

Review Article

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Role of Chymases in Human Biology: Significance, Mechanism and Functions

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ABSTRACT

Chymases belong to family of serine proteinases that are abundantly produced by mast cells in the blood vessels and the myocardium, Recent biochemical reports suggested that chymase is involved in a number of important physiological processes, such as inflammation, stimulation of sub mucosal gland secretion, processing of hormones and cytokines, release of growth factor, parasite expulsion and tissue remodeling. They show broad peptidolytic activity and are involved in a variety of functions. Chymase provides an important mechanism to maintain steady state AngII concentration in tissues but not in plasma. The review also gives information regarding various roles of chymase and its significance in heart modelling, chymase is a plays important role in inflammation and remodeling in the asthmatic lung. Chymase is the major chymotryptic proteinase of human mast cells, can be released in substantial quantities by mast cell activation, Chymase inhibitor ameliorates hepatic steatosis and fibrosis on established non-alcoholic steatohepatitis in hamsters fed a methionine- and choline-deficient diet . chymase as an important target for preventing complications of metabolic syndrome .Chymase plays a crucial role in angiotensin II formation in various tissues. Chymase converts angiotensin I to angiotensin II and it can also convert precursors of TGF- β and MMP-9 to their active forms. Chymase has various applications few are mentioned in this review.

Keywords

Chymases, Angiotensin-II, remodeling, inflammation, proteinase.

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Introduction

Chymase

Chymase is a chymotrypsin like serine protease mainly localized to the secretory granules of mast cells. Recent biochemical reports suggested that chymase is involved in a number of important physiological processes, such as inflammation, stimulation of sub mucosal gland secretion, processing of hormones and cytokines, release of growth factor, parasite expulsion and tissue remodeling. They show broad peptidolytic

activity and are involved in a variety of functions (Huang *et al.*, 2003). Human chymase belongs to protease family, it is synthesized in the form of inactive enzyme and has currently been characterized at atomic resolution (Reiling *et al.*, 2003). The reconstructed ancestral molecule of chymase had already substantial angiotensin II-forming activity pointing to a long evolutionary history suggested by exciting phylogenetic information (Chandrasekharan

et al., 1996, Trapani *et al.*, 2001). The chymase locus consists of a number of serine protease enzymes, here no enzymatic activity in the normal state is seen but are activated immediately upon release into the extracellular matrix. The leader peptide is clipped off and they are then secreted. Among the family of mast cell serine proteases chymase appears to be the different one. They display greatly selective hydrolysis of definite peptides in the instance of chymase PHE-8, HIS-9 in angiotensin I to form angiotensin II.

Actions of chymase

The high expression of chymase is evidently seen in mast cells, and an association between genetic variants of mast cell chymase and skin disease, e.g., eczema, has been noted (Mao *et al.*, 1996). Chymase plays a role in angiogenesis by upregulating VEGF (Katada *et al.*, 2002). Angiogenesis (Tsunemi *et al.*, 2002), including angiogenesis triggered by basic fibroblast growth factor, is inhibited by chymase inhibitors (Muramatsu *et al.*, 2002). The expression of chymase is visible in damaged vessels. e.g., after balloon injury. This type of vascular proliferation is inhibited both by angiotensin II receptor antagonists and by specific chymase inhibitors, suggesting that the effect is mediated via ACE-independent generation of angiotensin II (Takai and Miyazaki, 2002).

Techniques employed to study Chymases

Apart from determining chymase activity or ACE-independent generation of AngII, a number of interesting tools have recently become available. Wei *et al.* (2002) studied mice with homozygous disruption of the ACE gene, heterozygous mice and wild-type mice. AngII concentrations and AngII/AngI ratios in the kidney did not differ among

genotypes while plasma AngII concentration was extremely low. This observation suggests that chymase provides an important mechanism to maintain steady state AngII concentration in tissues but not in plasma. Conversely transgenic mice produced by microinjection of chymase mRNA into the heart and other tissues with selective over expression by a promoter-fusion gene had excessive AngII concentrations in the heart causing significantly increased metalloproteinase (MMP-9) activities and decreased collagen I synthesis (Chen *et al.*, 2002). This observation illustrates the potential role of chymase in heart remodelling. Finally, while chymostatin has long been known to selectively inhibit chymase activity, a whole new generation of orally active low molecular weight chymase inhibitors has recently become available, e.g., TEI-E-54 which was shown to increase survival of hamsters with myocardial infarction despite having no effect on the infarction size (Hoshino *et al.*, 2003); BCEAB, which improved indices of LV function in cardio myopathic hamsters (Takai *et al.*, 2003a, 2003b) possibly by interfering with the activation of transforming growth factor; and finally NK-3201, which was shown to suppress intimal hyperplasia after balloon injury (Takai *et al.*, 2003) or to prevent peritoneal adhesions after intra abdominal trauma (Okamoto *et al.*, 2002). The availability of such powerful tools will help to resolve some of the remaining uncertainties about the in vivo role of chymase.

Role of Chymases in humans

For a long time non ACE-dependent pathways of generation of AngII have been suspected. As a case in point a recent study on human internal mammary arteries evaluated the conversion of AngI to Ang II in the presence of captopril with or without a

chymase inhibitor (Voors *et al.*, 2003). The long and the short of it was that AngI-mediated effects were more effectively inhibited by AngII antagonism than by ACE inhibition, pointing to the presence of alternative AngII forming enzymes. The field is rendered complex by substantial species and organ differences. For instance, tsumeni *et al.* (2002) examined the ratio of ACE-dependent to chymase-dependent AngII formation in various species.

The relative importance of chymase may further be altered by vascular and tissue pathology. As a case in point, Ohishi *et al.* (1999) examined the expression of AngII in atherosclerotic plaques and their relation to ACE and chymase respectively using immunohistochemistry. Chymase be expressed in the cytosol of mast cells but immune double staining did not illustrate co localization of AngII and chymase (Ohishi *et al.*, 1999), illustrating the difficulty of drawing conclusions about a functional role from immunostaining, a caveat that may also apply to the study in diabetic kidney disease. In view of the species differences it is important that an *in vivo* role of chymase in humans has been documented in a simple but informative system, i.e. veno constriction of the vessel hand vein with a peptide analogue of AngI in the presence or absence of captopril. Using the ACE-resistant peptide, which is a specific substrate for chymase, the authors arrived at the conclusion that a non-ACE pathway capable of generating AngII exists in human veins *in vivo*.

The various studies on chymase reveal few of the worked aspects on chymase and their significance:

EM Hossny *et al* 2008, investigated that mast cell chymase is a mediator of inflammation and remodeling in the

asthmatic lung. Although a range of studies have examined the association between the -1903 G/A single nucleotide polymorphism (SNP) in the mast cell chymase gene (CMA1) and allergic phenotypes, the results have been inconsistent. A (TG)_n(GA)_m repeat polymorphism 254 base pairs downstream of CMA1 has been reported in adult asthmatics. They investigated the relationship between these CMA1 genetic variants and childhood asthma in Egyptian children. The methodology of a case-control study was undertaken in 15 children (6-10 years old) with bronchial asthma enrolled consecutively during exacerbation and 15 age-matched and sex-matched non asthmatic control subjects. Genotyping was performed by polymerase chain reaction (PCR) restriction fragment length polymorphism to search for polymorphisms in the CMA1 gene promoter region (1903 G/A) and PCR amplification followed by sequencing to detect the (TG)_n(GA)_m repeat 254 base pairs downstream of the gene. The results obtained revealed that a positive association between the CMA1 -1903 G/A SNP and asthma in children was seen. The G allele was detected in 70% of patients while the A allele was more frequent in the controls (83.3%). Concerning the (TG)_n(GA)_m repeat, allele 39 was only present in asthmatics while allele 37 was more common in controls. Their studies concluded the association of the -1903 G/A CMA1 SNP and (TG)_n(GA)_m repeat polymorphism with bronchial asthma in a group of Egyptian children. These polymorphisms are possible determinants of asthma susceptibility and may be involved in regulating immunoglobulin E level (Alan *et al.*, 1995).

Alan *et al.*, (1995) Investigated that Chymase is the major chymotryptic proteinase of human mast cells, can be released in substantial quantities by mast

cell activation. As this enzyme is stored in the secretory granules in its fully active form, they found various factors which might regulate its activity in storage and upon release. Chymase was purified from human skin by high salt extraction, cetylpyridinium chloride precipitation, heparin agarose affinity chromatography and gel filtration. Neither the addition of Mg²⁺ or Ca²⁺ (0.3-10 mM) nor their sequestration by EDTA had any effect on the rate of cleavage of the synthetic substrate N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide. Monovalent cations (Na⁺, K⁺) improved enzyme activity, but only at non-physiological concentrations (0.5-3.0 M), suggesting an ionic strength effect. At constant I = 0.15, enzyme activity was sturdily pH-dependent: at pH 5.5 (the approximate pH of the mast cell granule) the activity was only 10% of that at pH 7.5 (the approximate pH of the extracellular space). Heparin, which is stored with chymase in the mast cell granule, accentuated this difference by enhancing activity at pH 7.5 by 33% and depressing it at pH 5.5 by 40%. Histamine at concentrations up to 50 mM (I = 0.15) had small effect on chymase activity at either pH, although high concentrations did attenuate the actions of heparin. They concluded that pH and the interaction with heparin are central to the regulation of chymase activity within the granule and subsequent release.

Takai *et al.*, (2012) investigated chymase as an important target for preventing complications of metabolic syndrome. Chymase plays a crucial role in angiotensin II formation in various tissues. Angiotensin II induces gene expressions of transforming growth factor (TGF)- β and matrix metalloproteinase (MMP)-9, and chymase also converts precursors of TGF- β and MMP-9 to their active forms. All of

angiotensin II, TGF- β and MMP-9 are considered to be closely involved in the development and progression of metabolic syndrome and its complications. In a diabetic animal model, chymase induced pancreatic disorganization via attack of oxidative stress induced by augmentation of chymase-forming angiotensin II. In atherosclerotic lesions in patients, accumulation of chymase-positive cells was observed, and chymase inhibition prevented the development of atherosclerosis in an animal model. In Apo E-deficient mice, chymase inhibition prevents the development of angiotensin II-induced abdominal aneurysmal aorta (AAA). In this model, the AAA development on an increase in MMP-9 activities induced by angiotensin II, but the inhibition of MMP-9 activation by chymase inhibitor resulted in attenuation of the AAA development. Cardiac dysfunction after myocardial infarction was also attenuated by chymase inhibition. In this review, they propose the significance of chymase as a target to prevent complications of metabolic syndrome.

Takai *et al.* (2012) worked on the Angiotensin II role in regulating blood pressure. Moreover, angiotensin II directly promotes organ damage by inducing expression of various genes, such as transforming growth factor (TGF)- β and matrix metalloproteinase (MMP)-9 precursors. Blockade of angiotensin II has been shown to not only lower blood pressure, but also to prevent cardiovascular and renal dysfunction and fibrosis. Inhibition of TGF- β and MMP-9 has also been shown to prevent cardiovascular and renal damage. A mast cell-produced enzyme, chymase, generates angiotensin II and also converts precursors of TGF- β and MMP-9 to their active forms. On the other hand, chymase inhibitors, unlike

angiotensin-converting enzyme inhibitors and angiotensin II blockers, have no blood pressure-lowering effect despite blocking angiotensin II formation. Thus, chymase inhibitors may be useful for preventing damage to various organs via multiple mechanisms without lowering blood pressure.

Tashiro *et al.* (2010) studied that Chymase inhibitor prevents the nonalcoholic steatohepatitis in hamsters fed a methionine- and choline-deficient diet. Mast cells may be involved in the pathogenesis of nonalcoholic steatohepatitis (NASH). The mast cell protease chymase contributes to the formation of angiotensin II and matrix metalloproteinase (MMP)-9, both of which are intimately involved in liver fibrosis. Therefore, they hypothesized that chymase plays an important role in the development of NASH. Hamsters were fed a methionine- and choline-deficient (MCD) diet for 8 weeks. These animals were divided into two groups and received either TY-51469 (1 mg/kg per day). A third group was fed a normal diet as a control. Total plasma bilirubin, triglycerides, and hyaluronic acid levels were significantly higher in the MCD diet-fed hamsters than in the normal diet-fed hamsters, but the levels were significantly lower in chymase inhibitor-treated MCD diet-fed hamsters. Using histological analysis, marked steatosis and fibrosis were observed in MCD diet-fed hamsters, but these changes were significantly attenuated by treatment with the chymase inhibitor. Increases in mast cells and chymase-positive cells were observed in the liver after the MCD diet, but the increases disappeared in the chymase inhibitor-treated group. The significant increase observed in chymase activity in liver tissue extract from the MCD diet-fed group was also reduced by treatment with the chymase inhibitor. Chymase inhibition significantly reduced

not only angiotensin II expression but also matrix metalloproteinase 9 activity in MCD diet-fed hamsters. These findings demonstrated that the mast cell protease chymase may play a crucial role in the development of NASH in hamsters.

Hoshino *et al.*, (2003) investigated the effects of chymase inhibitor on angiotensin II-induced abdominal aortic aneurysm development in apolipoprotein E-deficient mice. By targeting that Chymase may play an important role in abdominal aortic aneurysm (AAA) development through matrix metalloproteinase (MMP)-9 activation. The purpose of this study was to determine whether chymase is involved in angiotensin (Ang) II-induced AAA development in apolipoprotein E (apoE)-deficient mice. In this study, Ang II (1000 ng/kg/min; vehicle group) or saline (saline group) was administered to 16-week-old, male, apoE-deficient mice for 4 weeks. To examine the effects of chymase inhibition on AAA development, oral NK3201 (30 mg/kg/day) was given for the same period as the Ang II infusion. AAAs developed at the suprarenal region of the abdominal aorta in the Ang II-treated vehicle group, but they were not observed in the saline group. On the other hand, the severity and luminal area of the AAAs in the Ang II-treated vehicle group were significantly suppressed by NK3201 treatment. MMP-9 activity was significantly lower in the Ang II-treated+ NK3201-treated group than in the Ang II-treated vehicle group.

Velayudham (2003) worked on VSL type 3 probiotic treatment attenuates fibrosis without changes in steatohepatitis in a diet-induced nonalcoholic steatohepatitis model in mice. Nonalcoholic fatty liver disease (NAFLD) and its advanced stage, nonalcoholic steatohepatitis (NASH), are the most common causes of chronic liver

disease in the United States. NASH features the metabolic syndrome, inflammation, and fibrosis. Probiotics exhibit immunoregulatory and anti-inflammatory activity. We tested the hypothesis that probiotic VSL type 3 may ameliorate the methionine-choline-deficient (MCD) diet-induced mouse model of NASH. MCD diet resulted in NASH in C57BL/6 mice compared to methionine-choline-supplemented (MCS) diet feeding evidenced by liver steatosis, increased triglycerides, inflammatory cell accumulation, increased tumor necrosis factor alpha levels, and fibrosis. VSL type 3 failed to prevent MCD-induced liver steatosis or inflammation. MCD diet, even in the presence of VSL type 3, induced up-regulation of serum endotoxin and expression of the Toll-like receptor 4 signaling components, including CD14 and MD2, MyD88 adaptor, and nuclear factor kappaB activation. In contrast, VSL#3 treatment ameliorated MCD diet-induced liver fibrosis resulting in diminished accumulation of collagen and alpha-smooth muscle actin. They identified increased expression of liver peroxisome proliferator-activated receptors and decreased expression of procollagen and matrix metalloproteinases in mice fed MCD+VSL#3 compared to MCD diet alone. MCD diet triggered up-regulation of transforming growth factor beta (TGFbeta), a known profibrotic agent.

Takai and Miyazaki (2003) worked on application of a chymase inhibitor, NK3201, for prevention of vascular proliferation. NK3201 is an orally active chymase inhibitor. Its inhibitory activity leads to formation of acyl-intermediate between active serine residue of the enzyme and di-ketone structure of NK3201. NK3201 inhibits human, dog and hamster chymases with IC(50) of 2.5, 1.2, and 28 nM, respectively. On the other hand, NK3201

does not inhibit other types of serine proteases, trypsin, thrombin, elastase, plasmin, and plasminogen activator. In dogs, at 8 h after oral administration of NK3201, 1 mg/kg, the drug levels in plasma, heart, and aorta reached 470, 195, and 78 nM, respectively. In a dog model NK3201, 5 mg/kg/day, increased chymase activity in grafted veins, and suppressed vascular proliferation. After balloon injury in dog vessels, chymase activity was increased locally, in the injured artery, and NK3201, 1 mg/kg/day was effective in preventing vascular proliferation. On the other hand, NK3201, unlike angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, did not affect blood pressure. These findings indicate that local angiotensin II production by chymase is involved only in vascular proliferation, as seen in the injured vessels. Therefore, NK3201 may be useful for preventing vascular proliferation without affecting blood pressure.

Takai and Miyazaki (2002) studied the effects of chymase inhibitor on vascular proliferation. In vascular tissues, angiotensin II is potentially cleaved from angiotensin I by chymase and angiotensin-converting enzyme (ACE). In the normal state, vascular ACE regulates local angiotensin II formation and plays a crucial role in the regulation of blood pressure, whereas chymase is stored in mast cells and has no enzymatic activity. Chymase is activated immediately upon its release into the extracellular matrix in vascular tissues after mast cells have been activated by stimuli such as vessel injury by grafting or a balloon catheter. In dog grafted veins, chymase activity is increased, and the vascular proliferation is suppressed by either a chymase inhibitor or an angiotensin II receptor blocker. After balloon injury in dog vessels, chymase activity is significantly

increased in the injured artery, and a chymase inhibitor is effective in preventing the vascular proliferation, but an ACE inhibitor is ineffective. Chymase plays an important role in the development of vascular proliferation via the induction of local angiotensin II formation in injured vessels

Gilles *et al.* (2001) Developed a new assays and improved procedures for the purification of recombinant human chymase .Chymase mediates a major alternative way of angiotensin II production from angiotensin I beside angiotensin converting enzyme in the final step of the renin-angiotensin system. This enzyme is also involved in other physio-pathological processes such as angiogenesis, atherosclerosis and inflammation. Several purification attempts of natural or recombinant chymase were reported in the literature. Most of these reports were not successful in obtaining the recombinant enzyme in a highly active form and in large quantity. This study describes a facile route for the Purification of the human recombinant chymase. Chymase being produced as inactive prochymase, to be cathepsin C-activated, newly raised anti-chymase Ig were used to follow the purification. In order to complete the available tools for the search of chymase inhibitors, they developed and assessed a new 96-well plate based assay for the measurement of enzyme activity, as well as a low throughput, HPLC-based one. The assays used an original derivative of angiotensin I, or the native hormone. Chymase was produced in CHO cells and appropriately matured. The amount of enzyme obtained at the end of the process is compatible with the medium-throughput screening (up to 10,000 points per day), about 800 $\mu\text{g}\cdot\text{L}^{-1}$ of culture medium with a specific activity of 6.16 mmol of angiotensin I cleaved per minute per mg of

protein. All the biological and technical tools are now available for the discovery of new classes of chymase inhibitors.

Takai *et al.* (2002) studied the Oral administration of a specific chymase inhibitor, NK3201, inhibits vascular proliferation in grafted vein by in-vitro experiments, but the role of chymase in vivo has been unclear. In this study, they investigated the effect of a novel chymase inhibitor, NK3201, on this proliferation in dog grafted veins. NK3201 inhibited human and dog chymases, but not rabbit ACE. NK3201 suppressed the Ang I-induced vascular contraction in isolated dog arteries in the presence of an ACE inhibitor, and the IC50 value of chymostatin and NK3201 in dog artery was 320 nM. In dog, the concentration of NK3201 in blood was about 10 μM at 24 h after oral administration of the drug (5 mg/kg). In the group treated with NK3201, each dog was administered orally 5 mg/kg per day from 5 days before to the day before the removal of the grafted veins. Each dog underwent right common carotid artery bypass grafting with the ipsilateral external jugular vein. By 28 days after grafting, a significant vascular proliferation was observed in the grafted veins and the chymase activity was also increased significantly. Treatment with chymase inhibitor significantly suppressed the proliferation of the grafted veins and the increased chymase activity. The study demonstrates for the first time that oral administration of a specific chymase inhibitor, NK3201, appears useful for preventing vascular proliferation.

Nakakuboi *et al.* (2000) succeeded in expressing in a *Pichia pastoris* (*P. pastoris*) host a cDNA encoding a mature human chymase (h-chymase) which was secreted directly into the culture medium. Recombinant human heart chymase (rh-

chymase) was purified from the culture medium via a single one-step heparin agarose column chromatography tracing, using succinyl-Ala-Ala-Pro-Phe-para-nitroanilide (Suc-AAPF-pNA) hydrolysing activity. On SDS polyacrylamide gel electrophoresis (SDS-PAGE), the rh-chymase showed a diffused protein band with molecular weight of 32-37 kDa. After deglycosylation, however, rh-chymase changed to a sharp protein band with molecular weight 28 kDa, which is equal in size to deglycosylated h-chymase. The rh-chymase had an activity to convert one of the natural substrates angiotensin I to angiotensin II. Double reciprocal plot analysis revealed that the K (m) value of rh-chymase against Suc-AAPF-pNA was approximately 5.1 mM, which is close to that of purified h-chymase.

Nakakuboi *et al.*(2000) investigated Functional reconstitution of an active recombinant human chymase from *Pichia pastoris* cell lysate .In earlier reports efficient production of mature human chymase (h-chymase) using an original system of expression in *Pichia pastoris* was done successfully , whereby recombinant h-chymase (rh-chymase) was secreted as a mature form with the correct N-terminal amino acid sequence and was easily purified. In the course of investigation of secretory rh-chymase. They also found large amounts of chymase to be present in insoluble form in the transformant cell. Although the cellular rh-chymase had no proteolytic activity, its chymotryptic activity was restored in a reconstitution process utilizing guanidine and glutathione. As with secretory rh-chymase, efficient purification was possible by heparin affinity chromatography. The purified cellular rh-chymase showed the same mobility as secretory rh-chymase in sodium dodecyl sulphate-polyacrylamide gel electrophoresis

(SDS-PAGE) before and after deglycosylation. N-terminal amino acid sequence analysis revealed that the signal peptide had been correctly removed. K(m) value (5.93 mM), as well as pH profile and inhibition profile toward protease inhibitors of reconstituted cellular rh-chymase, indicated that the rh-chymase enzymatically closely resembles native h-chymase. Furthermore, it showed a greatly restricted proteolytic activity towards Ang I, and formed Ang II without the further cleavage which is a feature of h-chymase. It was thus found that the insoluble rh-chymase stored in the cells could be solubilized and reconstituted to give the same structure as h-chymase, not only in terms of enzyme active site but also of substrate recognition site.

Waern *et al.*, (2009) Investigated that mast cells mast cells have a harmful role in asthma, for example by secreting various pro-inflammatory substances stored within their secretory granule. They studied that one of the substances stored within mast cell granule chymaseinfact has a protective role in allergic airway inflammation, indicating that mast cell may possess both harmful and protective activities in connection with this type of disease. Wild-type mice and mice lacking mouse MC protease 4 (mMCP- 4), a chymase that is functionally homologous to human chymase were sensitized and challenged with OVA, followed by the assessment of airway physiology and inflammatory parameters. Their show that the airway hyper responsiveness was significantly higher in mMCP-4 as compared with Wild type mice. Moreover, the degree of lung tissue inflammation was markedly higher in mice lacking mMCP-4 than in WT controls. Histological analysis revealed that OVA sensitization/challenge resulted in a marked increased in the thickness of the smooth muscle cell (SMC) layer and, notably, that the degree of SMC

layer thickening was more pronounced in mMCP-4 animals than in WT controls, thus indicating that chymase may have an effect on airway SMCs. In support of this, mMCP-4-positive MCs were located in the close vicinity of the SMC layer, mainly in the upper airways, and mMCP-4 was shown to be the major chymase expressed in these MCs. Taken together, our results indicate that chymase present in the upper airways protects against allergic airway responses, possibly by regulating SMCs.

Alan *et al.*, (1995) Chymase, the major chymotryptic proteinase of human mast cells, can be released in substantial quantities following mast cell activation. As this enzyme is stored in the secretory granules in its fully active form, we have investigated various factors which might regulate its activity in storage and upon release. Chymase was purified from human skin by high salt extraction, cetylpyridinium chloride precipitation, heparin agarose affinity chromatography and gel filtration. Neither the addition of Mg²⁺ or Ca²⁺ (0.3-10 mM) nor their sequestration by EDTA had any effect on the rate of cleavage of the synthetic substrate N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide. Monovalent cations (Na⁺, K⁺) enhanced enzyme activity, but only at non-physiological concentrations (0.5-3.0 M), suggesting an ionic strength effect. At constant I = 0.15, enzyme activity was strongly pH-dependent: at pH 5.5 (the approximate pH of the mast cell granule) the activity was only 10% of at pH 7.5 (the approximate pH of the extracellular space). Heparin, which is stored with chymase in the mast cell granule, accentuated this difference by enhancing activity at pH 7.5 by 33% and depressing it at pH 5.5 by 40%. Histamine at concentrations up to 50 mM (I = 0.15) had little effect on chymase activity at either pH, although

high concentrations did attenuate the actions of heparin. It is concluded that pH and the interaction with heparin are central to the regulation of chymase activity within the granule and following release.

HE Shao-Heng *et al.*, (2003) investigated the actions of protease inhibitors on the enzymatic activities of trypsin and chymase in similar experimental systems. The summed up methodology used is Human lung trypsin and human skin chymase were purified by a similar procedure involving high salt extraction of trypsin, heparin agarose affinity chromatography, and S-200 Sephacryl gel filtration chromatography. Actions of protease inhibitors on trypsin and chymase activities were examined by enzyme assays. The specific the outcome of these studies say that activities of trypsin and chymase were 2.1 kU/g protein and 4.9 kU/g protein, respectively.

Both preparations showed a single diffuse band on SDS-PAGE. Among non-native protease inhibitors, N-(1-hydroxy-2-naphthoyl)-L-arginyl-L-prolinamide hydrochloride (HNAP), leupeptin, antipain, benzamide, and protamine inhibited more than 90 % enzymatic activity of trypsin, whereas soy bean trypsin inhibitor (SBTI), Z-Ile-Glu-Pro-Phe-CO₂Me (ZIGPPM) and chymostatin inhibited more than 95 % enzymatic activity of chymase. Native protease inhibitors α 1-antitrypsin and secretory leukocyte protease inhibitor (SLPI) inhibited more than 90 % enzymatic activity of chymase, but lactoferrin appeared to enhance chymase enzymatic activity. All the 3 inhibitors had weak inhibitory actions on trypsin. The studied done concluded that protease inhibitors tested had relatively good selectivity to either trypsin or chymase.

In conclusion, chymase is involved in the synthesis of angiotensin II (Ang II), but the Ang II formed in this pathway is probably not involved in the short-term regulation of blood pressure. Ang II from the chymase pathway is, probably involved in the structural remodeling associated with disease of the cardiovascular system. Chymase has many other actions including degrading the extracellular matrix, activating transforming growth factor- α (TGF- α), and promoting the synthesis of 1 – 31 amino acid-length endothelins, all of which may contribute to the vascular response to injury. Pathophysiological role of chymase on the vasculature. Chymase is a mast-cell-specific serine protease that is stored within secretory granules and released together with heparin and histamine in response to allergen challenge or other stimuli. Recent studies have shown that chymases possess processing activity for biological peptides and cytokines implicated in a variety of diseases. For example, the chymases of primates and dogs have highly specific angiotensin II (Ang II) generating activity, and the results of animal studies suggest that chymase contributes to the pathogenesis of cardiovascular diseases via Ang II generation. This protease has been implicated in defense against helminthic parasites, allergic reactions, cardiovascular disease and chronic inflammatory diseases. Actions which have been attributed to chymase include the conversion of angiotensin I to angiotensin II (with more efficiency and greater specificity than angiotensin converting enzyme) the induction of microvascular leakage and neutrophil accumulation stimulation of mucus secretion the activation or inactivation of cytokines, tissue degradation and cleavage of apolipoprotein B-100, an early step in the aetiology of atherosclerotic plaques. Chymase inhibitors have emerged as potential therapeutic agents for treating

various inflammatory, allergic, cardiovascular, and renal disorders. Application of a chymase inhibitor, NK3201, for prevention of vascular proliferation. Therapeutic applications of chymase inhibitors in cardiovascular diseases and fibrosis.

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