Biochemical Pharmacology, Vol. 37, No. 8, pp. 1473–1480, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

IDENTIFICATION OF HUMAN LUNG MAST CELL KININOGENASE AS TRYPTASE AND RELEVANCE OF TRYPTASE KININOGENASE ACTIVITY*

DAVID PROUD,† EDWARD S. SIEKIERSKI and GRAHAM S. BAILEY‡
Division of Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, MD 21239, U.S.A.

(*Received* 22 *May* 1987; *accepted* 1 *September* 1987)

Abstract—We have described previously the IgE-mediated release of kininogenase activity from purified human lung mast cells. Using supernatant fractions from mast cells stimulated with anti-IgE in the presence of deuterium oxide, we have purified this kininogenase to homogeneity by gel filtration and heparin-agarose chromatography and have demonstrated that it is identical to tryptase, the major neutral protease of human lung mast cells. Thus, tryptase and kininogenase activities co-chromatographed through both purification steps with equivalent yields. The final purified kininogenase was free of detectable chymotryptic and carboxypeptidase activities and was identified as tryptase on the basis of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), amino acid composition and inhibition profile. Three such preparations of tryptase were all capable of releasing kinin from each of two different preparations of purified, single-chain, human low molecular weight kininogen. Interestingly, kinin generation was optimal at pH 5.5 and was enhanced by heparin, which has been reported to stabilize tryptase. SDS–PAGE analysis of kininogen hydrolysis by tryptase revealed the formation of a diffusely stained region in the molecular weight range of 60,000–65,000, rather than a discrete heavy chain band. Under optimal conditions, the three tryptase preparations released 10–12 μg kinin/hr/mg but released only 2 μg kinin/hr/mg at pH 7.2. HPLC analysis revealed that the kinin released was bradykinin. We conclude that the kininogenase activity from human lung mast cells is attributable to tryptase. The unique pH optimum of this reaction of a serine protease, however, raises doubts as to the physiologic significance of this activity.

It is generally accepted that the pathogenesis of the allergic response probably results from the combined actions of several inflammatory mediators. In recent years, evidence has begun to accumulate to support the hypothesis that kinins may be among the important mediators of allergic reactions. It has been shown that high molecular weight (HMW) kininogen is consumed during anaphylactic reactions in man [1]. More directly, it has been demonstrated that the potent vasoactive peptides bradykinin and lysyl-bradykinin are generated during both the immediate and late responses to nasal challenge with allergen [2, 3], and that levels of immunoreactive kinins in bronchoalveolar lavage fluids from symptomatic asthmatics, or from asthmatics responding to allergen challenge, are significantly higher than in fluids from normal individuals [4]. In addition, administration of bradykinin to the lower airways induces bronchoconstriction in asthmatics [5–7], while nasal provocation with bradykinin induces symptoms of rhinitis regardless of atopic status [8].

These studies have focused attention upon the enzymes that may be responsible for kinin generation during the allergic response. It has been shown that concentrations of tissue kallikrein in airway secretions increase in both upper and lower airways during the immediate allergic response [4, 9], while tissue kallikrein is present in late-phase blister fluids of some allergics [10]. In addition, plasma kallikrein activation also occurs during the allergic response in the upper airways [11]. Other possible candidates to play important roles in kinin generation, however, are cellular proteases. A kallikrein-like enzyme has been reported to be released from basophils [12] while, more recently, we have described the IgE-mediated release of a kininogenase from purified human lung mast cells [13]. In this paper we describe the characterization of this mast cell kininogenase and show that it is identical to tryptase, the predominant protease of human lung mast cells [14].

MATERIALS AND METHODS

Materials. The following materials were purchased: PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]), sodium heparin, chymopapain, elastase type I, deuterium oxide, bovine gamma globulin, diethylstilbesterol, diisopropylfluorophosphate (DFP), tosyl-L-lysine-chloromethyl-

* Publication No. 707 from the O'Neill Research Laboratories at The Good Samaritan Hospital, 5601 Loch Raven Boulevard, Baltimore, MD 21239. This work was supported by Grant HL32272 from the National Institutes of Health.

† All correspondence and reprint requests should be addressed to: Dr. David Proud, The Johns Hopkins University School of Medicine at The Good Samaritan Hospital, 5601 Loch Raven Boulevard, Baltimore, MD 21239.

‡ Current address: Department of Chemistry, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, Essex, U.K.

ketone(TLCK),tosyl-L-phenylalanine chloromethylketone (TPCK), benzamidine, soybean trypsin inhibitor (SBTI), pepstatin, 1,10-phenanthroline, iodoacetamide, heparin-agarose (Sigma Chemical Co., St. Louis, MO); MES (2-[*N*-morpholino]-ethanesulfonic acid), Pronase and DNase (Calbiochem, Los Angeles, CA); RPMI-1640 medium with 25 mM HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid), Hanks' solution, kanamycin (Gibco, Grand Island, NY); Trasylol (Bayer AG, Wuppertal, West Germany); human serum albumin (Miles Laboratories, Elkhart, IN); Sephacryl S-200, Percoll (Pharmacia, Piscataway, NJ); bradykinin, lysylbradykinin, methionyllysylbradykinin, Tyr^8-bradykinin (Peninsula Laboratories, Belmont, CA); PEG 8000 (Fisher Scientific Co., Pittsburgh, PA); and acrylamide, *N,N,N',N'*-tetramethylethylenediamine (TEMED), ammonium persulfate, affinity-purified goat anti-human IgE (Bio-Rad Laboratories, Rockville Center, NY). D-Pro-Phe-Val chloromethylketone, Phe-Ala-Lys chloromethylketone and D-Phe-Phe-Arg chloromethylketone were gifts from Dr. Elliott Shaw (Friedrich Miescher Institute, Basel, Switzerland).

Human low molecular weight kininogen was purified from fresh human plasma by a modification of the method of Pierce and Guimaraes [15] as previously described [13]. Two such preparations were used in the present studies. Each preparation contained approximately 0.95 mol kinin/mol and was predominantly (>90%) single chain as judged by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and by treatment with pepsin [13].

Buffers. PIPES-albumin (PA) buffer contains (g/l): PIPES, 7.6; NaCl, 6.4; KCl, 0.37; 10 N NaOH, 4.2 ml/l; and human serum albumin, 30 mg. The pH was adjusted to pH 7.4. PACM is PA with 1 mM $CaCl_2$, $2H_2O$ and 1 mM $MgCl_2 \cdot 6H_2O$ added. For release experiments, PACM in which 88% of the water was replaced with deuterium oxide (88% D_2O/PACM) was used.

Mast cell purification. Human lung tissue was obtained from patients undergoing thoracotomy and lung resection, usually for carcinoma of the bronchus or other neoplasms. Macroscopically normal lung tissue was dissected free from pleura, bronchi and blood vessels, minced into 5- to 10-mg fragments, and enzymatically dispersed into single cell suspensions. Mast cells were purified further, as previously described in detail [16, 17], by countercurrent centrifugation elutriation and, in some instances, by centrifugation over discontinuous gradients of Percoll. Mast cells were quantified after staining with alcian blue [18].

Mast cell activation. Purified mast cells were suspended in 88% D_2O/PACM at a concentration of 8 to 10×10^6/ml and were challenged with the appropriate dose of anti-IgE (usually 10 μg/ml final concentration). Incubations were performed for 30 min at 37°, at which time cells were sedimented by centrifugation, and the supernatant fraction was removed. Samples (10 μl) of the cell suspension and of supernatant fractions after challenge were added to tubes containing 1 ml of 2% perchloric acid and were diluted as appropriate for determination of histamine content, which was measured by an automated fluorometric technique [19].

Kininogenase assay. For routine assays, 50 μl of supernatant fraction from stimulated mast cells or 50 μl of column fraction was mixed with 10 μl (10 μg) of kininogen and 50 μl of buffer containing heparin (heparin was selected to give a tryptase/heparin ratio of approximately 1:10 by weight). The buffer was selected so that the pH of the reaction was pH 5.5. Incubations were performed at 37° for the appropriate time (selected to ensure that kinin generation remained linear with time) before terminating the reaction by snap-freezing on dry ice. Generated kinin was determined by rat uterus bioassay as previously described [20]. Samples to be determined were stored at −80° and thawed immediately prior to addition to the organ bath.

TAME-esterase activity. Tryptase was assayed based on its ability to hydrolyze *N*-α-*p*-tosyl-L-arginine methyl ester (TAME). TAME-esterase activity was measured by following the hydrolysis of [^{3}H]TAME according to the method of Imanari *et al.* [21]. Hydrolysis of substrate liberates tritiated methanol which is partitioned into a toluene-based scintillation fluid and counted. The assay is standardized using purified human urinary kallikrein, and 1 TAME unit (TU) is defined as that amount of enzyme that hydrolyzes 1 μmol TAME/min at pH 8.0 and 30°.

Chromatographic procedures. Purification of the mast cell kininogenase (tryptase) was performed as follows. The supernatant fraction from a suitable number of stimulated mast cells was concentrated using an Amicon PM-10 membrane to a volume of 2 ml and was made 3 M in NaCl and incubated for 2 hr at 4°. The supernatant fraction was then applied to a calibrated column (1.6 × 91 cm) of Sephacryl S-200 equilibrated in 0.1 M Tris/1.0 M NaCl, pH 7.4. The column was eluted at a flow rate of 12 ml/hr, and 2-ml fractions were collected. Fractions were monitored for absorbance at 280 nm (A_{280}), kininogenase activity, and TAME-esterase activity. The fractions containing kininogenase/tryptase activity were pooled, concentrated, and dialyzed overnight against 10 mM MES/2 mM $CaCl_2$, pH 6.2. The sample was then applied to a 3-ml bed volume (1.5 × 1.7 cm) of heparin-agarose equilibrated in the same buffer. Sample application was performed in 3 × 0.8 ml portions, with a 15-min period between portions to allow interaction with the heparin. The column was then eluted with equilibration buffer until the A_{280} was less than 0.003 and was then eluted, in a stepwise fashion, with buffer containing 0.1 M NaCl and then with buffer containing 1.0 M NaCl. Fractions were again monitored for A_{280}, kininogenase activity, and TAME-esterase activity. Fractions containing kininogenase/tryptase activity were pooled, concentrated, dialyzed against 0.05 M Tris/0.1 M NaCl, pH 7.4, and stored in aliquots at −80°.

Analytical procedures. SDS–PAGE, with or without reduction, was performed in 10% polyacrylamide gels according to the method of Laemmli [22]. Gels were stained with either Coomassie Blue R-250 or a silver stain [23].

Amino acid analysis of the purified enzyme was

performed on aliquots containing 0.027 A_{280} units. Each aliquot was dialyzed against distilled water, lyophilized and hydrolyzed *in vacuo* with constant boiling 6 N HCl at 110°. Hydrolysis times of 24 and 48 hr were used, and variable sized aliquots of each hydrolyzate were employed for analysis using the picotag method.

Kinin analysis. To determine which kinin was generated by tryptase, 2 μg of purified enzyme was incubated with 20 μg of purified kininogen for 2 hr at 37° and pH 5.5. The sample was then extracted by a modification of the method of Carretero *et al.* [24]. In brief, the sample was made 80% in ethanol and incubated at 4° for 30 min. The sample was then spun in a microfuge for 1 min, and the supernatant fraction was removed. The supernatant fraction was concentrated to approximately 0.2 ml using a Savant Speed Vac Concentrator (Savant Instruments Inc., Hicksville, NY) and was applied to a 0.8 × 2 cm column of QAE-Sephadex A-50 equilibrated in 0.0075 M Tris/0.025% NaN_3, pH 8.0. Kinins were eluted from the column using the same buffer. A total volume of 8 ml was collected to ensure that the ethanol content of the eluate was low enough not to affect Sep-Pak extraction. Kinins were then concentrated using a Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA) and were eluted from the cartridge using 2 ml of 50% Solution A/50% Solution B (Solution A = 0.05% trifluoroacetic acid/0.025% triethylamine, pH 2.45; Solution B = 80% acetonitrile in Solution A). The Sep-Pak eluate was concentrated to approximately 200 μl using the Speed Vac Concentrator and an aliquot was analyzed by HPLC as previously described [2]. HPLC fractions were monitored for kinin content by radioimmunoassay [2]. Controls of tryptase + buffer and buffer + kininogen were incubated, extracted and analyzed in the same way.

RESULTS

It has been shown previously that D_2O enhances IgE-mediated release from human lung mast cells [25]. In the present studies, therefore, we took advantage of this property to obtain supernatant fractions containing higher levels of kininogenase activity. The purification of kininogenase and tryptase activities from one such supernatant fraction is summarized in Table 1. The supernatant fraction was derived from 34 × 10^6 mast cells of 45% purity which released 55% of their cellular histamine upon stimulation in the presence of D_2O. Following a 2-hr incubation at 4° in the presence of 3 M NaCl to help dissociate protease–proteoglycan complexes, the supernatant fraction was applied to a column of Sephacryl S-200 equilibrated in high salt buffer. Kininogenase and tryptase activities coeluted at an apparent molecular weight of 145,000 with equivalent yields (Fig. 1). Fractions 39–48 were pooled, concentrated to approximately 2.5 ml, and dialyzed overnight. The sample was then applied in three portions to a column of heparin-agarose. A 15-min period was allowed to elapse between the addition of portions to the column to ensure adequate time for interactions with the column to occur. When all three portions were applied, the column was eluted as shown in Fig. 2. Tryptase and kininogenase activities again coeluted when 10 mM MES/2 mM $CaCl_2$/1.0 M NaCl, pH 6.2, was used as eluate. The yields of tryptase and kininogenase activities were again similar. Through the two chromatographic steps, tryptase was purified 5.4-fold with 56% yield, while kininogenase activity was purified 5.6-fold in 60% yield. In two other purifications, yields of 53 and 60% were achieved with, again, no significant differences in recovery of tryptase and kininogenase activities.

These data strongly suggested that the kininogenase activity from human mast cells was attributable to tryptase. To verify this suggestion the purified material was analyzed further. On SDS–PAGE, the protein appeared as a diffuse band with an apparent molecular weight of 34,000–35,000 (Fig. 3), consistent with previously reported behavior of tryptase [26]. A comparison of the inhibition profiles for the kininogenase and TAME-esterase activities (Table 2) of this material showed that inhibitors of acidic proteases, thiol proteases and metalloproteases had no effect on either activity, while the profiles of inhibition of both activities by a panel of serine protease inhibition were essentially identical. The minor difference observed with trasylol almost certainly reflects the poor binding of this inhibitor under acidic conditions such as those used in the kininogenase assay. The final means used to confirm that the purified kininogenase was, indeed, tryptase was amino acid analysis (Table 3). Data are expressed as residues/mol based on a subunit molecular weight of 31,250 for comparison with the

Table 1. Purification of mast cell kininogenase/tryptase

		[^{3}H]TAME activity			Kininogenase		
	A_{280} units	Activity (TU)	Specific activity†	Yield (%)	Activity (ng/hr)	Specific activity†	Yield (%)
Supernatant	5.68	390	69	100	4270	752	100
S-200	1.88	305	162	78	3202	1703	75
Heparin-Agarose	0.60	219	365	56	2562	4270	60

† Specific activities are given in units/A_{280}. Based on literature extinction coefficient (1 mg/ml = 2.81 A_{280}), final material had specific activities of 1025 TU/mg and 12 μg kinin generated/hr/mg. One TAME unit (TU) is the amount of enzyme that hydrolyzes 1 μmol TAME/min at pH 8.0 and 30°.

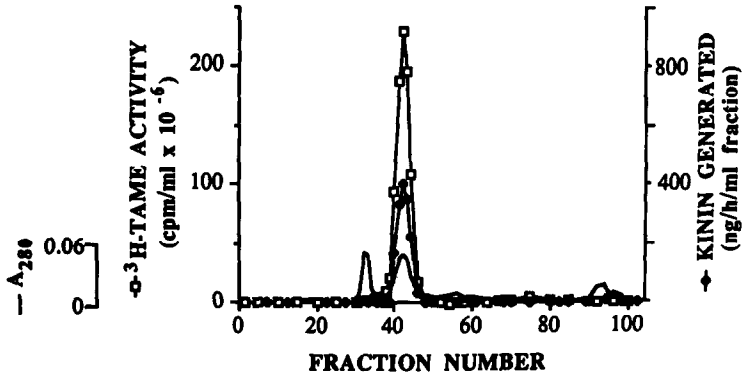

Fig. 1. Sephacryl S-200 gel filtration of a supernatant fraction from stimulated mast cells. The supernatant was made 3 M in NaCl and incubated for 2 hr at 4° prior to application to a column (1.6 × 91 cm) of Sephacryl S-200 equilibrated in 0.1 M Tris/1.0 M NaCl, pH 7.4. The column was eluted at a flow rate of 12 ml/hr, and 2-ml fractions were collected.

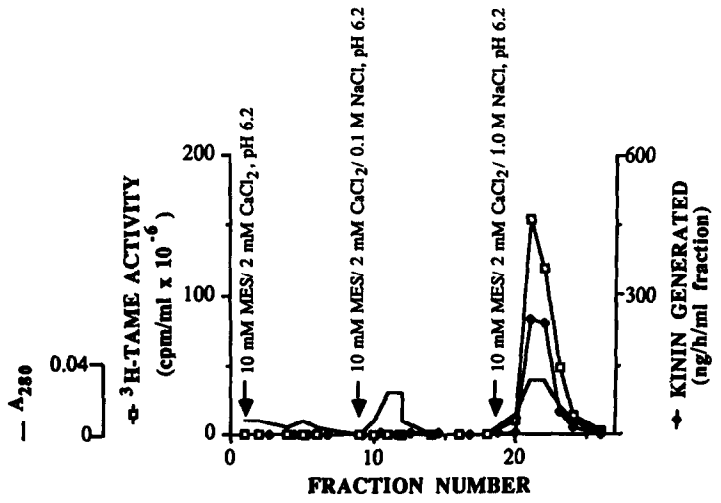

Fig. 2. Heparin-agarose chromatography of mast cell kininogenase. Pooled fractions from gel filtration were concentrated and dialyzed overnight against 10 mM MES/2 mM $CaCl_2$, pH 6.2. The sample was applied to a 3-ml column (1.5 × 1.7 cm) of heparin-agarose equilibrated in the same buffer. The column was eluted successively with buffer containing increasing salt concentrations as shown, and fractions of 2-ml volume were collected.

Table 2. Inhibition profiles of kininogenase and tryptase

Inhibitor*	Concn (M)	Kininogenase	Tryptase
D-Pro-Phe-Val·CH_2Cl	10^{-4}	–	–
Phe-Ala-Lys·CH_2Cl	10^{-6}	++	++
D-Phe-Phe-Arg·CH_2Cl	10^{-6}	++	++
DFP	10^{-3}	++	++
TLCK	10^{-3}	++	++
TPCK	10^{-3}	–	–
Benzamidine	10^{-3}	++	++
Trasylol	10^{-5}	–	±
SBTI	10^{-5}	–	–
Pepstatin	10^{-6}	–	–
Iodoacetamide	10^{-3}	–	–
1,10-Phenanthroline	5×10^{-3}	–	–

* Inhibitor was preincubated with the enzyme for 30 min at room temperature prior to assay.

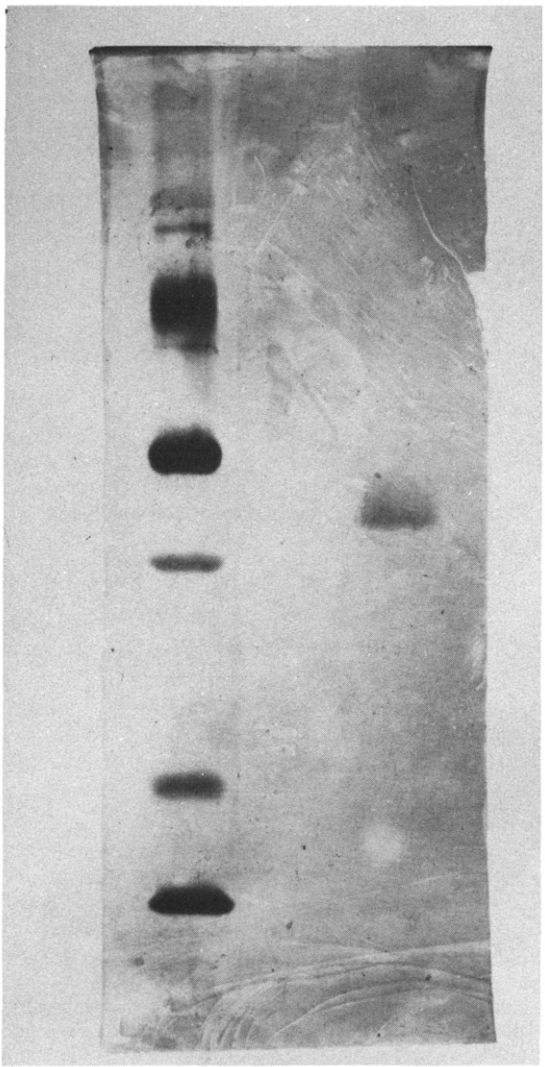

Fig. 3. SDS–PAGE of purified kininogenase/tryptase. The gel was run without reduction and was stained using a silver stain. The left-hand column shows the following molecular weight markers (from the top): phosphorylase *b* (94K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (30K), soybean trypsin inhibitor (20K) and α-lactalbumin (14.5K). The right-hand column shows 1.5 μg of tryptase with an apparent molecular weight of 34–35K.

previously published data of Smith *et al.* [27] and Schwartz and coworkers [14]. The amino acid composition of the material purified in the present work leaves little doubt that the protein is, indeed, tryptase. Analysis shows that comparisons with compositions reported by both Schwartz *et al.* and Smith *et al.* satisfy the Cornish–Bowden "strong" test for relatedness [28].

The confirmation that mast cell kininogenase activity is attributable to tryptase leads to controversy, since it has been reported previously that tryptase is devoid of kinin-generating activity [29, 30]. We, therefore, examined more closely the interaction of purified tryptase with kininogen. Each of three preparations of tryptase was capable of generating kinin from two different preparations of human low molecular weight kininogen. This interaction is unusual for a serine protease, however, in that purified tryptase generated kinin optimally at pH 5.5 in the presence of a 1:10 weight ratio of using crude supernatant fractions from stimulated mast cells [13]. SDS–PAGE analysis of this interaction revealed further anomalies. Tissue kallikrein hydrolyzes low molecular weight kininogen releasing kinin. Upon reduction, heavy and light chains are separated such that the intact molecule at 68,000 molecular weight is replaced by a heavy chain of 64,000 molecular weight and a 4,000 dalton light chain, which runs at the dye front [13]. Purified tryptase, however, did not generate a discrete heavy chain band but rather produced a diffusely strained area in the molecular weight range of 60,000–65,000 (Fig. 5), presumably reflecting multiple hydrolysis points within the heavy chain. Despite this diffuse pattern, however, kinin liberation clearly occurred, and HPLC analysis of the liberated peptide indicated that it was bradykinin (Fig. 6).

It has been shown previously that heparin stabilizes tryptase [26]. Kininogenase activity of the enzyme was similarly affected with kinin generation over a 1-hr period being reduced to approximately 50% of optimum in the absence of heparin. At pH 5.5 in the presence of a 1:10 weight ratio of tryptase:heparin, three tryptase preparations displayed kininogenase activities in the range of 10–12 μg kinin generated/hr/mg. At pH 7.2, however, activity was reduced by more than 6-fold.

DISCUSSION

The ability of mast cells or basophils to release high molecular weight proteases in response to IgE-mediated challenge has become the focus of

Table 3. Comparison of amino acid composition of purified tryptase with published data*

	Residues/mol		
	Schwartz *et al.*†	Present work	Smith *et al.*‡
Asp	25	27	25
Thr	11	12	13
Ser	19	19	13
Glu	30	36	26
Pro	24	26	26
Gly	30	31	27
Ala	17	19	16
Cys	1	1	9
Val	20	26	25
Met	3	3	4
Ile	8	10	12
Leu	21	23	26
Tyr	6	11	11
Phe	5	6	5
His	10	11	10
Lys	9	6	12
Arg	13	15	14
Trp	ND	ND	9

* Residues/mol based on a subunit molecular weight of 31,250 (see Ref. 27). ND = not determined.

† Ref. 14.

‡ Ref. 27.

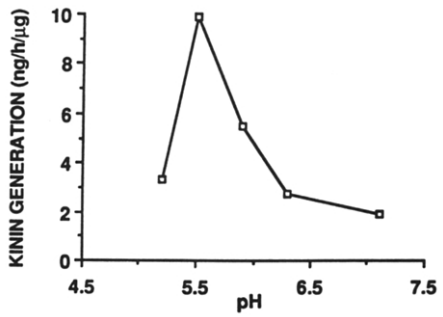

Fig. 4. pH dependence of kinin generation by tryptase. Aliquots of tryptase (1 μg) were incubated with 10 μg of kininogen in the presence of heparin at the pH points indicated.

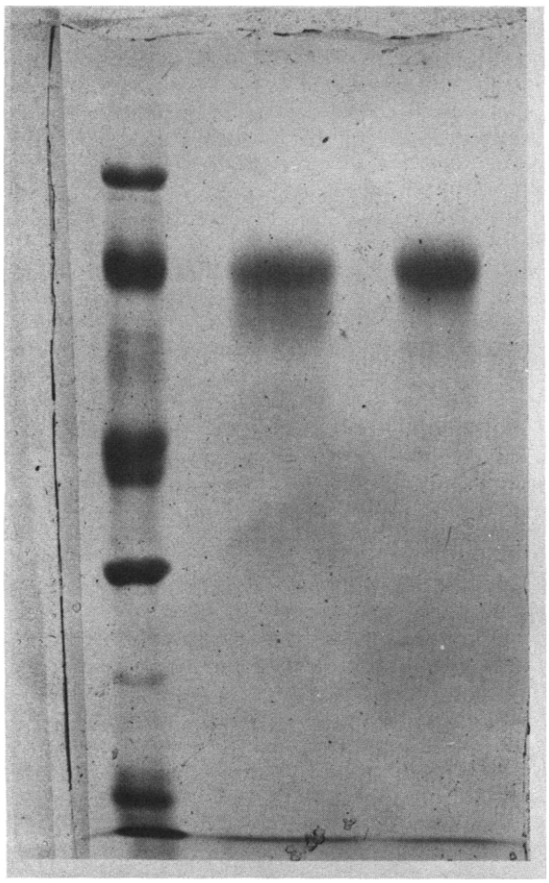

Fig. 5. SDS–PAGE analysis of the effects of tryptase on low molecular weight kininogen (LMWK). The gel was run under reducing conditions and was stained with Coomassie Blue R-250. The left-hand column shows the same molecular weight standards used in Fig. 3. The center column shows the pattern obtained when 10 μg LMWK was incubated with 1 μg tryptase in the absence of heparin. The right-hand column shows the pattern obtained when LMWK was incubated in buffer for the same time.

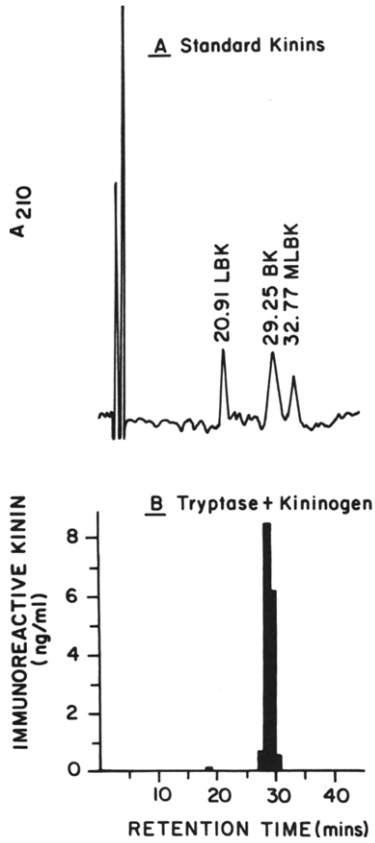

Fig. 6. HPLC analysis of the kinin generated upon incubation of tryptase with LMWK. Tryptase (2 μg) was incubated with LMWK (20 μg) for 2 hr. Generated kinin was extracted as described under Materials and Methods, and an aliquot was applied to a μBondapak C_{18} column (Waters Associates, Milford, MA). Kinins were eluted using isocratic conditions (25%B/75%A: A is 0.05% trifluoroacetic acid/0.025% triethylamine in water; B is 80% acetonitrile in A) at a flow rate of 1 ml/min. Fractions were collected and monitored for kinin content by radioimmunoassay. The upper panel shows the elution profile of standard kinins as monitored by absorbance at 210 nm. The lower panel shows the retention time of the immunoreactive kinin generated by tryptase, corresponding to that for bradykinin.

increased study over the past few years. In addition to tryptase, human mast cells have been shown to possess both a chymotrypsin-like enzyme [31] and a carboxypeptidase [32] and have been reported to contain elastase [33] and a prekallikrein activator [34]. The description of a kininogenase from human lung mast cells appeared to elongate this list [13]. The purpose of the present study was to characterize this enzyme and to assess its likely importance in kinin generation during allergic events.

The use of D_2O to enhance IgE-mediated release of the enzyme from mast cells provided a starting material consisting, presumably, primarily of mast cell granule contents but in higher amounts than would be obtained without D_2O. By contrast, while lysis would release 100% of granule contents, it would also result in a much higher contamination with a non-mast cell protein. The use of this starting material allows purification of the enzyme to homogeneity in two simple steps.

Treatment of the sample with high salt was used because it has been shown previously to dissociate mast cell proteases from proteoglycan [31, 35]. Gel filtration in high salt resulted in a single peak of

TAME-esterase activity at a molecular weight of 145,000, representing tryptase. It has been shown that mast cell chymotryptic and carboxypeptidase enzymes both elute at molecular weights around 30,000 in this system [31, 32]. The coelution of kininogenase and tryptase activities with equivalent yields was surprising, since tryptase is a neutral protease which has been reported to be devoid of kinin-generating activity [29, 30], but could have been coincidental. The subsequent parallel chromatography of both activities on heparin-agarose, again with equivalent yields and degrees of purification, however, strongly suggested that the kininogenase was, indeed, tryptase. By beginning with supernatant fractions from stimulated mast cells, only a 5.5-fold purification relative to the starting material was required to obtain tryptase which was free of chymotryptic and carboxypeptidase activities and was pure by electrophoretic and amino acid composition criteria. The simple, two-step procedure, which results in yields up to 55 or 60%, is not always adequate to achieve purity when whole cell lysates are used. Indeed, with whole cell lysates, we have occasionally seen a second kininogenase [36]. At first it was thought that this may also be mast cell-derived but the inconsistent observation of this enzyme in lysates, together with its total absence in 10 supernatant fractions from stimulated cells, and its ability to generate lysyl-bradykinin now lead us to believe that this enzyme may represent tissue kallikrein derived from another cell type in our lung preparations [4].

The single kininogenase purified from supernatant fractions of stimulated mast cells was clearly identical to tryptase in terms of its inhibition profile (Table 2). Although tryptase has been reported to appear as a doublet on SDS-electrophoresis when examined using densitometry [14], in our hands a single diffuse band was usually seen but was in an identical molecular weight range to that previously reported for tryptase [14, 26]. Similarly, although we were unable to perform separate analyses for cysteine and tryptophan, the amino acid composition obtained for our purified material was in good agreement with other published data [14, 27].

Although it has been reported previously that tryptase does not generate kinin from low molecular weight kininogen [29], these results are not necessarily incompatible with our own. These earlier studies used much lower levels of tryptase, lower concentration of kininogen and shorter incubation times than employed in our studies. We selected our kininogen concentrations to approximate to those present in plasma and used considerably higher tryptase concentrations since it has been suggested that in the skin, for example, tryptase concentrations may reach 800 nM [30]. In our hands, it is clear that tryptase can generate bradykinin from single chain kininogen (Fig. 6). The interaction of tryptase and kininogen, however, is clearly unusual in that, unlike tissue kallikrein, tryptase appeared to hydrolyze several different bonds in the heavy chain such that, upon reduction, a diffuse staining pattern was seen (Fig. 5). In addition, the pH optimum for this reaction, occurring at pH 5.5, is unusual for the interaction of a neutral serine protease with kininogen. At this point it is unknown whether this occurs as a result of enhanced binding to kininogen at this pH or if it reflects some conformational change in either tryptase or kininogen which favors kinin formation. Even under optimal pH conditions and in the presence of heparin as a stabilizing agent [26], tryptase is less than 5% as efficient as tissue kallikrein in generating kinin. Thus, in this respect we agree with the results of Schwartz *et al.* [29]. It is interesting to speculate, however, that this unusual pH optimum may have implications for the interaction of tryptase with other possible substrates and, since the pH of the mast cell granule is in the range of pH 5.5 to 6.5 [37], it is possible that tryptase may retain some activity towards an as yet unidentified substrate at the intracellular level.

In conclusion, a previously reported human mast cell kininogenase has been shown to be identical to tryptase. Although some role of the enzyme in kinin generation in local acidic environments cannot be ruled out, the unusual pH optimum for the enzyme and its relatively low efficiency as a kininogenase raise doubts regarding its widespread physiological importance in kinin generation during allergic events.

Acknowledgement—The authors thank Ms. Bonnie Evans for excellent secretarial assistance.

REFERENCES

1. P. L. Smith, A. Kagey-Sobotka, E. R. Bleecker, R. Traystman, A. P. Kaplan, H. Gralnick, M. D. Valentine, S. Permutt and L. M. Lichtenstein, *J. clin. Invest.* **66**, 1072 (1980).
2. D. Proud, A. Togias, R. M. Naclerio, S. A. Crush, P. S. Norman and L. M. Lichtenstein, *J. clin. Invest.* **72**, 1678 (1983).
3. R. M. Naclerio, D. Proud, A. G. Togias, N. F. Adkinson, Jr., D. A. Meyers, A. Kagey-Sobotka, M. Plaut, P. S. Norman and L. M. Lichtenstein, *New Engl. J. Med.* **313**, 65 (1985).
4. S. C. Christiansen, D. Proud and C. G. Cochrane, *J. clin. Invest.* **79**, 188 (1987).
5. H. Herxheimer and E. Stresemann, *J. Physiol., Lond.* **158**, 38 (1961).
6. H. S. Varonier and R. Panzani, *Int. Archs Allergy* **34**, 293 (1968).
7. R. W. Fuller, C. M. S. Dixon, F. M. C. Cuss and P. J. Barnes, *Am. Rev. resp. Dis.* **135**, 176 (1987).
8. D. Proud, C. J. Reynolds, S. LaCapra, A. Kagey-Sobotka, L. M. Lichtenstein and R. M. Naclerio, *J. Allergy clin. Immun.* **79**, 254 (1987).
9. C. R. Baumgarten, R. C. Nichols, R. M. Naclerio and D. Proud, *J. Immun.* **137**, 1323 (1986).
10. W. Dorsch, J. Ring, H. J. Reiman and R. Geiger, *J. Allergy clin. Immun.* **70**, 236 (1982).
11. C. R. Baumgarten, R. C. Nichols, R. M. Naclerio, L. M. Lichtenstein, P. S. Norman and D. Proud, *J. Immun.* **137**, 977 (1986).
12. H. H. Newball, R. W. Berninger, R. C. Talamo and L. M. Lichtenstein, *J. clin. Invest.* **64**, 457 (1979).
13. D. Proud, D. W. MacGlashan, Jr., H. H. Newball, E. S. Schulman and L. M. Lichtenstein, *Am. Rev. resp. Dis.* **132**, 405 (1985).
14. L. B. Schwartz, R. A. Lewis and K. F. Austen, *J. biol. Chem.* **256**, 11939 (1981).
15. J. V. Pierce and J. A. Guimaraes, in *Chemistry and Biology of the Kallikrein–Kinin System in Health and Disease* (Eds. J. J. Pisano and K. F. Austen), p. 121. DHEW Pub. No. 76-791, Washington, DC (1976).
16. E. S. Schulman, D. W. MacGlashan, Jr., S. P. Peters,

R. P. Schleimer, H. H. Newball and L. M. Lichtenstein, *J. Immun.* **129**, 2662 (1982).
17. T. Ishizaka, D. H. Conrad, E. S. Schulman, A. R. Sterk and K. Ishizaka, *J. Immun.* **130**, 2357 (1983).
18. H. S. Gilbert and L. Ornstein, *Blood* **46**, 279 (1975).
19. R. P. Siraganian, *Analyt. Biochem.* **57**, 383 (1974).
20. D. Proud, J. V. Pierce and J. J. Pisano, *J. Lab. clin. Med.* **95**, 563 (1980).
21. T. Imanari, T. Kaizu, H. Yoshida, K. Yates, J. V. Pierce and J. J. Pisano, in *Chemistry and Biology of the Kallikrein–Kinin System in Health and Disease* (Eds. J. J. Pisano and K. F. Austen), p. 205. DHEW Pub. No. 76-791, Washington, DC (1976).
22. U. K. Laemmli, *Nature, Lond.* **227**, 680 (1970).
23. C. R. Merril, D. Goldman, S. A. Sedman and M. H. Ebert, *Science* **211**, 1437 (1980).
24. O. A. Carretero, N. B. Oza, A. Piwonska, T. Ocholik and A. G. Scicli, *Biochem. Pharmac.* **25**, 2265 (1976).
25. S. P. Peters, E. S. Schulman, R. P. Schleimer, D. W. MacGlashan, Jr., H. H. Newball and L. M. Lichtenstein, *Am. Rev. resp. Dis.* **126**, 1034 (1982).
26. L. B. Schwartz and T. R. Bradford, *J. biol. Chem.* **261**, 7372 (1986).
27. T. J. Smith, M. W. Hougland and D. A. Johnson, *J. biol. Chem.* **259**, 11046 (1984).
28. A. Cornish-Bowden, *Analyt. Biochem.* **105**, 233 (1980).
29. L. B. Schwartz, M. Maier and J. Spragg, in *Advances in Experimental Medicine and Biology* (Eds. L. M. Greenbaum and H. S. Margolius), Vol. 198A, p. 105. Plenum Press, New York (1986).
30. M. Maier, J. Spragg and L. B. Schwartz, *J. Immun.* **130**, 2352 (1983).
31. B. U. Wintroub, C. E. Kaempfer, N. M. Schechter and D. Proud, *J. clin. Invest.* **77**, 196 (1986).
32. S. M. Goldstein, C. E. Kaempfer, D. Proud, L. B. Schwartz, A-M. Irani and B. U. Wintroub, *Clin. Res.* **35**, 249A (1987).
33. H. L. Meier, L. W. Heck, E. S. Schulman, D. W. MacGlashan, Jr., L. M. Lichtenstein, C. G. Cochrane, H. H. Newball and A. P. Kaplan, *Fedn Proc.* **42**, 445 (1983).
34. H. H. Newball, H. L. Meier, A. P. Kaplan, S. D. Revak, C. G. Cochrane and L. M. Lichtenstein, *Trans. Ass. Am. Phycns* **94**, 126 (1981).
35. L. B. Schwartz, R. A. Lewis and K. F. Austen, *J. Immun.* **126**, 1290 (1981).
36. D. Proud and L. M. Lichtenstein, *Fedn Proc.* **43**, 1807 (1984).
37. D. Lagunoff and A. Rickard, *Expl Cell Res.* **144**, 353 (1983).