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Research Article

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A Human Lung Mast Cell Chymotrypsin-like Enzyme

Identification and Partial Characterization

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Abstract

We have used a high performance liquid chromatography assay, which detects chymotryptic cleavage of the phe$_8$-his$_9$ bond of angiotensin I to yield angiotensin II, in order to examine human lung mast cells for the presence of chymotryptic activity. Mast cells, purified from human lung by enzymatic dispersion, countercurrent elutriation, and Percoll gradient centrifugation, were lysed or challenged with goat anti-human IgE. In multiple experiments angiotensin II–converting activity was detected in lysates of 10–99% pure mast cell preparations. Regression analysis of net percent release values of histamine and the angiotensin I–converting activity from dose-response experiments demonstrated a correlation between the two parameters, indicating that the chymotrypsin-like enzyme is a constituent of the mast cell secretory granule. The chymotryptic activity was completely inhibited by 10^{-3} M phenylmethylsulfonylfluoride but not by 10^{-3} M Captopril, and the pH optimum of activity was 7.5–9.5. Gel filtration of released material separated the activity from tryptase and demonstrated an approximate molecular weight of 30–35,000. The mast cell enzyme, like a human skin chymotrypsin-like proteinase, can be distinguished from leukocyte cathepsin G by lack of susceptibility to inhibition by bovine pancreatic trypsin inhibitor. Thus, an enzyme with limited chymotryptic specificity is present in human lung mast cells. The Michaelis constant of the enzyme for angiotensin I of 6.0×10^{-5} M is similar to that of endothelial cell angiotensin-converting enzyme and is consistent with a reaction of physiologic importance.

Introduction

Proteolytic enzymes of mast cells have been studied in cells from a variety of sources, including human lung. The predominant enzyme of the human lung mast cell is tryptase (1, 2), a molecule with a molecular weight of approximately 145,000, which has been localized to the secretory granule. In contrast, a chymotrypsin-like enzyme (rat mast cell proteinase I or chymase) is the major proteolytic enzyme of the rat serosal mast cell (3, 4). Similar chymotrypsin-like proteinases have been detected in mastocytoma tissue of dogs (5), and granule-associated serine neutral proteases have been detected in mouse bone marrow–derived mast cells (6). Recently, a 30–35,000-mol-wt chymotrypsin-like activity has been isolated from high ionic strength extracts of human skin (7) and found in 15-fold higher levels in skin of patients with mastocytosis (7). Like other enzymes with chymotryptic specificity, the human skin chymotryptic proteinase hydrolyzes the phe$_8$-his$_9$ bond of the decapeptide angiotensin I (AI)[1] to form angiotensin II (AII) (8, 9). It is of interest that the human skin enzyme carries out this reaction as efficiently as angiotensin-converting enzyme (ACE) (8).

Using synthetic substrates, chymotrypsin-like activity has been sought but not identified in preparations of human lung mast cells. Inefficient cleavage of synthetic substrates by a putative chymotrypsin-like enzyme is a possible explanation of this failure. By employing hydrolysis of the phe$_8$-his$_9$ bond of AI as a more sensitive assay, we have detected and partially characterized a chymotrypsin-like enzyme in preparations of purified human lung mast cells. Physicochemical and functional data distinguish this enzyme from tryptase and cathepsin G and show that it is similar to the human skin chymotryptic proteinase.

Methods

(Ile^5) AI and (Ile^5) AII were obtained from Vega Biotechnologies, Inc., Tucson, AZ; des-leu$_{10}$-AI, asp-arg-val-tyr, and ile-his-pro-phe from Peninsula Laboratories, Inc., Belmont, CA; $NaClO_4$, 85% H_3PO_4, and CH_3CN (high performance liquid chromatography (HPLC) grade) from Fisher Scientific Co., Allied Corp., Pittsburgh, PA; porcine pancreatic α-chymotrypsin (C_{Di}, 49 U/mg) from Worthington Diagnostics Div., Millipore Corp., Freehold, NJ; benzoyl-L-tyrosine ethyl ester (BTEE), soybean trypsin inhibitor (SBTI), lima bean trypsin inhibitor (LBTI), and bovine pancreatic trypsin inhibitor (BPTI) from Sigma Chemical Co., St. Louis, MO; Percoll from Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ; [^{3}H]tosyl-L-arginine methyl ester ([^{3}H]TAME) from Amersham Corp., Arlington Heights, IL; Captopril (Squibb E. R., & Sons, Squibb Corp., Princeton, NJ); EDTA, 1,10-phenanthroline, phenylmethylsulfonyfluoride (PMSF), piperazine-*N-N-bis*(2-ethanesulfonic acid), chymopapain, and elastase type 1, from Sigma Chemical Co.; collagenase from Worthington Diagnostics; pronase and Dnase from Calbiochem-Behring Corp., La Jolla, CA; crystallized human serum albumin from Miles Ames Div., Miles Laboratories Inc., Elkhart, IN; RPMI 1640 medium with 25 mM Hepes, Hanks' solution, and garamycin from Gibco, Grand Island, NY; gelatin from Difco Laboratories Inc., Detroit, MI; Percoll, Sephacryl S-200 from Pharmacia; and goat IgG from Cappel Laboratories Inc., Cochranville, PA. Goat anti-human IgE was kindly provided by Dr. Kimishige Ishizaka, Good Samaritan Hospital, Baltimore, MD. This was affinity-purified and contained no

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1. *Abbreviations used in this paper:* AI, angiotensin I; AII, angiotensin II; BPTI, bovine pancreatic trypsin inhibitor; BTEE, benzoyl-L-tyrosine ethyl ester; HPLC, high performance liquid chromatography; LBTI, lima bean trypsin inhibitor; PAGCM, piperazine-*N-N-bis*(2-ethane sulfonic acid), 7.6; NaCl, 6.4; KCl, 0.37; $CaCl_2$, 0.14; $MgCl_2$ $6H_2O$, 0.2; glucose 1.0; 10 N NaOH, 4.2 ml/liter; and human serum albumin, 30 mg/liter; PMSF, phenylmethylsulfonyl fluoride; SBTI, soybean trypsin inhibitor; TAME, tosyl-L-arginine methyl ester.

detectable activity when tested in any of the enzyme assays used in this work.

Mast cell purification. Human lung tissue was obtained from patients undergoing thoracotomy and lung resection, usually for carcinoma of the bronchus or other neoplasms, and mast cells were purified as described previously (10, 11). In brief, macroscopically normal lung tissue was dissected free from pleura, bronchi, and blood vessels, minced into 5–10-mg fragments and dispersed into single cell suspensions using two 30-min incubations with pronase and chymopapain followed by two additional 30-min incubations with elastase and collagenase. Mast cells were further purified by countercurrent centrifugation elutriation and by centrifugation over discontinuous gradients of Percoll. Mast cells were quantified at each stage of the purification procedure following staining with Alcian Blue (12).

Mast cell release and extraction. Mast cell release and extraction procedures were performed in a buffer which contained (grams/liter): piperazine-*N*-*N'bis*(2-ethanesulfonic acid), 7.6; NaCl, 6.4; KCl, 0.37; $CaCl_2$, 0.14; $MgCl_2$ $6H_2O$, 0.2; glucose, 1.0; 10 N NaOH, 4.2 ml/liter; and human serum albumin, 30 mg/liter (PAGCM). Final pH was adjusted to 7.4. In some dose-response experiments 0.1% bovine serum albumin was also added to help stabilize the chymotryptic enzyme.

Purified mast cells were suspended in PAGCM at a concentration of 4–8 × 10^6/ml and were challenged with the appropriate doses of anti-IgE (usually in the range of 1–10 μg/ml) or else with normal goat IgG (as a control). Incubations were performed for 30 min at 37°C, at which time cells were sedimented by centrifugation and the supernatant removed. The residual cell pellet was then resuspended to the same cell concentration in PAGCM and was extracted by freeze-thawing (× 4) on dry ice/acetone followed by centrifugation. Whole cell lysates were obtained in the same manner.

Histamine assay. Samples (10 μl) of the cell suspension and of supernatants after challenge were added to tubes containing 1 ml of 2% perchloric acid and were diluted as appropriate for determination of histamine, which was measured by an automated fluorometric technique (13). Histamine release was expressed as net percent of the content of whole cell lysates.

Enzyme assays. AI, AII, and des-leu_{10}-AI cleavage was measured by an isocratic HPLC assay as described (14). HPLC was performed on a model 144 system (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) using a 4.6 × 250 mm-Ultrasphere 5-μm ODS C18 column (Altex Scientific, Inc., Berkeley, CA) employing an isocratic perchlorate ion pair reverse-phase system using 40% CH_3CN and a flow rate of 1.0 ml/min. Peptides were monitored by absorbance at 200 nm, identified by retention time, and quantified by integration of peak area using an HP 3390a integrating recorder (Hewlett-Packard Co., Palo Alto, CA). Asp-arg-val-tyr-ile-his-pro-phe-his-leu (AI) eluted at ~11 min. Des-leu_{10}-angiotensin I eluted at ~4.5 min. Asp-arg-val-tyr-ile-his-pro-phe (AII) is formed by phe_8-his_9 bond cleavage of AI and elutes at ~7.0 min. Asp-arg-val-tyr is formed by tyr_4-ile_5 and phe_8-his_9 bond cleavage of AI and it elutes at ~5.6 min. His-leu, formed by cleavage of the phe_8-his_9 bond of AI, elutes at ~2.5 min. Synthetic standards for each peptide were used to calibrate the HPLC assay. Unless otherwise noted, enzymes were incubated with 5 × 10^{-5} M AI at 37°C in 500 μl 0.01 M Tris, pH 7.4, 0.15 M NaCl. Because the AI concentration was sixfold lower than the Michaelis constant (K_m) for this reaction, a constant incubation time of 15 min was used for enzyme activity determinations. Reactions were stopped by addition of an equal volume of HPLC solvent, and samples were then stored on ice. The term AI conversion refers only to reactions in which AII and his-leu are formed by phe_8-his_9 bond cleavage of AI in the absence of further degradation of AII.

Mast cell tryptase in supernatants or gel filtration fractions was assayed by its ability to liberate tritiated methanol from [^{3}H]-tosyl-L-arginine methyl ester essentially according to the method of Imanari et al. (15). A 10-μl aliquot of appropriately diluted sample and 30 μl of 0.05 M Tris, pH 8.0, 0.1 M NaCl, were added to a 1.5-ml microtube. A 10-μl vol (~2 × 10^5 dpm) of [^{3}H]-TAME was then added with mixing and the tube was placed in a counting vial containing 10 ml of toluene-based scintillation cocktail and 50 μl of stop solution (1 vol glacial acetic acid/9 vol 0.02 M TAME). The counting vial was then tightly capped and, after 30 min, the reaction was stopped and the liberated methanol partitioned into the toluene-based cocktail by vigorous shaking. The vials were then counted for 4 min in a liquid scintillation spectrometer. Human urinary kallikrein of known activity was used as a quality control in each assay.

BTEE hydrolysis was measured by a spectrophotometric assay as described (16). The assay was standardized with bovine α-chymotrypsin. Lactic dehydrogenase (17) was measured as described.

Kinetic constants were determined using Lineweaver-Burk analysis of the reciprocal of the reaction velocity and substrate concentration.

Enzyme purification. Human lung mast cell chymotrypsin-like enzyme was partially purified by Sephacryl S-200 gel filtration of whole mast cell lysates or from supernatants of mast cell preparations obtained following challenge with anti-IgE. In either case, enzyme sources were made 3 M NaCl before 2-h incubation on ice to dissociate the activity from insoluble cell constituents and/or proteoglycans. After centrifugation for 15 min at 10,000 *g*, supernatants were filtered through a 1.6 × 91-cm Sephacryl S-200 column run at 4°C in 0.1 M Tris, pH 7.4, 1.0 M NaCl at 12 ml/h. Fractions of 2-ml vol were collected, screened for protein by absorbance at 280 nm, and assessed for activity. Active fractions were pooled and concentrated to starting volume using a positive pressure system (Amicon Corp., Scientific Systems Div., Danvers, MA) and a YM-5 membrane.

Human leukocyte cathepsin G was purified to homogeneity from the granules of fresh human peripheral blood polymorphonuclear leukocytes by Aprotinin-Sepharose affinity chromatography and carboxymethyl cellulose cation exchange chromatography (18, 19). Human skin chymotryptic proteinase was highly purified from human skin by 2.0 M KCl extraction, Sephacryl S-200 gel filtration, and D-tryptophan methyl ester-Sepharose affinity chromatography (7). Its concentration was measured by active site titration with tritium-labeled diisopropylfluorophosphate.

Results

Detection of a chymotrypsin-like enzyme in human lung mast cell preparations. Purified human lung mast cell preparations (10–65% pure) were examined for chymotrypsin-like activity by 30-min incubation of 10-μl samples of whole cell lysates with AI and assay by HPLC for AI conversion to AII. In each case, phe_8-his_9 bond cleaving activity was detected by HPLC identification of the products AII and his-leu (Table I).

The time course of AI conversion by a 65% pure mast cell preparation which contained 5.5 × 10^5 mast cells/ml was examined by incubating 10 and 25 μl of the whole cell lysate with AI for various times at 37°C. AII formation proceeded rapidly (Fig. 1) and the constituent tetrapeptides of AII, asp-arg-val-tyr and ile-his-pro-phe, were not observed. The dose-response of AI conversion was studied by incubation of various amounts of the same mast cell preparation for 15 min with AI under identical reaction conditions. AII generation increased in a dose-depen-

Table I. Generation of AII from AI by Lysates of Preparations of Highly Purified Human Pulmonary Mast Cells

Preparation	Purity	Cell number	AI-converting activity
	%	*mc/ml*	*μmol AII/ml*
1	10	6 × 10^6	3.2
2	13	8 × 10^6	0.375
3	65	5.5 × 10^6	2.45

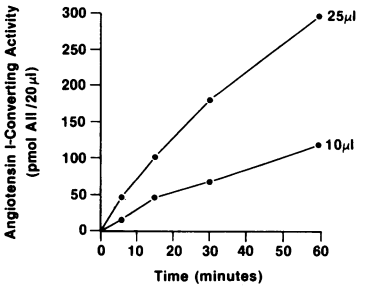

Figure 1. Time course of AI conversion by lysates of purified human lung mast cells. Mast cells (5.5×10^5 cells/ml) were 65% pure. The rate of AI conversion was ~2.1 μmol/10^6 mast cells/h.

dent fashion (Fig. 2). The pH optimum of the mast cell preparation AI-converting activity was determined by incubating 10-μl aliquots of the mast cell lysate with AI for 15 min in various mixtures which differed by 1.0 pH units from 3.5–10.5. The pH optimum was 7.5–9.5 (Fig. 3). Similar data for time course, dose dependence, and pH optimum were obtained with two additional mast cell preparations of 10 and 13% purity.

The susceptibility of the mast cell–derived AI-converting activity to a panel of enzyme inhibitors was assessed. Inhibitors included Captopril, pepstatin, PMSF, EDTA, and mersalyl acid. Aliquots (10 μl) of the 65% pure mast cell preparation, (5.5×10^5) mast cells/ml, were exposed to each inhibitor (10^{-3} M) for 15 min at 37°C before assay of AI conversion. PMSF (10^{-3} M) completely inhibited the AII-generating activity (Table II). Captopril, an inhibitor of the dicarboxylcarboxypeptidase angiotensin-converting enzyme, did not inhibit AI conversion by the mast cell enzyme. Taken together the results indicate that mast cell AI conversion is carried out by a chymotrypsin-like serine endopeptidase which cleaves the phe_8-his_9 bond of AI.

Localization of the chymotrypsin-like enzyme to the mast cell. Since mast cell preparations contained other cell types, experiments were carried out to identify the cell which contained the chymotrypsin-like AI-converting activity. A preparation of mast cells of >99% purity (10^6 cells/ml) was examined for AI-converting activity and challenged with goat anti-human IgE antibody. Supernatants from unchallenged and challenged cells and the residual cell lysate were examined for AI-converting activity and for the mast cell secretory granule marker, histamine (Table III). The chymotrypsin-like activity was detected in the >99% mast cell preparation and was released by IgE-mediated challenge in a manner similar to histamine.

Net percent release values of histamine and the chymotrypsin-like activity from nine preparations of human lung mast cells of 10–99% purity were determined following challenge with anti-IgE. Net percent release values for immunologic challenge of each preparation and at all doses of antibody in a dose-response experiment for the chymotrypsin-like activity were plotted against those of histamine (Fig. 4) and the data was analyzed by linear regression. There was a linear relation by regression

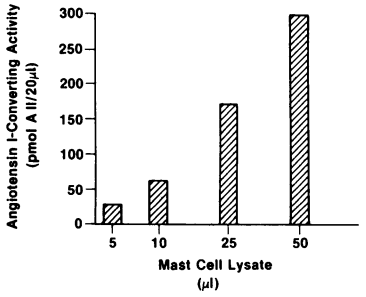

Figure 2. Dose response of AI conversion by lysates of purified human lung mast cells. Mast cells (5.5×10^5 cells/ml) were 65% pure.

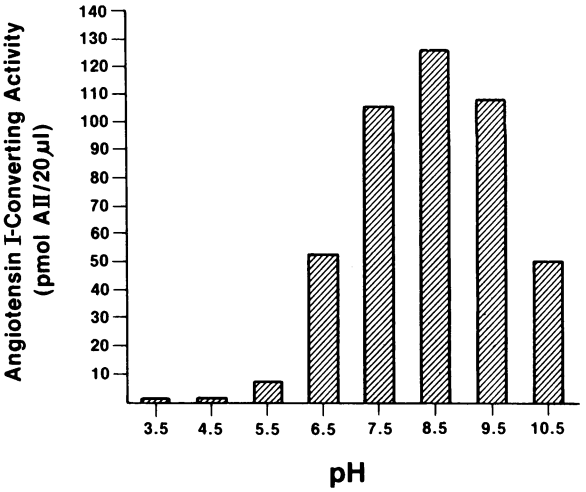

Figure 3. pH optimum of AI conversion by lysates of purified human lung mast cells. Mast cells (5.5×10^5 cells/ml) were 65% pure. Determinations were carried out at AI concentrations sixfold lower than 1 cm for practical reasons. Thus, data may result partially from variations in pH-dependent affinity of enzyme for substrate. $r = 0.9$; $n = 19$.

analysis with $r > 0.89$ and 99.8% confidence limits of 0.82 ($P < 0.01$). The intercept did not differ significantly from zero. The net percent release value of the chymotrypsin-like activity relative to histamine was 0.64.

Separation of the chymotrypsin-like enzyme from mast cell tryptase and relationship to leukocyte cathepsin G and the human skin chymotrypsin-like enzyme. To determine the relationship of the chymotrypsin-like enzyme to human mast cell tryptase, supernatants ($n = 4$) obtained after IgE-mediated challenge of mast cell preparations were made 3 M NaCl, incubated on ice for 2 h, and then subjected to Sephacryl S-200 gel filtration. Column fractions were examined for AI conversion and tryptase activity (Fig. 5). A single region of AI-converting activity with an approximate molecular weight of 30,000–35,000 was detected. The activity was distinct from tryptase, which eluted at an apparent mol wt of 145,000. The recovery of angiotensin-converting activity varied from 45 to 50%. Column fractions which contained AI-converting activity were pooled and concentrated and used in subsequent experiments as partially purified chymotrypsin-like enzyme.

Experiments were designed to determine the functional relationship of the human lung mast cell chymotrypsin-like en-

Table II. Inhibition of Human Lung Mast Cell AI-converting Activity

Inhibitor concentration	Angiotensin-converting activity
	pmol AII/20 μl
Control	49.9
PMSF (10^{-3} M)	0
Pepstatin (10^{-3} M)	55.8
EDTA (10^{-3} M)	43.9
Captopril (10^{-3} M)	45.7
Mersalyl acid (10^{-3} M)	55.3

Table III. Detection of AI-converting Activity and Histamine in a >99% Pure Mast Cell Preparation

	AI-converting activity		Histamine	
	pmol AII/20 μl	%	*μg/10⁶ mc*	%
Supernatant, control	8.2	3.5	0.03	1
Supernatant, challenged	89.1	39.5	1.31	45
Cell residual, challenged	131.0	57	1.57	54

zyme to pure human leukocyte cathepsin G and to the chymotrypsin-like enzyme isolated from human skin. The profile of inhibition of each enzyme by various concentrations of BPTI, SBTI, and LBTI and by PMSF was examined. Enzymes were utilized in amounts which gave similar AI-converting activities, falling within the linear range of the assay. Each proteinase was exposed to each inhibitor for 15 min at 37°C in 0.01 M Tris, pH 7.4, 0.15 M NaCl before incubation with AI. Cathepsin G was inhibited by BPTI while the lung mast cell enzyme and human skin chymotrypsin-like proteinase were not (Table IV). Likewise, the lung mast cell and skin chymotrypsin-like proteinases were distinguished from cathepsin G with respect to susceptibility to LBTI and SBTI. All three enzymes were similarly susceptible to PMSF.

Partially purified mast cell chymotrypsin-like enzyme was examined for ability to hydrolyze BTEE, a synthetic substrate of cathepsin G and the skin proteinase. 25-, 50-, and 100-μl samples of partially purified lung mast cell enzyme displayed BTEE hydrolyzing activity equivalent to 0.095, 0.1875, and 0.350 mg of chymotrypsin. Amounts of each enzyme with identical activities on BTEE were then evaluated for ability to convert AI to AII. Cathepsin G, skin chymotryptic proteinase, and lung mast cell enzyme activities were 2.80, 8.95, and 8.82 nmol AI-converted/chymotrypsin equivalent, respectively.

Specificity and affinity of mast cell chymotrypsin-like enzyme for AI as substrate. The specificity of the mast cell enzyme for cleavage of the phe_8-his_9 bond of AI was studied by incubation of 20 μl of partially purified chymotrypsin-like enzyme with AI, des-leu_{10} AI, or AII, each at 5×10^{-5} M. Reactions were carried out at 37°C in 500 μl 0.1 M Tris, pH 7.4, 0.15 NaCl. 50-μl samples were removed from each mixture at various times up to 60 min and assessed for cleavage by HPLC assay. Phe_8-his_9 bond cleavage was detected only when AI served as substrate (Fig. 6). Cleavage/degradation of des-leu_{10}-AI or AII were not detected.

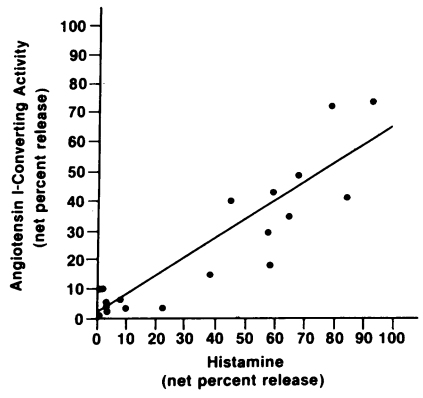

Figure 4. Relationship of the net percent release values of the AI-converting activity and histamine in preparations of human lung mast cells challenged by goat anti-human IgE.

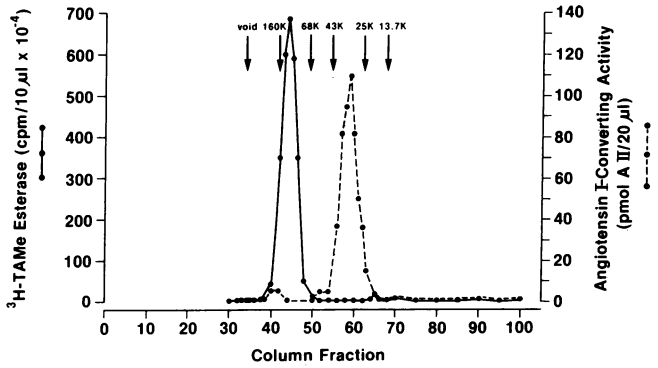

Figure 5. Sephacryl S-200 gel filtration of the supernatant of a preparation of human lung mast cells challenged with goat anti-human IgE. Mast cells (13×10^6 cells/ml) were 83% pure. K, molecular weight.

Finally, the K_m of the lung mast cell chymotryptic enzyme for AI was determined by incubating 2.5, 5.0, and 10.0 μl of partially purified enzyme (n = 3 preparations) with 1.25, 2.5, 5.0, 10.0, and 20.0×10^{-5} M AI for 10 min at 37°C in 0.01 M Tris, pH 7.4, 0.15 M NaCl. A Lineweaver-Burk plot of the reciprocal of the reaction velocity and substrate concentration showed the K_m to be 6.0×10^{-5} M, a value similar to that published for the skin proteinase (6.6×10^{-5} M) (8) but different from that of cathepsin G (2.2×10^{-4} M) (20).

Discussion

High salt extracts of dispersed human lung mast cell preparations contain a chymotrypsin-like enzyme which cleaves the phe_8-his_9 bond of AI (Figs. 1 and 2; Table I) to form AII. This enzyme is

Table IV. Percent Inhibition of Various Human Chymotryptic Proteinase

Inhibitor	Lung mast cell enzyme	Skin proteinase	Cathepsin G
M			
BPTI			
3.0×10^{-5}	0	0	65
1.5×10^{-5}	3.6	1.7	58
3.0×10^{-6}	0	0	3.7
SBTI			
1.0×10^{-5}	100	99	100
1.0×10^{-6}	91	87	100
1.0×10^{-7}	52	38	90
LBTI			
1.0×10^{-7}	63	46	79
1.0×10^{-8}	10	0	9
1.0×10^{-9}	0	0	0
PMSF			
1.0×10^{-4}	90	76	81
1.0×10^{-5}	16	5	13
1.0×10^{-6}	0	0	5

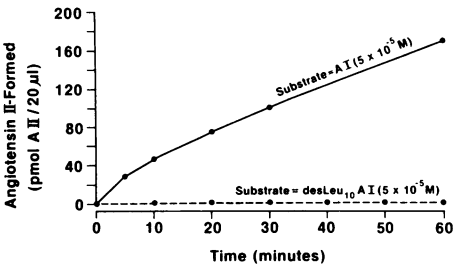

Figure 6. Specificity of human lung mast cell chymotrypsin-like enzyme for the phe_8-his_9 bond of AI.

active at neutral pH (Fig. 3) and is inhibited by PMSF but not by Captopril (Table II). The chymotryptic activity is apparently preformed as it was detected in whole lysates. It was released by challenge of a 99% pure mast cell preparation with anti-IgE (Table III) and protease release paralleled histamine release. Regression analysis of the net percent release of the enzyme, when plotted against those of histamine, yielded a straight line which passed through the origin (Fig. 4). The observation that the slope of this line is 0.64 requires comment. The slope may be due to: (*a*) the high affinity of the enzyme for heparin, analogous to those previously reported for carboxypeptidase A and chymase in preparations of rat peritoneal mast cells (21) or (*b*) partial localization of enzyme in a nonreleasable compartment. Since the net percent release values for histamine and lung enzyme were similar in a preparation of mast cells which was greater than 99% pure, the slope may also result from adherence of released enzyme to contaminating cells or inhibition of the enzyme by an inhibitor from contaminating cells. As the total activity in challenged cells was consistently 90–110% of activity in unchallenged cells, decay of enzyme activity is an unlikely possibility. These experiments indicate that the chymotrypsin-like enzyme is a preformed mast cell secretory granule, neutral, serine endopeptidase.

To characterize the chymotrypsin-like enzyme, supernatants from challenged mast cells were subjected to Sephacryl S-200 gel filtration. This procedure clearly separated the chymotryptic activity from mast cell tryptase and demonstrated an approximate molecular weight of 30,000–35,000 (Fig. 5). The enzyme was similar to a chymotrypsin-like enzyme isolated from human skin with respect to susceptibility to inhibitors (Table IV), relative activity on the synthetic substrate BTEE, and affinity for AI substrate. It was distinguished from leukocyte cathepsin G by the same criteria. The partially purified enzyme displayed limited chymotryptic specificity as it hydrolyzed only the phe_8-his_9 of AI but failed to hydrolyze the same bond of des-leu_{10}-AI or the tyr_4-$ileu_5$ bond of AII (Fig. 6). Thus, the lung mast enzyme is a previously unrecognized human chymase and is probably identical to the human skin chymotrypsin-like proteinase which is also thought to be mast cell associated (7).

Chymotrypsin-like proteinases have been previously identified in mast cells from a variety of sources, including rat peritoneum (3, 4) and dog (5) and mouse (22) mastocytoma tissues. In addition such enzymes have been isolated from rat (23, 24) and human (7, 25) skin. The major neutral protease of the rat peritoneal mast cell is rat mast cell proteinase I (chymase) (3, 4), while tryptase is thought to be the major protein constituent of the human mast cell secretory granule (1) and is present at levels of 10–20 $\mu g/10^6$ mast cells (1). While a chymotrypsin-like activity has been sought in dispersed human lung mast cell preparations using BTEE as substrate (1), the relative inability of the human lung mast cell chymotrypsin-like enzyme to cleave this substrate prevented its detection. The use of AI as substrate made the detection of this activity possible. Available data does not permit quantification of the amount of the human chymotrypsin-like enzyme in lung mast cells; however, the activity is easily detected in lysates of 10^3 mast cells using AI as substrate.

The functional role of mast cell enzymes in vivo has not been identified. It is possible that, when released as constituents of the mast cell secretory granule, they may act as mediators so as to directly affect tissue targets, or they may act indirectly to generate bioactivities from fluid phase or tissue substrates. In vitro data suggest that both mechanisms are possible. Human skin chymotrypsin-like proteinase cleavage of the cutaneous epidermal-dermal junction at the lamina lucida (26) is an example of a direct action of a mast cell enzyme, while mast cell kinin-generating activity (27) and tryptase cleavage of the third component of complement (28) and fibrinogen (29) illustrate indirect actions of mast cell enzymes. If the human mast cell chymase is indeed identical to the human skin chymotrypsin-like proteinase, the K_m and K_{cat} for AI conversion would be 6.6×10^{-5} M and 50 s^{-1}, respectively (8). These kinetics are consistent with a reaction of potential biologic significance. This pathway may then provide a unique mechanism through which the vasoactive peptide AII may be generated in perivascular sites in close proximity to vascular smooth muscle targets. Such extra vascular pathways for the generation of vasoactive peptides may provide a hitherto little appreciated mechanism for control of local tissue blood flow.

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