-64d is known to inhibit calcium-dependent cysteine proteinases (calpains) within cells (32), we examined the effects of EGTA (1 and 5 mM), a general calcium-dependent proteinase inhibitor, and other metalloproteinase inhibitors, EDTA (1 and 5 mM) and o-phenanthroline (100 μM), on the processing of PR-3. These inhibitors did not block the processing ruling out the possibility that calpain-type proteinases are involved.

DPP-I Is Not Involved in the Processing of Pro-PR-3—We next determined the role of DPP-I in the processing of PR-3. Cells were preincubated with 10 μM GF-CHN2, a covalent inhibitor of DPP-I (33), prior to pulse labeling with [3H]isoleucine and chased for 4 h in the continuous presence of the inhibitor. Under these conditions, DPP-I was inhibited >95%. In the presence of GF-CHN2, pro-PR-3 was processed to the mature proteinase as indicated by the radioactive isoleucine at position 3. Since E-64d is known to inhibit calcium-dependent cysteine proteinases (calpains) within cells (32), we examined the effects of EGTA (1 and 5 mM), a general calcium-dependent proteinase inhibitor, and other metalloproteinase inhibitors, EDTA (1 and 5 mM) and o-phenanthroline (100 μM), on the processing of PR-3. These inhibitors did not block the processing ruling out the possibility that calpain-type proteinases are involved.

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In striking contrast, and consistent with the results obtained by McGuire et al. (29), pro-Cat G was not processed in the presence of GF-CHN2, as evidenced by the presence of radioactive isoleucine at positions 3 and 4. Similar to PR-3, the processing of pro-HLE was not inhibited by GF-CHN2 (data not shown). These results demonstrate that the processing of PR-3 (and HLE) is distinct from that of Cat G.

**Discussion**

We have previously reported the identification (1), biochemical characterization (3), and gene structure (34, 35) of PR-3, an elastolytic PMNL serine proteinase. The present investigation focused on the biosynthesis, processing, and intracellular transport of the enzyme. The biosynthesis studies showed that PR-3 is initially detectable as a 35-kDa form and over 3 h is converted to a 29-kDa protein, a size identical to the mature enzyme. The mass of the larger precursor was comparable with the 33-kDa size deduced from the cDNA, for PR-3 comprised 256 amino acids plus the two carbohydrate chains at the Asn-linked glycosylation sites. However, our data, which include 1) the failure to immunoprecipitate the precursor with an antibody directed to the NH2-terminal Ile of the active enzyme, 2) the radiolabelling results that identified Ile in the position 3 of the 35-kDa precursor, demonstrate that removal of the 25-amino acid signal peptide occurs early in the biosynthesis of the enzyme probably in a co-translational fashion. The amino-terminal cleavage occurs between Ala-3 and Ala-2 and fits the rules predicted for signal peptidases according to von Heijne (36). The signal peptide cleavage results in the earliest identifiable precursor being a zymogen containing a dipeptide (Ala-2-Glu-2) before the NH2-terminal Ile of the active enzyme. This, however, leaves a disparity between the observed molecular mass of the earliest precursor (35 kDa) and the predicted molecular mass (30.6 kDa) of the glycosylated protein minus the signal peptide. This difference may be explained by the reduced mobility of the protein in SDS-PAGE due to its cationic nature and by “trimming” of the Asn-linked oligosaccharides (37).

Following trimming of the oligosaccharides, the maturation of pro-PR-3 to the biologically active proteinase proceeds via excision of the dipeptide (Ala-2-Glu-2) at the acidic residue. This dipeptide is homologous to other proteinases of hematopoietic origin including HLE and Cat G (28, 29, 38), human mast cell chymase (39), mouse mast cell chymase (40), and mouse cytotoxic lymphocyte granzyme B (41). In the case of Cat G and suggested for the other hematopoietic cell-derived serine proteinases, the cysteine exopeptidase DPP-I has been reported to be the processing enzyme involved in the excision of the dipeptide (29). This prompted us to investigate its role in the processing of PR-3 (and HLE). When DPP-I was inhibited by GF-CHN2, the processing of pro-Cat G was prevented, but pro-PR-3 and pro-HLE were processed at their acidic residues. In addition, alkalinization of the secretory granules with NH4Cl, which should prevent the dipeptide removal by DPP-I, since the enzyme requires an acidic milieu for activity, had no effect on the processing of pro-PR-3, pro-HLE, or pro-Cat G. In contrast to the results of the present investigation, Lindmark et al. (42) reported that NH4Cl inhibited the processing of both HLE and Cat G in U937 cells. However, the site of inhibition in the processing pathway was not determined, and prevention of the dipeptide removal was not demonstrated.

Chloroquine, which has a similar effect on the pH of the granules as NH4Cl, surprisingly showed differential effects on the processing of the PMNL serine proteinases. The processing of pro-Cat G was inhibited, but that of pro-PR-3 and pro-HLE was not. This raises the possibility that the observed effect of both the DPP-I inhibitor and chloroquine on the processing of pro-Cat G may be related to the blockage of the transport of the enzyme through the subcellular organelles rather than inhibition of DPP-I and that the transport of Cat G may be different from that of PR-3 and HLE. While it is clear from our data that DPP-I is not involved in the processing of PR-3 or HLE, inhibition of the dipeptide excision by E-64d establishes that a cysteine proteinase, perhaps related to DPP-I, is involved in...
this processing step. Ongoing work in our laboratory is aimed at identifying the processing enzyme.

In the present investigation, analysis of the COOH terminus established arginyl residues as the final two amino acids of that mature PR-3 Arg221-Arg222). Thus PR-3 is shorter by seven amino acid residues on the COOH-terminal side than would be predicted from the cDNA. The COOH termini for HLE and Cat G are monoarginyl residues (Arg219 and Arg223, respectively, Fig. 1). This implicates a basic amino acid directed endoproteinase in the processing of the COOH termini of these enzymes. Recently several mammalian subtilisin-like convertases have been identified that cleave at dibasic and monobasic sites of proproteins (43–46). Among these enzymes, furin would be a candidate enzyme for processing of PMNL proteinases. However, furin is localized in the Golgi compartment (47).

While studies by Salvesen and Enghild (28) on the processing of Cat G are consistent with removal of its COOH-terminal extension peptide in the Golgi, our results suggest that the COOH-terminal extension of PR-3 is likely removed co-translationally, indicating that its processing enzyme is probably localized to the endoplasmic reticulum. In addition, it has been shown that furin requires a precursor protein comprising the consensus cleavage site Arg-Xaa-(Lys/Arg)-Arg or Arg-Xaa-Xaa-Arg (47–50), indicating importance for an Arg at P4 position. None of the PMNL proteinases contain Arg at the P4 position. Thus basic amino acid directed endoproteinases other than furin likely are involved in processing the PMNL proteinases. In preliminary studies, Nα-p-tosyl-L-lysine chloromethyl ketone and phenylmethanesulfonyl fluoride did not inhibit the processing of PR-3.

The azurophilic granules of PMNL are considered analogous to lysosomes (51) of nonmyeloid cells. In the present investigation, we compared the targeting of PR-3 to granules with sorting of lysosomal enzymes. Following synthesis, lysosomal enzymes in nonmyeloid cells are transported from endoplasmic reticulum to Golgi, during which time high mannose side chains are modified and mannose 6-phosphate residues are added as a recognition marker for receptor targeting to the lysosome. In the acidic intralysosomal environment, the lysosomal enzyme dissociates from the receptor and the proenzyme is proteolytically processed. We found that PR-3 zymogen was not processed to the mature form when we treated cells with BFA, an agent that specifically blocks protein transport distal to the Golgi, suggesting that the PR-3 precursor is transported beyond the Golgi compartment before processing of the propeptide. The cleavage of the propeptide from the PR-3 precursor in the presence of tunicamycin suggests that the transport of PR-3 to the proper cell compartment is not mannose 6-phos-
Our results show that the processing of PR-3 in U937 cells is and seven-amino acid-long peptide COOH-terminal to Arg222. In additional cleavages, the dipeptide (Ala-Glu)10. Gabay, J. E., and Almeida, R. P. (1993) "Actions on the storage granules and processing of PR-3. These findings are consistent with results from previous investigations of various azurophilic granule proteins such as myeloperoxidase (52) and defensins (53), indicating that the sorting mechanisms are distinct from those of typical lysosomal enzymes (54).

In summary, the processing of PR-3 in U937 cells requires three proteolytic cleavages, two on the amino-terminal side and one on the carboxyl-terminal side of 256-amino acid precursor PR-3. The initial amino-terminal cleavage results in the removal of a 25-amino acid endoplasmic reticulum-targeting signal sequence. In additional cleavages, the dipetide (Ala-Glu)10. Gabay, J. E., and Almeida, R. P. (1993) "Actions on the storage granules and processing of PR-3. These findings are consistent with results from previous investigations of various azurophilic granule proteins such as myeloperoxidase (52) and defensins (53), indicating that the sorting mechanisms are distinct from those of typical lysosomal enzymes (54).