Dog Mast Cell α-Chymase Activates Progelatinase B by Cleaving the Phe^{88}-Gln^{89} and Phe^{91}-Glu^{92} Bonds of the Catalytic Domain*

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Kenneth C. Fang‡§†, Wilfred W. Raymond, John L. Blount, and George H. Caughey‡§‖

From the §Department of Medicine, University of California, San Francisco, California 94143-0911

In prior work we showed that a metalloelastase is secreted from mast cells and directly activated by exocyotosed mast cell α-chymase. The current work identifies the protease as a canine homologue of progelatinase B (92-kDa gelatinase, MMP-9), determines the sites cleaved by α-chymase, and explores the regulation of gelatinase expression in mastocytoma cells. To obtain a cDNA encoding the complete sequence of mastocytoma gelatinase B, a 2.3-kilobase clone encoding progelatinase was isolated from a BR mastocytoma library. The sequenced cDNA predicts a 704-amino acid propeptide domain sequence, PRCGVPD, which correspond to residues 89–92 of the cDNA predicted sequence, respectively. Thus, α-chymase cleaves the catalytic domain of gelatinase B at the Phe^{88}-Gln^{89} and Phe^{91}-Glu^{92} bonds. Like BR cells, the C2 line of dog mastocytoma cells constitutively secrete gelatinase B, which is activated by α-chymase. By contrast, non-chymase-producing C1 cells secrete gelatinase B (which remains in its proform) only in response to 12-O-tetradecanoylphorbol-13-acetate. Whereas 12-O-tetradecanoylphorbol-13-acetate stimulation of BR cells produced a 15-fold increase in gelatinase B mRNA expression, decomethasone down-regulated its expression. Thus, extracellular stimuli may regulate the amount of mast cell progelatinase B expressed by mast cells. These data further support a role for mast cell α-chymase in tissue remodeling involving gelatinase B-mediated degradation of matrix proteins.

Gelatinase B is a matrix-degrading Ca^{2+}- and Zn^{2+}-dependent metalloenzyme secreted as an inactive zymogen by a variety of inflammatory, tumor, and epithelial cells (1–7). Like other members of the matrix metalloproteinase (MMP) family, gelatinase B must first be processed to an active form before it can degrade its preferred matrix substrates. Whether initiated by reagents such as chaotropes, oxidants, or proteases, MMP activation proceeds along a putative common pathway which involves disruption of an intramolecular interaction between a propeptide Cys and Zn^{2+} in the active site, a mechanism which has been termed the cysteine switch (8).

Gelatinase B, like other MMP's, appears to be biologically ubiquitous. It has been identified in all major organ systems and implicated in numerous homeostatic and pathological processes. Wound injury models suggest a unique role for the enzyme in remodeling of basement membranes, which are composed mainly of collagen IV, its principal collagenous substrate (9–12). While mechanisms regulating MMP activation in vivo remain unclear, activation pathways involving proteases are likely. Proteolytic cleavage on the COOH-terminal side of the propeptide domain sequence, PRGVPD, disrupts the cysteine switch and permits further zymogen processing by autocatalytic cleavages. Autolysis truncates the proenzyme at both the NH_{2} and COOH termini to yield enzymatically active forms (8).

Serine proteases, such as plasmin and furin (13–15), as well as certain members of the MMP family (14, 16–18) are among the proposed physiologic activators of pro-MMP's.

Mast cells are widespread, extravascular mononuclear cells which release serine proteases during degranulation. They produce and store tryptic and chymotryptic enzymes (tryptases and chymases, respectively) which have been implicated in MMP activation pathways (19–24). We previously reported that BR dog mastocytoma cells constitutively release a 92-kDa gelatinolytic protease similar to gelatinase B, but secrete its activator, α-chymase, only in response to a degranulating stimulus (24). Those data predicted that activation of gelatinase by α-chymase would occur in the setting of mast cell degranulation by agents such as anaphytoxins, substance P, or antigen-bound IgE (19). Once released into the neutral extracellular environment, α-chymase readily activates gelatinase B without intermediary proteases or cofactors (24). In the present work, we identify the sites in progelatinase B cleaved by α-chymase and explore the expression of mast cell gelatinase B.

EXPERIMENTAL PROCEDURES

Cloning and Sequencing of Gelatinase cDNA—To screen the dog BR mastocytoma λgt10 cDNA library (25), a 235-base pair fragment of human gelatinase B corresponding to nucleotides 1291–1526 (26) was fluorescein-labeled in a random primed reaction using fluorescein-11-dUTP and exonuclease-free Klenow fragment (Amersham). This fragment, which encodes a portion of the α_{2}-collagen V-like region that is unique to gelatinase B, was a gift of R. Dehiya. Phage from the library

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† Recipient of Individual National Research Service Award HL-09133 and Mentored Clinical Scientist Development Award HL-03845 from the National Institutes of Health. To whom correspondence and reprint requests should be addressed: Box 0911, Cardiovascular Research Institute, University of California, San Francisco, CA 94143-0911. Tel.: 415-502-7938; Fax: 415-476-9749; E-mail: kfang@itsa.ucsf.edu.

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1 The abbreviations used are: MMP, matrix metalloproteinase; IL-1, interleukin-1; SBTI, soybean trypsin inhibitor; TPA, 12-O-tetradecanoylphorbol-13-acetate; CAPS, 3-cyclohexylamino)-1-propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.
were plated in agarose, incubated at 37 °C for 12 h, and then transferred to Hybond N+ nylon membranes (Amersham). The membranes were pre-hybridized in buffer containing: 0.75 mM NaCl, 0.05 mM NaH2PO4 (pH 7.4), and 5 mM EDTA (5× SSPE), 0.1% bovine serum albumin (wt/v), 0.1% Ficoll, 0.1% polyvinylpyrrolidone (5× Denhardt's reagent); and 10 mg/ml salmon sperm (Sigma) for 1 h at 65 °C. Imobilized phage DNA was then hybridized to 100 ng of denatured fluorescein-labeled probe in the same solution for 16 h at 65 °C. Following hybridization, the membranes were washed twice in 2× SSPE with 0.1% SDS for 10 min at 20 °C, and twice in 1× SSPE with 0.1% SDS for 15 min at 50 °C.

For signal generation, 150 μg/ml of the renatured salmon sperm (Sigma) for 1 h at 65 °C. To immobilize phage DNA from several clones was purified and rescreened. Phage DNA from several clones was purified by BR or C2 cells were incubated with 40 nM 

The largest oligonucleotide fragment was ligated into the EcoRI site of phagemid SK+ phagemid (Stratagene). pBluescript T3 and T7 primers or synthetic oligonucleotides (Biomolecular Resource Center, University of California, San Francisco) based on previously determined sequences were used to prime the sequencing reactions. Sequences were analyzed and aligned using GeneWorks software (Intelligenetics).

Cell Culture—BR dog mastocytoma cells were cultured in Dulbecco's modified Eagle's medium-H16 medium supplemented with 10% calf serum and harvested as described previously, C2.5. C1 and C2 dog mastocytoma cells were harvested by centrifugation at 500 × g for 5 min, washed three times in Ca2+- and Mg2+-free PBS, and then resuspended in serum-free media to a final concentration of 2 × 106 cells/ml. Cells were incubated in the presence of 10-8 M 12-O-tetradecanoylphorbol-13-acetate (TPA) or 10-8 M soybean trypsin inhibitor (SBTI) at 37 °C. The conditioned medium was harvested after 24 h and centrifuged at 500 × g for 5 min to remove cells and debris, and then stored at −20 °C. Aliquots of conditioned medium were concentrated to 10-fold using a Centricon-10 concentrator (Amicon). Gelatinolytic activity was assayed by gelatin substrate zymography performed as described previously.

To generate medium rich in unactivated progelatinase B, BR and C2 cells were co-incubated in the presence of 3 mM phenylmethylsulfonyl fluoride and 10-8 M TPA at 37 °C for 18 h. The conditioned medium was harvested after 18 h and centrifuged at 500 × g to remove cells and debris; decanted supernatant was stored at −20 °C.

For isolation of gelatinase B mRNA transcripts, BR cells were resuspended in serum-free Dulbecco's modified Eagle's medium-H16 medium at a concentration of 1 × 106 cells/ml and incubated alone or with either 10-8 M TPA, 10-10 M dexamethasone, or 10-10 M interleukin (IL)-1β for 6 h at 37 °C. Cells were then washed three times in phosphate-buffered saline and cultured for 18 h more in Dulbecco's modified Eagle's medium-H16 medium either alone or with the same concentrations of TPA, dexamethasone, or IL-1β. After centrifugation at 500 × g for 5 min, the cell debris was removed at various intervals and the reactions were terminated by addition of 62.5 mM SBTI, 3 mM aprotinin, or 10 μg/ml A-Pho-Fr-Pro-Pho chloromethylketone (Sigma) and placed at 0 °C. The gelatinase samples were diluted in 25 mM Tris-HCl (pH 7.6) with 5 mM CaCl2, 150 mM NaCl, 0.02% NaN3, and 2 mg/ml ovalbumin and then added to an equal volume of [3H]gelatin. After incubation at 37 °C for different time periods, the reactions were stopped by the addition of an equal volume of 50% trichloroacetic acid. Aliquots of supernatants were analyzed by liquid scintillation spectrometry to detect acid-solubilized gelatin peptides. One unit (U) of gelatinolytic activity was defined as 1 μg of gelatin digested per min at 37 °C.

RNA Blotting—Poly(A)+ RNA was isolated from BR mastocytoma cells incubated alone or in the presence of TPA, dexamethasone, or IL-1β using a poly(A)+ RNA extraction kit (Micro-Fast Track, Invitrogen). After denaturation in 5% formaldehyde, 50% formamide at 85 °C for 15 min, poly(A)+ RNA was size-fractionated on a 1% agarose gel containing 6.1% formaldehyde, transferred to Nytran Plus nylon membrane (Schleicher and Schuell), and vacuum-baked at 80 °C. The membrane was prehybridized for 2 h at 42 °C in 50% formamide containing 5 × Denhardt's reagent and 5 × SSPE with 0.1% SDS and 150 mM sodium-sulphite DNA. The 2.3-kilobase dog gelatinase B cDNA was random-prime labeled with [3H]deoxyATP (Amersham) and hybridized to the filter at 42 °C overnight. The filters were washed twice in 6× SSPE with 0.1% SDS at room temperature for 15 min, twice in 1× SSPE with 0.1% SDS at 37 °C for 15 min, and once in 0.1× SSPE with 0.1% SDS at 55 °C for 15 min. To remove previously bound probe, blots were incubated in 5 mM Tris (pH 8.0), 0.2 mM EDTA, 0.05% pyrophosphate, and 0.1% Denhardt's reagent at 65 °C for 5 h. Densitometric data were analyzed by computer analysis of autoradiographs generated using a laser densitometer. The data were then compared with control values obtained with the γ-actin probe. Densitometric data were then compared with control values obtained with the γ-actin probe.

RESULTS

Cloning of Dog Mastocytoma Gelatinase B—Screening of ~106 plaques of the BR dog mastocytoma λgt10 cDNA library with a human gelatinase B cDNA fragment yielded 16 hybridizing clones. A 2.3-kilobase cDNA isolated from these clones contains an open reading frame of 2115 bases that encodes a 704 amino acid protein. A search in a database using the method of BLAST indicates that none of these sequences share significant similarity with any of the mammalian, avian, or insect types of collagenase, and the cDNA sequence is not translated at any of the start codons present in the cDNA sequence.

The 3′-region of the cDNA sequence was then compared with human collagenase B cDNA sequence.

Processing of a 19-residue signal peptide yields a cDNA-predicted proenzyme NH2-terminal sequence that differs by one residue from the previously reported dog mastocytoma NH2-terminal sequence.
radation of the purified enzyme yielded the sequence, APXNPKTVVVFP, with an indeterminate residue in position 3. The dog cDNA predicts the sequence, APRPHKPTVVVFP, which includes His at position 5. This is consistent with a less intense His peak also previously detected by Edman degradation (24).

The dog nucleotide and predicted amino acid sequences are 78 and 80% identical, respectively, to the corresponding human gelatinase B sequences. Whereas the dog protein sequence is 70% identical to mouse and rat sequences, it is 81% identical to the rabbit sequence (29–31). Regions critical for MMP active site latency and structural stability are highly conserved. As seen in Fig. 2, the dog propeptide sequence, PRCGVPDLG (78–86), and catalytic domain sequence, HEFGHALGLDHSSVPE (382–392), are identical to the corresponding mammalian sequences. These regions contain the Cys and His residues essential for ligation of the catalytic Zn$^{2+}$ in MMP's (32). The dog and human peptide sequences also share 19 conserved Cys residues as shown in Fig. 2. Two of these residues, Cys446 in the collagen V-like region unique to gelatinase B and Cys652 at the COOH terminus, do not occur in other types of MMP's. The dog enzyme also shares 2 of 3 putative N-glycosylation sites identified in other gelatinase B's. As seen in Fig. 2, the dog consensus site, Asn19, is shared by the human, mouse, and rabbit sequences, but not by the rat sequence. The dog enzyme lacks another potential glycosylation site at residue 101 shared by other species. The dog cDNA contains a unique 9-base pair in-frame deletion in the 54 residue collagen V-like region that predicts the absence of 3-amino acid residues beginning at Pro$^{338}$ as indicated in Fig. 2.

**Mast Cell Gelatinase B Expression**—In addition to the BR line of dog mastocytoma cells, several other mast cell lines expressed gelatinase B. As seen in Fig. 3, crude media conditioned by C1 and C2 dog mastocytoma cells demonstrated gelatinolytic activity with an electrophoretic profile characteristic of gelatinase B. Expression of gelatinase B by C2 cells in the presence of TPA or SBTI was similar to that previously observed for BR cells (24). C1 cells expressed gelatinase B only in response to TPA stimulation. No gelatinolytic activity appeared at a molecular mass below 92 kDa in media conditioned by cells in the presence of TPA alone. These data suggest that progelatinase B secreted by C1 cells is inactive probably due to the absence of a-chymase. C2 cells release measurable amounts of a-chymase, but C1 cells do not (33). As shown in lane 5 of Fig. 3, incubation of purified BR or C2 progelatinase B and crude TPA-induced C1 progelatinase B with purified dog a-chymase generated lower molecular weight bands of gelatinolytic activity.

**Soluble Activity of Gelatinase B**—We previously determined that a-chymase cleavage of purified dog mastocytoma gelatinase B yields products which digest $^{3}H$-labeled gelatin substrate in solution (24). To investigate the effect of heparin on a-chymase-mediated activation of gelatinase B, soluble gela-

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**Fig. 1. Nucleotide sequence of dog gelatinase B.** The nucleotide sequence was determined on both strands of a full-length cDNA obtained by screening a dog Agt10 cDNA library using a human probe containing the a2-collagen V-like region unique to gelatinase B. The predicted amino acid sequence shown under the DNA sequence contains a 19-residue signal peptide. The amino terminus of the proenzyme is indicated by an asterisk. The putative AATAAA polyadenylation signal is underlined. The sequence is available under GenBank accession No. U89842.
tinase B activity was determined following proenzyme activation by either α-chymase alone or α-chymase reconstituted with heparin, as shown in Fig. 4. As previously shown, gelatinase activity detected at time 0 reflects the presence of active forms resulting from autoactivation during purification or storage. In the absence of heparin, α-chymase-activated gelatinase B activity increases; 1.8-fold at 3 h and then declines. As shown in Fig. 5, the maximal specific activity of α-chymase-activated gelatinase B is 735 units/mg which represents 55% of the maximal specific activity of trypsin-activated gelatinase B (1335 units/mg). By contrast, gelatinase B activity peaks at 1 h when α-chymase is reconstituted with heparin (in a 1:100 heparin:α-chymase mass ratio) and demonstrates a similar; 1.7-fold increase in activity. Increasing heparin:α-chymase mass ratios precludes detection of gelatinase B activity, suggesting rapid proenzyme activation and inactivation (data not shown).

### Chymase Cleavage Site of Gelatinase B

As seen in Fig. 6, incubation of progelatinase B with α-chymase results in a decrease in the proenzyme form and the appearance of two bands at 88 and 84 kDa. Addition of heparin in a mass ratio of 1:1 with α-chymase accelerated the cleavage of progelatinase B compared with α-chymase alone (data not shown). Increasing the enzyme:substrate ratio resulted in greater conversion of the proenzyme to the 84-kDa band, without any increase in the faint 92-kDa band as seen in lane 3. Whereas bands corresponding to the 92-kDa proform and the 84-kDa α-chymase cleavage product were visible on the membrane following blotting and detection by Coomassie Blue staining, the 88-kDa band was variably detected. NH₂-terminal sequencing of the 84-kDa band yielded two overlapping sequences offset by three residues, QTFEGDLK X and EGDLK X HHND, in a molar ratio of approximately 1:1.5. No residue was assigned in cycles 9 or 6, respectively, due to insufficient discrimina-

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**Fig. 2.** Amino acid alignment of mammalian gelatinase B. The dog protein sequence (U89842) was aligned with the predicted amino acid sequences of human (J05070), rabbit (L36050), mouse (D12712), and rat gelatinase B (U24441) using GeneWorks software (GenBank accession numbers in parentheses). Areas of identity are boxed. The amino terminus of the proenzyme is indicated by the number 1. Scissile bonds in the dog catalytic domain cleaved by α-chymase are indicated by arrows. The α2-collagen V-like region unique to gelatinase B, a frequent site of variability, is indicated by a black overbar. Note the unique deletion in this region of the dog sequence indicated by an open overbar. The dog sequence contains two of three potential glycosylation sites shown by a gray overbar. Cysteine residues are indicated by an asterisk (*).
FIG. 3. α-Chymase activation of mast cell gelatinase B. A, expression of gelatinase B by the BR, C1, and C2 lines of dog mastocytoma cells. Cells were incubated with medium alone (lane 1), medium plus 10⁻⁸ M TPA (lane 2), or with medium plus 10⁻⁸ M TPA and 12.5 nM SBTI (lane 3). After 24 h, medium was collected and subjected to gelatinzymography as described previously. Gelatinolytic activity was detected in medium conditioned by either BR or C2 cells incubated alone. TPA increased the intensity of multiple bands, while SBTI ablated activity of the lower bands. Medium conditioned by C1 cells incubated alone demonstrated no gelatinolytic activity, but incubation with TPA resulted in the appearance of a single band at ~92 kDa. SBTI had no effect on activity in medium conditioned by C1 cells. B, α-chymase activation of gelatinase B. Activity of the purified BR or C2 gelatinase B and the TPA-induced C1 gelatinase B demonstrates a single band of activity at ~92 kDa (lane 4). Incubation of aliquots with 40 nM purified dog α-chymase for 20 min at 37 °C resulted in the appearance of lower bands (lane 5). The size (in kDa) and elution position of marker proteins are indicated to the left of the gel.

FIG. 4. Effect of heparin on α-chymase activation of progelatinase B. Purified dog gelatinase B (6.8 nM) was incubated with α-chymase (0.13 nM) alone or with α-chymase plus heparin in a 100:1 (α-chymase:heparin) mass ratio at 37 °C. Aliquots were removed at the indicated time intervals and the reactions were stopped by the addition of SBTI (200 μM). Gelatinase activity in the aliquots was determined by incubation of samples with [3H]gelatin. Activity of gelatinase B incubated with α-chymase alone increased by ~1.8-fold at 3 h. Activation of gelatinase B by α-chymase reconstituted with heparin resulted in a similar increase in activity which peaked at 1 h.

α-chymase cleavage of chromatographic peak amplitudes from the prior and subsequent cycles. With the exception of the indeterminate residue, these sequences are identical to the amino acid sequence (residues 89–101) predicted by the dog gelatinase B cDNA, as seen in Fig. 1, and are situated on the COOH-terminal side of the propeptide Cys80. This residue has not been cleaved by any of the aminopeptidases used in this study, and the indeterminate residue is resolved as Trp303 in the propeptide. The amino acid sequence of the propeptide Cys80 is therefore the same as that reported by Beverley (31). The sequence of the propeptide Cys80 is also consistent with the sequence of the propeptide Cys80 of the human gelatinase B (26), which is the same as that of the dog gelatinase B.

Detection of Transcripts for Gelatinase B—Analysis of poly(A)+ RNA from unstimulated BR cells demonstrated basal production of mRNA encoding gelatinase B as shown in Fig. 7. Incubation of cells with TPA at 37 °C for 48 h resulted in a 15-fold increase in the gelatinase B signal. Stimulation with IL-1β resulted in a smaller increase in the gelatinase B signal. By contrast, incubation with dexamethasone down-regulated expression of gelatinase B by 5-fold.

DISCUSSION

We previously reported that degranulating mastocytoma cells release α-chymase, which cleaves and activates the pro-form of a secreted metalloelastase (24). The work here establishes the identity of the previously purified dog gelatinase B as a canine homolog of gelatinase B, identifies Phe88-Gln89 and Phe91-Glu92 as the sites in its catalytic domain cleaved by α-chymase, and explores induction and suppression of gelatinase B expression in mast cells.

Progelatinase B catalytic domain sites cleaved by α-chymase provide insights into the tertiary structure of the proenzyme. Cleavage at a Phe residue is consistent with substrate specificities previously established for mammalian chymases, which also cleave less preferentially at sites with the hydrophobic amino acid Lys which is substituted for Glu92 in the mouse sequence.
These data predict that α-chymase-activated gelatinase B might not be as active as mature forms of gelatinase B with a Phe residue as the NH₂ terminus. The maximum specific activity of α-chymase-activated gelatinase B represents 55% of that induced by trypsin. This degree of enzymatic activity compares favorably to that of chymotrypsin-activated stromelysin (20%) (37) and rat chymase 2-activated collagenase (35%) (35). Therefore, α-chymase activates gelatinase B, but the activity of the product is lower than that of the product generated by proteases which yield an NH₂-terminal Phe. Thus, although an NH₂-terminal Phe may be needed to achieve maximal activity of gelatinase B, one or more alternative NH₂ termini generated by α-chymase nonetheless yields an enzyme with substantial activity as revealed by zymography as well as by cleavage of gelatin in solution.

α-Chymase released from human mast cells exists in a high molecular weight complex with proteoglycans (41), suggesting that bound heparin is an intrinsic determinant of its physiologic activity. Compared with purified α-chymase alone, the addition of heparin in a 1:1 mass ratio increases the rate of α-chymase-mediated procollagenase activation (20). Similarly, heparin accelerates the rate of α-chymase-mediated activation of gelatinase B, but does not appear to alter the magnitude of the specific activity of the mature enzyme. Thus, these data predict that physiologic activation of MMPs mediated by α-chymase proceeds at a rapid rate immediately following mast cell activation and degranulation.

The overlapping double sequence identified in the ~84-kDa cleavage product suggests that α-chymase may induce either sequential or simultaneous cleavages in the gelatinase B catalytic domain. An initial cleavage by α-chymase at Phe68, Glu89 may facilitate a second cleavage at Phe91-Glu92. Alternatively, α-chymase may cleave both sites simultaneously, possibly at different rates given the unequal molar ratio of the two NH₂-terminal sequences. Several lines of evidence favor a mechanism involving two α-chymase cleavages. Activation of human gelatinase B by collagenase, stromelysin, matrilysin, or trypsin results from a single cleavage at Arg88-Phe89 (35). The α-chymase cleavage product suggests that α-chymase-mediated activation of gelatinase B may induce either a Phe88-Gln89 or Phe91-Glu92 cleavage (20). The preference of α-chymase for Leu instead of Phe or Trp may be partially explained by the presence of a Val residue in the P2 position which has been shown to increase the sensitivity of a P1 residue to α-chymase cleavage (34). It is likely that the tertiary structure of the MMP propeptide and catalytic domains, and the exposure of aromatic residues (which one would usually expect to be buried in the hydrophobic interior of the protein) on surface loops ultimately determine the favored site for α-chymase-mediated hydrolysis.

α-Chymase activation of progelatinase B removes a Phe, which is the new NH₂ terminus of the mature form following activation of the zymogen by certain other proteases (Fig. 8). Cleavage by α-chymase at either Phe68-Gln69 or Phe91-Glu92 in the catalytic domain yields mature gelatinase B with Glu89 or Glu92 as the new NH₂ terminus. By contrast, trypsin, collagenase (MMP-1, MMP-8), stromelysin (MMP-3), or matrilysin (MMP-7) all cleave progelatinase B at the same site, generating a product with Phe380 as the NH₂ terminus (17, 36). Activation studies of procollagenase and stromelysin demonstrate that intermediates or mutant forms lacking an NH₂-terminal Phe exhibit reduced activity compared with those which have a Phe at the NH₂ terminus (35, 37, 38). Crystalllographic analysis of collagenase suggests that the Phe ammonium group forms a salt linkage with the side chain carboxylate of an Asp residue in the catalytic domain. Absence of the Phe residue results in a disordered NH₂-terminal hexapeptide, loss of enzymatic efficiency, and alterations in interactions with substrates (39, 40).

![Fig. 7. Effect of phorbol, dexamethasone, and interleukin-1β on mastocytoma gelatinase B expression. Poly(A)⁺ RNA isolated from BR cells incubated in medium alone (Medium), or in the presence of 10⁻⁸ M TPA (+TPA), 10⁻¹⁰ M dexamethasone (+DEX), or 10⁻¹⁰ M interleukin-1β (+IL-1β), was separated on a 1% agarose gel containing 6.1% formaldehyde and transferred to nylon membrane. The blot was hybridized with a [³²P]-labeled probe for dog gelatinase B. Densitometric data were obtained by analysis of autoradiographic signals after 96 h and then normalized to values obtained with the γ-actin probe. The normalized values are expressed as a percentage of the control signal obtained from unstimulated cells.](image-url)
absence of heparin resulted in either the preservation of the proenzyme form or its complete conversion to the ~84-kDa band. Thus, the ~88-kDa band appears to be a transient intermediate and could not be isolated for NH₂-terminal sequence determination.

Differential expression of progelatinase B by C1 dog mastocyte cells illustrates the importance of α-chymase in the activation of mast cell gelatinase B. In contrast to BR and C2 cells, which constitutively release progelatinase B which is activated extracellularly by α-chymase, C1 cells secrete progelatinase B only in response to TPA and the proenzyme is not proteolytically activated after release. Lack of α-chymase production by C1 cells (33) accounts for the preservation of the secreted enzyme in its proform. From this, we predict that subsets of mast cells lacking α-chymase (such as human MC7 cells) will be incapable of activating gelatinase B by this mechanism.

Whereas activation by exocytosed α-chymase can acutely gelatinase B activity, the magnitude and persistence of its activity may depend on extracellular signals which determine the relative abundance of the available proform. Following tissue injury, expression of gelatinase B increases to a greater extent and normalizes more rapidly than that of gelatinase A. This noncoordinate manner of regulation suggests that gelatinase B acts early during basement membrane reassembly, while gelatinase A plays a chronic role in stromal remodeling (11). Induction of gelatinase B by phorbol and IL-1β suggests that regulation of expression of the dog enzyme may occur at the level of transcription in mast cells present in a milieu rich in proinflammatory cytokines (29, 44). Down-regulation of gelatinase B by dexamethasone suggests that corticosteroid therapy may suppress mast cell MMP production. Our prior work showed that progelatinase B secreted by mastocytes remains in its proform in conditioned medium, but prior work showed that progelatinase B secreted by mastocytes only in response to TPA and the proenzyme is not proteolytically activated after release. Lack of α-chymase production by C1 cells (33) accounts for the preservation of the secreted enzyme in its proform. From this, we predict that subsets of mast cells lacking α-chymase (such as human MC7 cells) will be incapable of activating gelatinase B by this mechanism.

In summary, our results demonstrate that α-chymase cleaves the highly conserved portion of the progelatinase B catalytic domain at two scissile bonds containing the P1 Phe residue preferred by chymases. Hydrolysis by α-chymase at these sites suggest that aromatic residues are unusually exposed in progelatinase B’s catalytic domain.

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FIG. 8. Comparison of sites cleaved by α-chymase in the catalytic domains of procollagenase (MMP-1) and progelatinase B (MMP-9). Portions of the propeptide and catalytic domains of human progelatinase (46) and dog and human (26) progelatinase B are shown. The dog and human cDNA predicted sequences were aligned with GeneWorks software. Regions of identity are boxed. The Cys residue involved in the cysteine switch mechanism is indicated by a dot. Sites of progelatinase B (16) or progelatinase B (36) cleaved by aminopeptidase mercuric acetate are indicated by an asterisk (*). Sites of progelatinase B cleaved by plasmin, kallikrein, stromelysin (MMP-3), (16), trypsin (47), rat chymase 2 (RC2) (35), and α-chymase (20) are indicated by an arrow. Sites of progelatinase B cleaved by stromelysin (36), collagenase, matrilysin (MMP-7), and trypsin (17) are also shown by an arrow. Dog α-chymase cleaves progelatinase B at the Phe61-Gln69 and Phe91-Glu92 bonds.
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