Human Mast Cell β-Tryptase Is a Gelatinase

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remodeling of extracellular matrix is an important component in a variety of inflammatory disorders and metabolic processes such as wound healing and angiogenesis. Previous investigations have identified the various matrix metalloproteinases, e.g., gelatinases A and B, as key players in the degradation of extracellular matrix under such conditions. Here we show that an additional enzyme, human mast cell β-tryptase, has potent gelatin-degrading properties, indicating a potential contribution of this protease to matrix degradation. Human β-tryptase was shown to degrade gelatin both in solution and during gelatin zymographic analysis. Further, β-tryptase was shown to degrade partially denatured collagen type I. β-Tryptase bound strongly to gelatin, forming high molecular weight complexes that were stable during SDS-PAGE. Mast cells store large amounts of preformed, active tryptase in their secretory granules. Considering the location of mast cells in connective tissues and the recently recognized role of mast cells in disorders in which connective tissue degradation is a key event, e.g., rheumatoid arthritis, it is thus likely that tryptase may contribute to extracellular matrix-degrading processes in vivo. The Journal of Immunology, 2003, 171: 1493–1499.

issue-remodeling processes take place in many physiological and pathological conditions, e.g., embryonic development, wound healing, rheumatoid arthritis, and tumor invasion. In these processes degradation of the extracellular matrix (ECM) is required to allow the proliferation and/or migration of different cell types. Among other mechanisms, ECM degradation is controlled by the fine balance between a number of proteases and their respective inhibitors (1). At present, matrix metalloproteinases (MMPs) are being recognized as the main players in the ECM degradation associated with various tissue-remodeling processes. The MMP family can be divided into several major categories depending on their preferential substrate, e.g., the collagenases, gelatinases, stromelysins, and membrane-type MMPs. Together the MMPs have the ability to degrade all the different types of macromolecules present in the extracellular matrix, e.g., collagen, laminin, fibronectin, and proteoglycans. MMPs are initially synthesized as proteolytically inactive proenzymes. The level of active MMPs in the tissue is carefully controlled by regulating their rate of synthesis, by controlling the maturation of pro-MMPs to active enzymes, and by the presence of endogenous MMP inhibitors such as α2-macroglobulin and tissue inhibitors of metalloproteinases (2-5).

In addition to MMPs, several serine proteases have been implicated in ECM degradation, e.g., the plasminogen activator/plasmin system (6), which is subject to regulation by the plasminogen activator inhibitors (7). Another group of serine proteases that may have the potential to participate in ECM degradation is the mast cell proteases. Mast cells synthesize and store large amounts of various serine proteases of two subclasses: tryptases and chymases. In connective tissue-type mast cells, both the chymases and tryptases are packaged in the secretory granules in tight complexes with heparin proteoglycan (8). When mast cells are activated, e.g., by cross-linking of surface-bound IgE molecules by specific Ag or by exposure to anaphylatoxins or neuropeptides, the mast cells degranulate and release large amounts of serine proteases and other inflammatory mediators, e.g., histamine and TNF-α (9). The large amounts of proteases that are being released by mast cells during inflammatory conditions together with the fact that the mast cell serine proteases are stored and released in their active form make these proteases likely candidates for involvement in connective tissue degradation. Indeed, it has been indicated previously that mast cell chymase may promote ECM degradation by processing pro-MMP-9 (pro-gelatinase B) to its active form (10, 11) and by directly degrading various connective tissue components, e.g., fibronectin (12).

In humans, several forms of tryptases are being expressed: the proteolytically inactive α-tryptase (13), the transmembrane γ-tryptase (14), and the closely related β-tryptases, βI, βII, and βIII (see Ref. 15 for a review). Of these, the β-tryptases constitute the main active form of tryptase that is stored and secreted by human mast cells. Earlier work established that human β-tryptase is a heparin-stabilized tetramer in its active form (16), and the following crystallization of the tetramer showed that the active sites are facing toward a narrow central pore (17). This unique organization explains the almost complete resistance of tryptase to various macromolecular protease inhibitors (18). Another consequence is that relatively few macromolecular substrates are able to enter the central pore, resulting in a narrow substrate specificity of tryptase. Accordingly, many of the known substrates for tryptase are small peptides, including, e.g., vasoactive intestinal peptide and calcitonin gene-related peptide (reviewed in Ref. 19). The relatively few known macromolecular substrates for human β-tryptase include fibrinogen (20), single-chain urinary-type PA (pro-urokinase) (21), and fibronectin (22). However, recent data indicate that human β-tryptase can undergo dissociation into proteolytically active monomers, and it was shown that only the monomeric form of β-tryptase degrades fibronectin (23). Previous

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reports have also suggested that β-tryp has activ proMMP-3 (24). To further investigate the possibility that β-tryp may have a role in connective tissue degradation during inflammatory conditions, we here investigated whether tryp may, similarly to, e.g., MMP-2 and -9, have gelatinase activity. The results presented here identify human β-tryp as a potent gelatin-degrading enzyme, suggesting that tryp may have a direct role in the various ECM remodeling processes that occur during different inflammatory conditions.

Materials and Methods

Reagents

All studies were performed using recombinant human β-tryp (rt-BT) purchased from Promega (Madison, WI). The expression and purification of the enzyme have been previously described (25). The enzyme preparation, supplied in 2 M NaCl/10 mM MES, pH 6.1, at a concentration of 2 mg/ml, was diluted to 0.1 mg/ml using the same buffer and was stored (−70°C) in 2-μl aliquots. Human β-tryp purified from lung tissue (HLT) was purchased from Calbiochem (San Diego, CA). This tryp preparation, supplied in 300 mM NaCl, 10 mM MES, 20 μM heparin (pH 6.1), and 0.02% NaN3 with a protein concentration of 0.54 μg/ml, was diluted to 10 μg/ml using PBS (10 mM phosphate, 137 mM NaCl, and 0.05% Tween 20) and stored (−20°C) in 1-μg aliquots. Pig mucosal heparin (Mr = 15,000) was a gift from E. Lindahl (Upssala University, Uppsala, Sweden). Porcine skin gelatin (type A; Sigma-Aldrich, St. Louis, MO) was dissolved in H2O at 37°C before use. Bovine native type I collagen, purchased from Biotrend (Köln, Germany), was dissolved in 0.1 M acetic acid (pH 3) and stored at 4°C. Bovine pancreatic trypsin inhibitor was purchased from Roche (Mannheim, Germany). Pefabloc SC was obtained from PENTAPHARM (Basel, Switzerland). The chromogenic sub- strate S-2288 ([H-o-Ile-Pro-Arg-p-nitroanilide] was obtained from Chromogenix (Molndal, Sweden). All buffer salts were provided by Merck (Darmstadt, Germany) or Sigma-Aldrich.

Enzymatic assays

Enzymatic assays were performed in 96-well microtiter plates. Trypsate activities were routinely assayed in a volume of 100 μl of PBS (pH 6.0). Enzymatic activity was recorded after addition of 20 μl of a 2.16-MM solution (in H2O) of the chromogenic substrate S-2288. The absorbance at 405 nm was monitored at room temperature with a Titer-Tek Multiscan spectrophotometer (Flow Laboratories, McLean, VA).

Size-exclusion gel chromatography

Size-exclusion gel chromatography was performed on a Superdex 200 column (10 × 300 mm) using a fast protein liquid chromatography system (Amersham Pharmacia Biotech, Arlington Heights, IL). A flow rate of 0.5 ml/min was routinely used. Molecular masses were calculated after calibration of the column with the following molecular mass standards (all from Sigma-Aldrich): carbonic anhydrase (29 kDa), BSA (66 kDa), alcohol dehydrogenase (150 kDa), and β-amylose (200 kDa). The void volume of the column was determined using blue dextran. Running buffers were either PBS (pH 6.0); with or without 10 μM heparin or PBS (pH 7.4), as specified in the figure legends. Calibration of the column with molecular mass standards was performed for all the running buffers used. Before runs, the column was pre-equilibrated with 2 column volumes of the respective buffer. In each experiment, 2 μl of rh-BT (in 20 μl of 2 M NaCl/10 mM MES, pH 6.1) was diluted with 180 μl of PBS (pH 6.0) and was analyzed either directly or after being subjected to the different treatments specified in the figure legends. Five hundred-microliter fractions were collected, from which 100-μl samples were analyzed for tryp activity (see above).

Gelatin zymography

Gelatin zymography assays were performed as previously described (26). Briefly, samples were subjected to nonreducing SDS-PAGE (at 4°C) on gels containing 0.1% gelatin and 6% polyacrylamide. After the electrophoresis, gels were washed twice with 50 mM Tris-HCl, pH 7.4, supplemented with 2% Triton X-100, and twice with 50 mM Tris-HCl, pH 7.4. Each wash was for 10 min with continuous shaking and was performed at room temperature. After the washes, the gels were incubated overnight at 37°C immersed in a substrate buffer (50 mM Tris-HCl (pH 7.4), supplemented with 1% Triton X-100, 5 mM CaCl2, and 0.02% NaN3). Afterward, the gels were stained with Coomassie Brilliant Blue R-250, and gelatinolytic activity could be detected as nonstained bands.

Immunoblot analysis

Trypsate samples subjected to the different treatments specified in the figure legends were separated at 4°C on 6% SDS-PAGE gels with or without 0.1% gelatin. Proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Polyscreen; NEN, Boston, MA; previously equilibrated −10 min in the transfer buffer according to instructions from the manufacturer) in 25 mM Tris buffer containing 192 mM glycine and 20% methanol, using a BlueBlot Wet/100 transfer system (Serva Electrophoresis, Heidelberg, Germany) at 4°C. After transfer (1 h, 200 mA), membranes were blocked in PBS (pH 7.4) containing 0.1% Tween 20 and 5% nonfat milk, overnight at 4°C. All the following steps were performed at room temperature. After blocking, the membranes were washed briefly with PBS/0.1% Tween 20 before addition of a polyclonal anti-human tryp Ab (generated in rabbits; Calbiochem, La Jolla, CA) diluted 1/1000 in PBS/0.1% Tween 20 containing 5% nonfat milk. Next, the membranes were washed three times for 15 min each time in PBS/0.1% Tween 20 and were then incubated for 1 h with HRP-conjugated anti-rabbit IgG (Amersham Pharmacia Biotech) diluted 1/5000 in PBS/0.1% Tween 20 containing 2% nonfat milk. After 2 h the membranes were washed three times for 15 min each time in PBS/0.1% Tween 20, and immunoreactive proteins were detected using the ECL system (Amersham Pharmacia Biotech).

Results

Gelatinolytic activity of β-tryp

The possibility that human β-tryp may possess gelatinolytic activity was investigated by gelatin zymography. As a positive control, conditioned medium from the human fibrosarcoma HT1080 cells, a cell line widely used as a source of the matrix metalloproteinases MMP-2 and MMP-9, was included. As shown in Fig. 1A (lane 1), two gelatinolytic bands were detected in HT1080-conditioned medium, with apparent molecular weights of 212 and 170 kDa (Fig. B, lane 1). When 100 ng of rh-BT was incubated in a substrate buffer that did not contain CaCl2 (without reducing agent) and subjected to gelatin zymography as described in Materials and Methods, a 10-μl sample of HT1080-conditioned medium, containing proMMP-2 and -9 was included as a control (lane 1). B. Samples were subjected to the same procedure as in A with the exception that 100 ng was incubated in a substrate buffer that did not contain CaCl2 but was supplemented with 20 mM EDTA. A 10-μl sample of HT1080-conditioned medium was also included as a control (lane 1).
consistent with proMMP-9 and proMMP-2, respectively. It was also apparent that gelatinolytic activity was present in the preparations of both rh-βT (Fig. 1A, lanes 2 and 3) and HLT (Fig. 1A, lanes 6 and 7). In all tryptase samples a high molecular weight (HMW) band, barely entering the gel, was observed. The HMW compound showed a slower migration velocity than standard laminin (data not shown), indicating a size >800 kDa. In addition, samples treated with heparin gave a second, low molecular weight (LMW), gelatinolytic band (<50 kDa; Fig. 1A, lanes 3, 5, 7, and 9). The LMW gelatinolytic band was absent in rh-βT tryptase samples that had not been treated with heparin (Fig. 1A, lane 2), but was detectable at a low level in HLT that had not been treated with heparin (Fig. 1A, lane 6). However, the HLT preparation used is supplied with heparin, and the appearance of the HMW band in the heparin-untreated HLT samples is thus probably due to the heparin present in the starting material. The LMW band in HLT samples was increased in intensity upon treatment with exogenous heparin (Fig. 1A, lane 7). The HMW compound, which apparently does not require heparin for activity, is considerably larger than the tryptase tetramer (~120 kDa), whereas the LMW, heparin-dependent compound has a size compatible with that of monomeric tryptase (~30 kDa), taking into account that molecular weight determinations are uncertain in gelatin-containing gels. Treatment of the tryptase samples with Pefabloc SC, a general inhibitor of serine proteases, caused essentially complete inhibition of the gelatinolytic activity in the HMW band (Fig. 1A, lanes 4, 5, 8, and 9). In contrast, the gelatinolytic activity in the LMW band was only moderately affected by Pefabloc SC (Fig. 1A, lanes 5 and 9). However, when 2 mM Pefabloc SC was included in the substrate buffer during the gel development, both the HMW and LMW gelatinolytic bands were abolished (not shown). Pefabloc SC did not produce any significant inhibition of MMP activities (data not shown). As shown in Fig. 1B, the absence of calcium/presence of EDTA in the substrate buffer completely abolished MMP activities (lane 1), but did not affect gelatinolytic activities in the tryptase samples. These data indicate that the gelatinolytic activities seen in the tryptase samples are due to serine protease activities and rule out the possibility of contamination of the tryptase preparations with metalloproteases.

Characterization of the HMW gelatinolytic activity

The low m.w. gelatinolytic activities (Fig. 1) are consistent with tryptase monomers that have been formed by the dissociation of tetramers by the SDS present in the gels. However, the nature of the HMW gelatinolytic band is intriguing, since such HMW, SDS-resistant forms of tryptase have not been described previously. Experiments were therefore performed to investigate whether the HMW band contained immunoreactive β-trypatse. As shown by immunoblot analysis (Fig. 2A), in the absence of gelatin in the gel, immunoreactive tryptase was found in LMW (monomeric) form only regardless of the various incubation conditions. In contrast, the inclusion of gelatin in the gel (Fig. 2B) resulted in the additional detection of immunoreactive tryptase (lanes 1 and 3) with a size corresponding to the HMW gelatinolytic bands observed in the zymographic analysis (see Fig. 1). We thereby conclude that the HMW gelatinolytic band observed on zymography is due to β-trypatse and not to any other contaminating serine protease activity. The HMW tryptase bands were not affected by the presence of reducing agent (Fig. 2B, lane 3), but were abolished by heat denaturation of the samples before SDS-PAGE (Fig. 2B, lanes 2 and 4).

The results presented above indicate that gelatin induces a HMW form of tryptase previously not described. One possibility would be that the HMW tryptase form is a result of tight (SDS-stable) complex formation between β-trypatse and gelatin. Alternatively, the HMW tryptase could be composed of several tryptase tetramers stacked together. In fact, the distinct migration position of the HMW tryptase would point to the latter possibility, i.e., a highly organized macromolecular tryptase:gelatin complex, since complexes of tryptase with gelatin would most likely show a broader distribution (smearing) on SDS-PAGE because gelatin is present in all parts of the gel. However, the distinct migration position at the very top of the gels could also be explained by immediate trapping of tryptase molecules after entering the gel, which is favored by the high concentration of gelatin. In the latter case, decreasing the gelatin concentration in the gels may result in a broader distribution of the tryptase on SDS-PAGE. Hence, gelatin zymography was performed at gradually lower concentrations of gelatin in the gels. As shown in Fig. 3, the gradual decrease in the gelatin concentration from 0.1% (Fig. 3A) to 0.007% (Fig. 3D) indeed resulted in a broader distribution of the tryptase at the obvious expense of the distinct HMW band (compare Fig. 3, lane 2 in A–D). This effect was more apparent for tryptase samples treated with heparin (compare Fig. 3, lane 2 with lane 3 in A–D). In contrast, gelatinolytic activity associated with the monomeric tryptase bands was not affected by the gelatin concentration in the gels. Further, the migration properties of standard MMP-2 and -9 present in conditioned media from HT1080 cells were not significantly affected by decreasing the gelatin concentration. Taken together, the results indicate that the HMW tryptase is a result of complex formation between tryptase and gelatin.

Tryptase binds to and is stabilized by gelatin in solution

From the experiments described above, it appears that tryptase forms tight complexes with gelatin within gels containing SDS.

**FIGURE 2.** Immunoblot analysis of rh-βT. A. One hundred-nanogram samples of rh-βT were incubated for 5 min at room temperature in the presence or the absence of 10 μM heparin in PBS (pH 6.0; total volume, 30 μl). Samples were mixed with 10 μl of 4× SDS-PAGE sample buffer (with or without reducing agent) and were either boiled (5 min) or not subjected to heat treatment. Next, samples were subjected to SDS-PAGE (6% gels) at 4°C. After the electrophoresis, proteins were blotted onto polyvinylidene difluoride membranes that were subsequently probed with an anti-human tryptase Ab as described in Materials and Methods. B. Five hundred-nanogram samples of rh-βT were diluted 6-fold in PBS (pH 6.0; total volume, 30 μl). Samples were mixed with 10 μl of 4× SDS-PAGE sample buffer (with or without reducing agent) and were either boiled (5 min) or not subjected to heat treatment. Next, samples were subjected to SDS-PAGE (6% polyacrylamide) at 4°C in a gel that contained 0.1% gelatin added to the resolving gel. After electrophoresis, proteins were blotted onto polyvinylidene difluoride membranes that were subsequently probed with an anti-human tryptase Ab as described in Materials and Methods.
Experiments were performed to explore whether this type of complex is also formed in solution and in the absence of SDS. To this end, tryptase:gelatin complex formation was studied using gel filtration chromatography. The results displayed in Fig. 4 show that tryptase incubated with gelatin in the presence of heparin generated a single peak of enzymatic activity, eluting at a position consistent with tryptase tetramers (Fig. 4, A and C), similar to that obtained when tryptase was incubated with heparin only (Fig. 4, A and C). In contrast, no tryptase activity was recovered after chromatography of tryptase that had been incubated in the absence of heparin (Fig. 4, B and D), in agreement with the well-known stabilization effect of heparin on the tryptase tetramer (16). However, gel chromatography of heparin-free tryptase that had been incubated with gelatin revealed two separate peaks, one corresponding to the tryptase tetramer and an additional HMW peak eluting in the void volume (Fig. 4, B and D). When gelatin (without tryptase or heparin) was subjected to size-exclusion gel chromatography under the same conditions, a single peak of absorbance (A280) was obtained in the void volume (not shown). Together these data indicate that treatment of tryptase with gelatin results in the formation of a HMW complex, consistent with the strong interaction of tryptase with gelatin observed in the zymographic analyses. Further, the data indicate that gelatin has the ability to cause stabilization of tryptase activity when heparin is not present. To test the specificity of the effects of gelatin toward tryptase, samples of tryptase were incubated in the presence of 0.1% of either BSA or

FIGURE 3. Gelatin zymographic analysis of rh-βT in gels with different gelatin concentrations. One hundred-nanogram samples of rh-βT were incubated for 5 min at room temperature in the absence or the presence of 10 μM heparin in PBS (pH 6.0; total volume, 30 μl). Samples were mixed with 10 μl of 4× SDS-PAGE sample buffer (without reducing agent) and subjected to gelatin zymography as described in Materials and Methods in gels that contained 0.1% (A), 0.05% (B), 0.01% (C), or 0.007% (D) gelatin (in the resolving gel). For each gel, a 10-μl sample of HT1080-conditioned medium was loaded as a control. Bars on the right of each gel indicate the positions of the molecular weight standards.

FIGURE 4. Size-exclusion gel chromatographic analysis of rh-βT. Two micrograms of rh-βT (in 20 μl of 10 mM MES (pH 6.1) and 2 M NaCl) was incubated together with 0.1% gelatin in the presence (C) or the absence (ΔΔ) of 10 μM heparin in PBS (pH 6.0) for 15 min at room temperature. As a control, tryptase was incubated without gelatin in the presence (square) or the absence (dashed) of 10 μM heparin. Samples were subjected to size-exclusion gel chromatography as described in Materials and Methods, using PBS, pH 6.0 (A and B), or PBS, pH 7.5 (C and D), as running buffer. Fractions of 0.5 ml were collected, and 100-μl samples from the fractions were assayed for tryptase activity using the chromogenic substrate S-2288. The indicated pH value refers to the pH of the running buffer used in the chromatography. T, tetramer peak; V0, void volume of the column. The elution positions of molecular weight markers (see Materials and Methods) are indicated by arrows.
fibrinogen, followed by gel filtration analysis. However, no stabilization or complex formation was observed when tryptase was treated with either of the latter proteins (not shown).

**Tryptase degrades gelatin and denatured type I collagen in solution**

Experiments were conducted to test whether tryptase degrades gelatin in solution. Gelatin, composed of a heterogeneous mixture of differently sized polypeptides (Fig. 5A, lane 1), was gradually degraded at both pH 6.0 (Fig. 5A, lanes 3–8) and, in a slightly less effective manner, at pH 7.5 (not shown) at room temperature. The somewhat higher activity at pH 6.0 than at pH 7.5 may be related to the higher stability of tryptase at acidic than at neutral pH (27) or to the effects of pH changes on gelatin conformation. No detectable degradation was observed for type I collagen when incubations were performed at room temperature (not shown). In contrast, when incubations were conducted at 37°C, a marked degradation of type I collagen was observed at pH 6.0 (Fig. 5B, lanes 3–8) and, to a lesser extent, at pH 7.5 (not shown). The degradation of type I collagen at 37°C, but not at room temperature, suggests that the elevated temperature causes partial unfolding of the collagen into a form that is susceptible to degradation by tryptase. This is in agreement with earlier reports that demonstrated denaturation of intact type I collagen at temperatures >36°C (28, 29). Moreover, control experiments showed that the type I collagen preparation was susceptible to degradation (at 37°C) by pancreatic trypsin, an enzyme that is not recognized as a collagenase (not shown). These results indicate that β-tryptase appears to have the ability to degrade partially denatured, but not native, type I collagen.

We have recently shown that human β-tryptase, when exposed to neutral pH and 37°C, can dissociate into heparin-dependent active monomers (23). We next wanted to test whether gelatin and type I collagen were substrates for both tryptase tetramers and monomers. Tetrameric and (active) monomeric tryptase were isolated by size-exclusion gel chromatography using a running buffer containing heparin, as previously described (23). Gelatin or type I collagen were incubated with running buffer only. Samples (30 μl) from the incubation mixtures were removed after the times indicated, mixed with 10 μl of 4× SDS-PAGE sample buffer (with reducing agent), and subjected to SDS-PAGE (8% gels) under reducing conditions. Proteins were visualized after silver staining.

**Discussion**

Mast cells have for a long time been linked to different conditions in which remodeling of the ECM takes place. For example, there...
is evidence indicating a role in tumor angiogenesis (9, 11, 30, 31) as well as in normal angiogenesis (9, 32). An involvement of mast cells in rheumatoid arthritis has been suggested by numerous studies (33–37) and was recently supported by a study using mast cell-deficient mice (38). However, the mechanisms underlying the contribution of mast cells to the ECM destruction observed in these cases are not elucidated. There are at least three possibilities of how mast cells could contribute to the ECM degradation. First, mast cell mediators could promote the recruitment and/or proliferation of other cell types with the capacity to produce the different proteases required for degradation of the ECM. For example, activated mast cells secrete histamine, heparin, TGF-β, TNF-α, and other mediators that promote proliferation of fibroblasts and endothelial cells (for a review, see Refs. 9 and 31), cell types known to secrete different ECM-degrading proteases. A second possibility would be the activation of latent ECM-degrading proteases to their active forms by the mast cell proteases. In fact, it has been reported that mast cell proteases may activate proMMP-3, proMMP-9, and pro-urokinase (10, 11, 21, 24). The third possibility could be the direct degradation of ECM components by the mast cell proteases. Indeed, both tryptase and chymase have been reported to degrade fibronectin (12, 22, 23), and tryptase has also been reported to degrade intact type VI collagen microfibrils (39).

In this report we provide evidence that tryptase binds to and degrades denatured collagens (gelatin). We show that both tetrameric and monomeric active tryptases degrade gelatin. Further, both tetrameric and monomeric tryptases were found to degrade type I collagen, although the results indicated that it was only the partially denatured type I collagen that was recognized as a substrate. Indeed, a failure of tryptase to degrade intact type I collagen is in agreement with a previous study (40).

The unique macromolecular organization of β-tryptase, with its active sites facing a narrow central pore, results in a quite restricted substrate cleavage profile of this enzyme, with most proteins being too large to gain access to the active sites. Therefore, it is not unexpected that intact collagen as well as, e.g., fibronectin (23) are prevented from entering the central pore of tetrameric tryptase. However, our results indicate that denatured collagen, obtained after unfolding of the triple helix, readily enters the central pore of tetrameric tryptase. It is important to note that the active tryptase monomers, similarly to the tetramers, were not able to degrade intact collagen type I. This indicates that the failure of tetrameric β-tryptase to degrade intact collagen may not only be a result of the restricted accessibility to its active sites, but may also be due to an absolute requirement for collagen unfolding to unmask susceptible peptide bonds. The present findings are thus in agreement with the well-established idea that only the collagenases, i.e., interstitial collagenase, neutrophil collagenase, collagenase-3, and cathepsin K, are able to destroy the highly ordered structure of fibrillar collagens, and that subsequent degradation is conducted by other proteases (2, 5) (41). We may thus propose that mast cell β-tryptase could act in a similar fashion as the previously described gelatinases (e.g., MMP-2 and -9), i.e., once collagen strands are disordered, tryptase would be able to continue the degradation process. Importantly, mast cells reside in the connective tissue and contain large stores of preformed active tryptase that, once released, could act early in the ECM degradation process. In contrast, unleashing of MMP activities may require recruitment of MMP-expressing cells, induction of MMP synthesis in these cells, and activation of proMMPs to their proteolytically active forms. It is also important to stress that MMPs are regulated markedly differently compared with β-tryptase. Whereas the MMPs are tightly controlled by various endogenous inhibitors, e.g., tissue inhibitors of metalloproteases, tetrameric β-tryptase is essentially resistant to all endogenous macromolecular protease inhibitors (18). Hence, degradation of ECM by tryptase may be a comparably long-lived event following mast cell degranulation, insensitive to, e.g., the protease inhibitors that escape into the tissue due to the plasma leakage that occurs during an inflammatory process.

The results presented here indicate that β-tryptase interacts strongly with gelatin, forming complexes. To our knowledge, such complexes between gelatin and any gelatin-degrading enzymes have not been described previously. For example, MMP-2 and -9 (42) as well as pancreatic trypsin (43, 44) generally show migration velocities in zymographic analysis that correspond to monomeric enzymes (small amounts of high m.w. forms of MMP-9 are sometimes observed in zymography, but such forms have not been characterized). The recovery of tryptase:gelatin complexes during zymography indicates that the interaction is remarkably resistant toward the strongly denaturing agent SDS. Complex formation was apparently dependent on the native conformation of tryptase (or gelatin), since heat treatment abolished the interaction. Complexes were not only formed during zymographic analysis, but also in solution, detected by gel filtration technique, demonstrating that the tryptase:gelatin complexes were not an artifact of the conditions prevailing in gelatin-containing SDS-PAGE gels. Interestingly, in the presence of heparin, complex formation was less pronounced in the zymographic analysis and was completely abolished when samples were analyzed by the gel filtration technique. Since heparin is highly negatively charged and binds strongly to tryptase (19), it appears likely that the binding of heparin to tryptase could result in the masking of potential binding sites in tryptase for gelatin. Heparin, the physiological ligand to tryptase in the secretory granule (9), is a well-known stabilizing agent for tryptase and has also been implicated in the assembly of the tryptase tetramer (45). The interaction of tryptase with heparin is promoted by the low pH that is present in the secretory granules (~5.5), and it is thought that exocytosed tryptase is regulated by its dissociation from heparin due to a higher extracellular pH, leading to tryptase monomerization. The released monomers will rapidly lose activity, although it is possible that they may possess enzymatic activity for a short time period (23). If, however, the monomers are rescued by binding to heparin, they will regain enzymatic activity (46, 47), predominantly by the generation of active tryptase monomers (23). According to the results presented here, tryptase may thus, after dissociation from heparin, interact strongly with collagens (or proteoglycans) in the ECM. A strong binding of tryptase to ECM is also supported by a previous study in which human lung tryptase was found to bind to bovine cartilage (47). This interaction could serve to target tryptase to its potential substrate in the ECM. Further, the interaction with partially denatured collagens may stabilize tryptase, making it independent of heparin that may have dissociated after exocytosis.

References


