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# Biological Functions of Serine Proteases in Mast Cells in Allergic Inflammation

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Serine proteases in mast cell granules, such as chymase, atypical chymase, and tryptase, which are major proteins in the granules, may play important roles in the process of immunoglobulin E (IgE)-mediated degranulation and in pathobiological alterations in tissues. Indeed, inhibitors of chymase, substrate analogs, and antichymase $F(ab')_2$, but not inhibitors of tryptase, markedly inhibited histamine release induced by IgE-receptor bridging but not that induced by Ca ionophore. In contrast, inhibitors of metalloprotease inhibited histamine release induced not only by IgE-receptor bridging but also by Ca ionophore. These results suggest that chymase and metalloprotease are involved at different steps in the process of degranulation. The extents of inhibition of histamine release were closely correlated with the amounts of the inhibitors of chymase accumulated in the granules. After degranulation, the released proteases may in part contribute to pathobiological alterations in allergic disorders through generations of C3a anaphylatoxin and thrombin by human and rat tryptase, respectively, and those of angiotensin II and a chemotactic factor of neutrophils by human and rat chymase, respectively. Moreover, chymase and atypical chymase from rat were shown to destroy type IV collagen, and human tryptase was found to hydrolyze various plasma proteins, such as fibrinogen and high-molecular-weight kininogen. The biological activities of tryptase and chymase from rat may be regulated by their dissociation from and association with trypstatin, an endogenous inhibitor of these proteases.

**Key words: chymase, atypical chymase, tryptase, trypstatin, histamine release, chemotaxis**

Mast cells play a central role in immunoglobulin E (IgE)-dependent and drug-induced immediate hypersensitivity reactions [1–3]. Two types of mast cells, termed connective tissue (normal) and mucosal (atypical) mast cells, have been distinguished in rodents [4–8] and humans [9] on the basis of differences in their compositions and histological, functional, and pharmacological properties. These cells as well as basophils have high-affinity plasma membrane receptors for IgE and show coupled activation-secretion on cross-linkage of these receptors. Mast cells contain a variety of vasoactive bronchoconstrictors, such as histamine, serotonin, and slow reacting substance of anaphylaxis; and chemotactic mediators; as well as enzymes and struc-

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tural proteoglycans. Activation of the mast cells leads to release of these preformed elements and to generation of potent biological substances. It is noteworthy that approximately 30% of the granular matrix of mast cells consists of protein and that serine proteases are major proteins in these granules [10–13].

Two serine proteases have been identified in rat connective tissue mast cells, and these have been named chymase (EC 3.4.21.39) [11], or mast cell proteinase I [14], and tryptase [15]. A chymotrypsinlike protease has also been identified in rat mucosal mast cells, and it has been named mast cell proteinase II [16] or atypical chymase [17]. Tryptase [18,19] and chymase [20] have also been found in human connective tissue mast cells, and tryptase has been identified in human mucosal mast cells. However, there is no report of atypical chymase in human mucosal mast cells or of tryptase in rat mucosal mast cells. These serine proteases are postulated to play important roles in the process of IgE-mediated degranulation and in pathobiological alterations in connective tissue around venules and in bronchial and gastrointestinal mucosa [1,21,22]. However, the actions of these proteases in allergic inflammation are not well understood.

Recently, serine protease inhibitors, substrates, substrate analogs, and antibodies of protease were shown to inhibit histamine release from IgE-activated mast cells, and the protease involved in the process of degranulation was identified [17,21–25]. Pathobiological alterations that occur in allergic disorders may in part be explained by these serine proteases secreted from activated mast cells. Endogenous regulators of the activities of these proteases, such as trypstatin [26] for rat tryptase [15], heparin for human tryptase [27] and for rat and human chymase [11,28,29], and phosphoglycerides and fatty acids for rat chymase [30,31], have been reported. This paper reviews current studies on the properties of serine proteases in mast cells and on the possible functions of these proteases in mast cells in degranulation and in allergic pathobiological alterations.

## PROPERTIES AND INHIBITORS OF SERINE PROTEASES FROM MAST CELLS

The chymotrypsinlike serine protease in connective tissue mast cells, named chymase, was first detected in rat peritoneal mast cells by Benditt and Arase [32]. This protease was purified and crystallized from rat skeletal muscle and liver as "group-specific protease" [33–35] and was later shown to be localized in connective tissue mast cells in these organs [36]. It has also been purified from rat peritoneal mast cells [11,29] and shown to have properties identical to those of chymase from rat skeletal muscle [37]. Human chymase was found in extracts from human skin [38,39] and was also purified [20].

Chymase is localized in the granules of connective tissue mast cells as shown by histochemistry studies [40,41], by immunofluorescence studies with monospecific antibody against purified chymase [42], and by immunoelectron microscopy [43]. It is localized in mast cell granules as a complex with heparin proleoglycan and is solubilized from granule fractions only at high ionic strength [11,28,41,42]. Although $\beta$-hexosaminidase (EC 3.2.1.30) and histamine are fully soluble on their release from mast cells into physiologic buffer, the complex of chymase and heparin proteoglycan is retained by the cells in the form of an insoluble complex exposed to the extracellular milieu [24,42]. The amino acid sequence of rat chymase from peritoneal mast cells

was determined [36,43]. The active enzyme contains 227 residues, including three corresponding to the catalytic triad characteristic of serine protease (His 517, Asp 102, and Ser 195 in α-chymotrypsin), as shown in Figure 1. The sequence shows 73% identity with that of rat atypical chymase from mucosal mast cells and 33% identity with that of bovine α-chymotrypsin. The isoelectric point of the chymase is 9.3, whereas that of α-chymotrypsin is 8.5 [29]. Unlike bovine α-chymotrypsin, which is composed of two protein chains linked by disulfide bonds and has a molecular weight of 25,000, rat and human mast cell chymases are single chain proteins with molecular weights of 24,000–29,000 [11,29,33,48] and 30,000 [20], respectively, as determined by electrophoresis in sodium dodecyl sulfate (SDS) polyacrylamide gel (Table I). The minimum molecular weight of rat chymase, calculated from amino acid sequence data, is 25,190 [43]. Like α-chymotrypsin, chymase catalyzes the cleavage of peptide and ester bonds on the carboxyl end of internal aromatic amino acids at pH 8–9 [33,48]. The substrate specificity of purified rat chymase toward a spectrum of synthetic peptide derivatives of p-nitroanilide (p-NA) revealed the preferred structure to be

$$\begin{array}{cccc} P_4 & P_3 & P_2 & P_1 \\ \downarrow & \downarrow & \downarrow & \downarrow \\ X & - Y - & Leu & - Phe - p - 6NA \end{array} \quad [49].$$

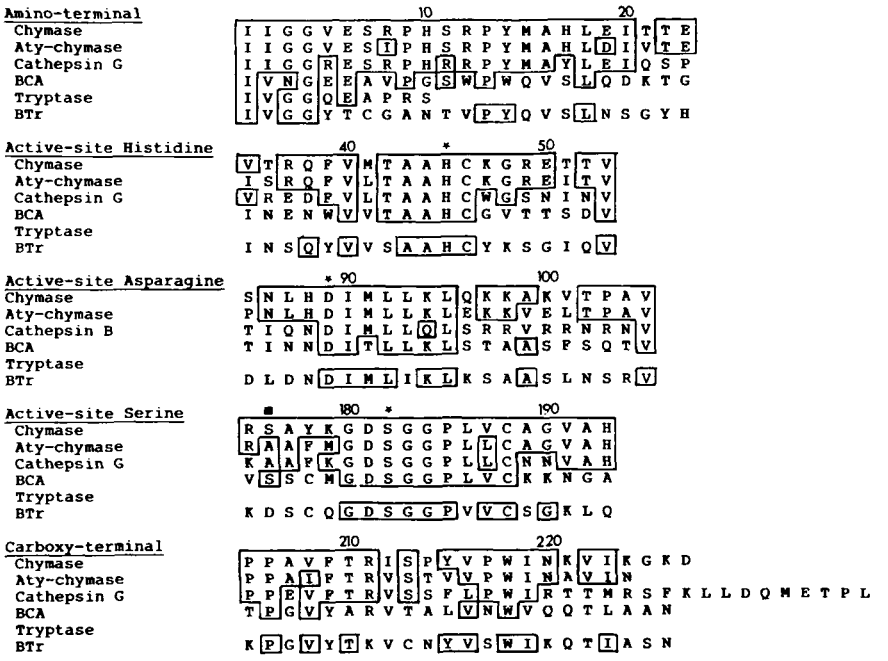


Fig. 1. Homologies of rat chymase [43], rat atypical (Aty) chymase [16], human cathepsin G (EC 3.4.21.20) [44], bovine α-chymotrypsin (BCA) [45], human tryptase [46], and bovine trypsin (BTr) (EC 3.4.21.4) [47]. Asterisks indicate components of the catalytic triad; the solid square indicates residue 176 in the putative binding pocket.

**TABLE I. Comparison of Properties of Human and Rat Chymase, Atypical Chymase, and Tryptase**

| Properties | Chymase | Atypical chymase | Tryptase |
|---|---|---|---|
| Localization | Granules (rat and human connective tissue mast cells) | Granules | (Rat and human connective tissue mast cells and human mucosal mast cells) |
| Crystal form | Long rods (rat) | Cubes (rat) | — |
| Molecular weight | 25,190 (rat)<br>30,000 (human) | 24,768 (rat) | 142,000 (rat)<br>144,000 (human) |
| Subunit composition | Monomer | Monomer | Tetramer<br>four subunits of 35,000 daltons (rat)<br>two subunits of 37,000 daltons and two subunits of 35,000 daltons (human) |
| Optimum pH | 8–9 | 8–9 | 8–9 |
| Inhibitors in serum and tissue | $\alpha_1$-antichymotrypsin (human, rat)<br>$\alpha_1$-antitrypsin (rat)<br>Phosphoglycerides (rat)<br>Fatty acids (rat)<br>Trypstatin (rat) | | Trypstatin (rat)<br>$\alpha_1$-antitrypsin (rat)<br>Aprotinin (rat) |

The best substrate found is N-Suc-Phe-Leu-Phe-p-NA, which has a $K_{cat}/K_m$ of about $1 \times 10^6\ M^{-1}\ sec^{-1}$ [49]. Cleavage of proteins, such as ornithine transaminase (EC 2.6.1.13) apoenzyme [50], glucagon and neurotensin [51], human plasma fibronectin [52], and type IV but not type I collagen [53], has been demonstrated to occur in vitro. Rat chymase activity is inhibited by various chymotrypsin inhibitors, such as chymostatin ($K_i = 61.9 \times 10^{-9}$ M), eglin c ($K_i = 23.2 \times 10^{-9}$ M), Kunitz soybean protease inhibitor ($K^i = 83.3 \times 10^{-9}$ M), Bowman-Birk soybean protease inhibitor (BBI; $K^i = 1\ 3.2 \times 10^{-9}$ M) and methoxysuccinyl (Meo-Suc)-Ala-Ala-Pro-(L)boro-Phe-OH ($K^i = 57.9 \times 10^{-9}$ M), a substrate analog [17,54]. Rat chymase is also markedly inhibited by membrane components, such as phosphoglycerides [30] and fatty acids with carbon chain lengths of 14–22 [31]. Phosphoglycerides specifically inhibit rat chymase and human granulocyte elastase but have no effect on $\alpha$-chymotrypsin, atypical chymase, or trypsin [30].

A chymotryptic protease in mucosal mast cells was first detected in rat small intestine and crystallized by Katunuma et al. [33]. The enzyme, originally called "group-specific protease," was shown to be localized in mucosal mast cells in the gastrointestinal and bronchial mucosa [55]. This enzyme (atypical chymase) was also detected in the granules of rat basophilic leukemia (RBL) cells [56,57], but its presence in human mucosal mast cells has not been reported. Antibodies against atypical chymase and chymase do not cross react. Connective tissue and mucosal mast cells in mice were recently shown to share a common stem cell and to diverge phenotypically because of differences in the tissue environments for their final differentiations [58,59].

The amino acid sequence of atypical chymase from rat small intestine was determined [16]. The enzyme contains 224 amino acid residues in a single polypeptide chain, has a minimum molecular weight of 24,768, has a calculated amino acid

sequence (Table I), and contains three disulfide bonds. Atypical chymase has nine fewer lysyl residues than chymase, which may account for the striking difference in the case of extraction of the two enzymes from mast cells; atypical chymase, which may be associated with proteoglycan [60], is readily extracted with physiological saline, whereas chymase, which interacts tightly with the more sulfated heparin in the granules, can be extracted only with 1 M salt [11,28,41,42]. Another structural difference of chymase from atypical chymase is the presence of a seryl residue in place of an alanyl residue at position 176, which is the putative substrate pocket of atypical chymase [43], as is shown in Figure 1. This difference may be correlated with the finding that the specificity constants, $K_{cat}/K_m$, for atypical chymase and cathepsin G are in general several orders of magnitude smaller than those for chymase and chymotrypsin [49]. The complete sequence of the cDNA and a corresponding genomic clone of atypical chymase from RBL cells were recently analyzed [61]. The results show that atypical chymase is synthesized as a precursor, with a signal peptide at the $NH_2$ terminus, as would be expected for a secreted protein, and an additional tripeptide sequence, Thr-Ser-Ser, at the COOH terminus. Analysis of RNA from RBL cells and from peritoneal mast cells indicated that atypical chymase and chymase are products of different genes, although their primary structures are strikingly similar, as is shown in Figure 1 [43]. Comparison of the exon–intron structure of the atypical chymase gene with those of genes of related serine proteases, such as chymotrypsin, elastase (EC 3.4.21.11), trypsin, and adipsin, shows that atypical chymase is a representative of a distinct gene family of serine proteases [61].

Tryptase, an endopeptidase that cleaves peptide and ester bonds on the carboxyl side of basic amino acids, was purified from human pulmonary mast cells [18] and also from rat peritoneal mast cells [15]. Trypsinlike protease activity was also found in a mouse mast cell tumor [61]. In humans, tryptase was shown immunohistochemically to be localized in secretory granules of human TC (tryptase-positive, chymase-positive) mast cells (connective tissue mast cells) and human T (tryptase-positive, chymase-negative) mast cells (mucosal mast cells) [13]. Tryptase from rat peritoneal mast cells was also suggested to be localized in the granules [15], but its localization in rat mucosal mast cells has not been reported.

The enzymes from humans and rats are tetramers, with apparent molecular weights of 144,000 and 142,000, respectively [15,18]. Human tryptase has two subunits of 37,000 daltons and two subunits of 35,000 daltons [18], and that from rat has four identical subunits of 35,000 daltons, each having one active site [18]. A human pituitary-derived serine protease, immunologically identical to human lung tryptase, was also purified [46]. The first 10 residues in the amino-terminal sequences of the subunits of the tryptases from human lung and human pituitary are the same [46]. The $NH_2$-terminal Ile-Val-Gly-Gly sequence of the tryptase from lung and pituitary is identical to that in trypsin and many other serine proteases, in which the $NH_2$-terminal isoleucine has been shown to form an initial salt bridge upon zymogen activation (Fig. 1). There are several differences between human and rat tryptases. Human tryptase was reported to be resistant to inhibition by aprotinin, soybean and lima bean trypsin inhibitor, any $\alpha_1$-trypsin inhibitor [18,63], whereas rat tryptase is inhibited by these inhibitors and by trypstatin [26], an endogenous inhibitor of tryptase. Human tryptase binds to heparin proteoglycan in physiologic buffer in vitro and may be present as a complex with heparin proteoglycan in vivo [18], whereas rat tryptase does not interact with heparin (H. Kido, unpublished data).

Trypsin inhibitor was detected in extracts of various organs, such as the lung, parotid gland, spleen, liver, pancreas, and seminal vesicles [64], and was shown to be localized in tissue mast cells by an indirect immunofluorescence technique with antibody against aprotinin [65].

We have purified a Kunitz-type protease inhibitor, named trypstatin, from rat peritoneal mast cells. Trypstatin inhibited blood coagulation factor Xa and tryptase most strongly, for which its $K_i$ values were 1.2 and $3.6 \times 10^{-10}$ M, respectively. Trypstatin also inhibited chymase ($K_i = 2.4 \times 10^{-8}$M) and chymotrypsin but not plasmin, urokinase, or papain [15]. The amino acid sequence of rat trypstatin was recently determined to be as shown in Figure 2 [26]. A computer search for homology indicated 70% sequence identity of its inhibitory domain with those of inter-α-trypsin inhibitors (bovine and human) [66,67] and 35.1% identity with that of aprotinin [68]. Human inter-α-trypsin inhibitor is identical to an endothelial cell growth factor 2b from human hepatoma and human glycopeptide urinary proteinase inhibitor [69]. These results suggest that trypstatin not only is a serine protease inhibitor but also act as a growth-stimulatory factor that is specific for endothelial cells.

## ROLE OF SERINE PROTEASES IN MAST CELL DEGRANULATION

Serine protease inhibitors and substrates of trypsin and α-chymotrypsin are reported to inhibit phospholipid methylation and so to inhibit increased $Ca^{2+}$ influx and histamine release [21]. These findings suggest that serine protease(s) in mast cells, such as chymase and/or tryptase, is activated by IgE-receptor bridging and plays some role in triggering the process of degranulation. To clarify which of these proteases is involved in the process of degranulation, we studied the effects of potent inhibitors, substrate analogs, and $F(ab')_2$ fragments of antichymase on histamine release from activated mast cells, and results are shown in Table II. Mast cells were activated by antirat IgE, compound 48/80, and Ca ionophore A23187, respectively, which are thought to act at different steps in the process of degranulation. Low-molecular-weight inhibitors of chymase, such as chymostatin and peptide boronic acids, which are substrate analogs [70], markedly inhibited histamine release

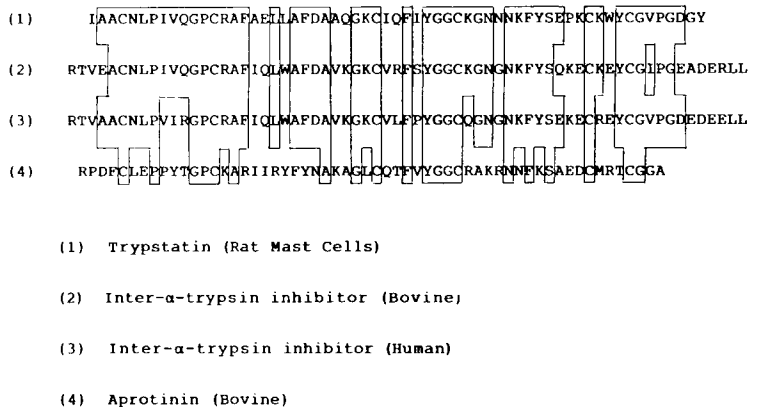


Fig. 2. Homologies of rat trypstatin, bovine inter-α-trypsin inhibitor [66], human inter-α-trypsin inhibitor [67], and bovine aprotinin [68].

**TABLE II. Effects of Inhibitors of Proteases on Histamine Release From Mast Cells***

| Inhibitors and antibody | Concentration | Percent of maximum histamine release | | |
|---|---|---|---|---|
| | | Anti-IgE (150 μg/ml) | Ca-ionophore A23187 (0.5 μg/ml) | Compound 48/80 (5 μg/ml) |
| None | | 100 | 100 | 100 |
| Aprotinin | 25 μM | 121 | 92 | 86 |
| Leupeptin | 25 μM | 80 | 106 | 94 |
| E-64 | 25 μM | 100 | 86 | 90 |
| 1,10-Phenanthroline | 25 μM | 23 | 82 | 47 |
| Phosphoramidon | 25 μM | 27 | 51 | 72 |
| Chymostatin | 25 μM | 27 | 62 | 100 |
| MeO-Suc-Ala-Ala-Pro-(L)boro-Phe-OH | 50 μM | 30 | | |
| MeO-Suc-Ala-Ala-Pro-(L)boro-Phe-pinacol | 50 μM | 25 | | |
| BBI | 25 μM | 74 | | |
| | 100 μM | 52 | | |
| Eglin C | 25 μM | 80 | | |
| | 100 μM | 62 | | |
| Antichymase $F(ab')_2$ | 25 μg | 97 | | |
| | 50 μg | 73 | | |
| | 100 μg | 45 | 100 | 120 |
| | 150 μg | 0 | | 115 |
| Control serum $F(ab')_2$ | 50 μg | 115 | | |
| | 100 μg | 91 | 100 | 100 |
| | 150 μg | 95 | | 105 |

*Mast cells were preincubated with protease inhibitors, or $F(ab')_2$ of antichymase, or nonimmunized $F(ab')_2$ for 10 min at 37°C, and histamine release was induced by antirat IgE, Ca ionophore A23187, or compound 48/80 [22]. Values are averages for triplicate samples.

[17,22,24] from rat mast cells induced by anti-rat IgE, but inhibitors of tryptase, such as aprotinin and leupeptin, caused little inhibition. Inhibitors of chymase with molecular weights of more than 6,000, such as BBI and eglin c, also inhibited histamine release dose-dependently, although they were less inhibitory than chymostatin and the substrate analogs. $F(ab')_2$ of antichymase, which specifically inhibits chymase activity, inhibited histamine release induced by anti-rat IgE dose-dependently, inhibition being complete with 150 μg/ml of the antibody [24]. However, it did not inhibit histamine release induced by Ca ionophore A23187 or compound 48/80. Nonimmunized $F(ab')_2$ was not inhibitory. E-64, an inhibitor of thiol protease, had no effect on histamine release. These results suggest that chymase in mast cell granules is involved in the process of IgE-mediated degranulation before a step of calcium entry but that tryptase is not involved. In contrast, the releases of histamine induced by anti-rat IgE, Ca ionophore A23187, and compound 48/80 were all inhibited by inhibitors of metalloprotease, such as 1,10-phenanthroline and phosphoramidon. Carboxypeptidase A in peritoneal mast cell granules [71,72] is reported to be inhibited by 1,10-phenanthroline [72]. These results suggest that another protease, which may be a carboxypeptidase A, is also involved in the process of degranulation at a step different from that at which chymase acts and possibly after a step of calcium entry, since histamine release induced by the Ca ionophore A23187 is blocked by inhibitors of metalloprotease but not by $F(ab')_2$ of antichymase. Similar results were obtained on

inhibition of the acetylcholine receptor-mediated process of catecholamine secretion from adrenal medullary cells [73,74]. Therefore, the chymotrypsinlike protease and metalloprotease in secretory granules of various cells seem to play some common roles in exocytosis.

For determination of which organelles in mast cells contain these potent inhibitors of chymase and antichymase $F(ab')_2$ that inhibit degranulation, radioiodinated antichymase $F(ab')_2$ and BBI were incubated with mast cells, and then their intracellular distributions were examined [25]. Results showed that these materials first bound to plasma membranes and then accumulated time- and dose-dependently in the granules of mast cells. The extent of inhibition of histamine release from IgE-activated mast cells was closely correlated with the amounts of these inhibitors accumulated in the granules but was not inhibited simply by their bindings to the plasma membrane [25]. Chymase could not be detected in the plasma membranes of mast cells by immunohistochemical staining (H. Kido, unpublished data).

The main questions that still remain to be answered are how chymase is activated by receptor bridging, what kind of biologically active peptides chymase and metalloprotease produce, and which process of degranulation the proteolytic products stimulate. Recently, stimulation of phospholipid methylation in rat mast cells [75] and phosphatidylinositol turnover in mast cell granules [76,77] after bridging of IgE receptors were demonstrated. In addition, phosphatidylinositol kinase was found in mast cell granules [77]. Phosphatidylinositol and phosphatidylserine and phosphatidic acid are potent inhibitors of chymase, whereas highly methylated phospholipids, such as phosphatidylcholine, are activators of chymase [30]. These facts suggest that changes in the phospholipid composition of the granules of mast cells induced by IgE-receptor bridging may alter the chymase activity in the granules and that after activation chymase may stimulates histamine release. Atypical chymase in the granules of mucosal mast cells and RBL cells may also have some role in the process of degranulation of these cells, but this possibility has not yet been investigated.

## ROLE OF SERINE PROTEASES IN MAST CELLS AFTER DEGRANULATION

After bridging of IgE receptors, histamine, tryptase, trypstatin, and $\beta$-hexosaminidase are released into the extracellular milieu, but released chymase is retained on the cell surface as a complex with heparin proteoglycan [24,42]. Therefore, the action of chymase may be restricted to a relatively small protein that can penetrate the chymase–heparin network [43]. The prolonged presence of heparin proteoglycan with chymase at the cell surface of activated mast cells may be relevant for the chronicity of the host response to mast cell activation.

Little is known about the function of chymase in vivo, but the enzyme from rat stimulates other mast cells in a microenvironment [78], generates a chemotactic factor of neutrophils [22] from IgG, and degrades basement membrane type IV collagen [53] and fibronectin but not type I, II, or III collagen [79] in vitro, and the enzyme from human converts angiotensin I to angiotensin II [80] (Fig. 3). Exposure of peritoneal mast cells to rat chymase or $\alpha$-chymotrypsin results in degranulation [78,81], and this degranulation is inhibited by enzyme inhibitors such as diisopropyl fluorophosphate and lima bean trypsin inhibitor [78]. The proteolytic product(s) of IgG formed by rat chymase has potent chemotactic activity on neutrophil leukocytes in vitro, and in vivo, whereas IgG itself has little chemotactic activity [22]. Mast cells

have been shown to have specific receptors for IgE and IgG [82]. Therefore, released chymase may cause limited hydrolysis of IgG and produce the chemotactic factor(s). The structural characteristics of the factor have not been elucidated. Chymase in mast cells of human skin has been shown to convert angiotensin I rapidly to angiotensin II, with only minor cleavage elsewhere in the molecule. This conversion was not inhibited by captopril, an inactivator of an angiotensin-converting enzyme [80].

Atypical chymase from rat mucosal mast cells, which is a highly soluble protein, is released into the blood circulation or gut lumen after intestinal anaphylaxis; thus the plasma or serum level of atypical chymase is correlated with events related to mucosal mast cells [83]. The enzyme released from mucosal mast cells has been suggested to play a role in generation of intestinal epithelial permeability [84] and to be involved at some level in the expulsion of intestinal parasites [85]. Atypical chymase has also been shown to degrate basement membrane type IV collagen in the same way as chymase does in vitro [53].

Various mediators of increase in permeability of venules, such as histamine, prostaglandin $D_2$, and the slow-reacting substances leukotrienes $C_4$ and $D_4$ [86], are released with tryptase from activated mast cells. Therefore, it is important to elucidate the effects of tryptase on plasma proteins. Human tryptase generates C3a from human C3 [87] and destroys high-molecular-weight kininogen [88] and fibrinogen [89] but has no effect on low-molecular-weight kininogen, bradykinin, plasma prekallikrein [90], or plasminogen [89] in vitro. These results show that human tryptase acts as an anticoagulant (Fig. 3). In contrast, rat tryptase converts prothrombin to thrombin [91] (Fig. 3). Its apparent $K_m$ for bovine prothrombin and its $K_{cat}$ are 2.3 $\mu$M and 46.3 $sec^{-1}$, respectively, suggesting that rat tryptase contributes to blood coagulation or the process of fibrin formation in tissues [91]. Trypstatin inhibits the activation of prothrombin by factor Xa and rat tryptase. The regulation and mechanism of dissociation and association of rat tryptase and trypstatin and those of human tryptase and heparin are not known. Trypstatin, which has 70% identity with inter-$\alpha$-trypsin

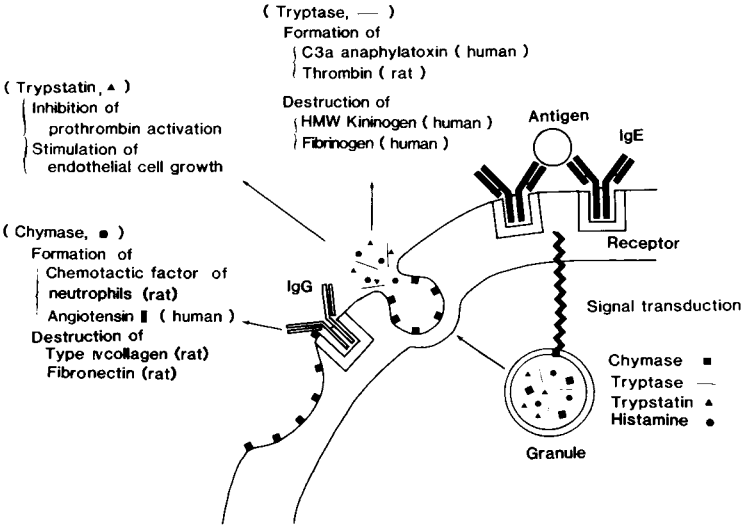


Fig. 3. Possible roles of serine proteases in connective tissue mast cells.

inhibitor as well as endothelial cell growth factor 2b, as shown in Figure 2, may act not only as an inhibitor of tryptase and chymase but also as a growth factor of endothelial cells.

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