Tryptase, the predominant neutral protease in human mast cell secretory granules, was purified to homogeneity from dissociated and concentrated pulmonary mast cells by sequential chromatography on Dowex 1-X2, DEAE-Sephadex, and heparin-agarose. Purified tryptase gave a single stained protein band on polyacrylamide gels after electrophoresis at pH 4.3 in the presence of 4 M urea. The enzyme has an apparent molecular weight of 120,000 to 140,000 by gel filtration chromatography. Electrophoresis of purified tryptase under denaturing conditions revealed subunits with molecular weights of 37,000 and 35,000 in a molar ratio of 1:1, consistent with a tetrameric subunit structure for the holoenzyme of \( M_r = 144,000 \). Both subunits bind \(^{3}{}_{H}\)diisopropyl fluorophosphate as assessed by the correspondence of radioactivity with the two stained protein bands in a polyacrylamide gel after electrophoresis of purified tryptase under denaturing conditions, indicating that all four subunits of the holoenzyme may have active site capacity. Purified tryptase has a specific activity for tosyl-L-arginine methyl ester of 97 units/mg (1 unit = 1 nmol of substrate cleaved/min at 22 °C). Human pulmonary mast cells contain tosyl-L-arginine methyl esterase at levels more than 100-fold higher than those of human neutrophils, eosinophils, and monocytes. One million mast cells contain about 1.1 units, or 6 to 19 \( \mu \)g of tryptase, and have the capacity to contribute dominant levels of this enzyme at tissue sites of mast cell degranulation.

A serine esterase capable of cleaving tosyl-L-arginine methyl ester was recently localized to the secretory granules of human pulmonary mast cells by its release into the extracellular milieu in a fixed ratio to histamine after IgE-dependent activation of the dispersed concentrated human pulmonary mast cells (1). Tryptic esterase activity had been previously localized to mast cells of human gingiva (2) and skin (3) by histochemical techniques and several proteases had been observed to be released from human lung fragments after IgE-dependent activation (4), but a cell source for these uncharacterized proteases has not been defined. The mast cell tryptase extracted from or released by IgE-dependent activation of dispersed and partially purified human lung mast cells was further characterized by inhibition of its esterase activity by diisopropyl fluorophosphate and tosyl-L-lysine chloromethyl ketone, but not by tosyl-L-phenylalanine chloromethyl ketone, \( \alpha \)-trypsin inhibitor, and several other trypsin-inhibitor proteins (1). Gel filtration chromatography of tryptase extracted in 1 M NaCl from human pulmonary mast cell preparations revealed a single peak of enzyme activity with an apparent molecular weight of 120,000 to 140,000. After further purification, the human pulmonary mast cell tryptase has been recognized as a major protein component of the secretory granule and characterized as a tetramer.

### EXPERIMENTAL PROCEDURES

**Materials**—Diethylaminoethyl-Sephadex (A-25), phosphorylase \( b \), bovine serum albumin, ovalbumin, carbonic anhydrase, \( \alpha \)-trypsin inhibitor, \( \alpha \)-lactalbumin, aldolase, and chymotrypsinogen A (Pharmacia); carboxypeptidase A (45.5 units/mg) and pyruvate kinase (164 units/mg) (Millipore); TAMe; sodium azide, and 2-(N-morpholino)ethanesulfonic acid (Sigma); heparin-agarose (Miles); acrylamide, bisacrylamide, ammonium persulfate, \( N,N',N' \)-tetramethylethylenediamine, (SDS), Coomassie brilliant blue, urea, Bromophenol blue, basic fuchsin, and Dowex 1-X2 (Bio-Rad); 1-[\(^{3}{}_{H}\)]DFP, (0.9 Ci/ mmol), Econofor, and Protocol (New England Nuclear); and Tris, sucrose, and trichloroacetic acid (Fisher) were obtained as indicated.

DEAE-Sephadex A-25 was suspended and washed three times in 3 to 5 volumes of 0.01 M Tris (pH 7.2, 4 °C) containing 2 mM CaCl\(_2\), stored at 4 °C with 0.02% (w/v) sodium azide and was deaerated under vacuum before use. Heparin-agarose was suspended in 3 to 5 volumes of 0.01 M Tris (pH 7.2, 4 °C) containing 2 mM CaCl\(_2\), washed once with suspension buffer, and stored at 4 °C. Dowex 1-X2 was prepared according to instructions of the manufacturer and was equilibrated in 10 mM Tris, pH 7.8, 2 mM CaCl\(_2\), 1 M NaCl and stored as above.

Salt concentrations were measured with a Radiometer Conductivity Meter CDM 3 after 200-fold dilutions with water. Absorbance measurements were performed with a Gilford 250 and 300-N-Micro-Sample spectrophotometers. Protein was measured by the method of Lowry et al. (6) with bovine serum albumin as a standard.

**Cell Isolation and Chromatographic Procedures**—Dispersed cell suspensions were obtained from surgical specimens of human lung and the mast cells were partially purified as previously described (6). Preparations of purified human neutrophils, eosinophils, and monocyes were supplied by A. Tauber, P. Weller, and L. Klickstein (Harvard Medical School, Boston, MA), respectively. Mast cell or leukocyte preparations were suspended in 10 mM Tris, pH 7.8, 2 mM CaCl\(_2\), 1 M NaCl at concentrations of 2 to 20 \( \times \) 10\(^6\) cells/ml and sonicated with a Branson Sonifier using a microtip attachment at power 3, 50% pulse cycle, 40 pulses in order to disrupt completely the plasma and perigranule membranes and to solubilize enzyme activity which was measured as previously described (1). Up to 50 \( \mu \)l of enzyme solution is added to 2 ml of 1 M TAMe, 40 mM Tris, pH 8.1, 10 mM CaCl\(_2\) at room temperature. The increase in absorbance at 247

* The abbreviations used are: TAMe, tosyl-L-arginine methyl ester; SDS, sodium dodecyl sulfate; DFP, diisopropyl fluorophosphate.

**Acknowledgments**

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nm is followed spectrophotometrically; ε = 540; 1 unit of enzyme activity converts 1 μmol of TAMe/min.

Mast cell sonicates were applied directly to columns of Dowex 1-X2 equilibrated in the sonication buffer (10 to 20 × 10^6 mast cell equivalents/ml of resin) and the column was washed with 3 volumes of the same buffer. Heparin-proteoglycan binds to Dowex 1-X2 under these conditions (7), whereas most of the protein is not bound. TAMe-esterase activity appears only in effluent fractions and these were combined and dialyzed against 10 mM Tris, pH 7.8, 2 mM CaCl_2 to less than 40 mM NaCl.

The dialyzed Dowex 1-X2 effluent was applied to A-25 equilibrated in 10 mM Tris, pH 7.8, 2 mM CaCl_2 (~1 mg of protein/ml of bed volume). The resin was washed with 5 column volumes of equilibration buffer. TAMe-esterase activity was eluted with a linear 0 to 1 M NaCl gradient in equilibration buffer. The gradient size was approximately 20 column volumes and gradient fraction volumes equivalent to 2 to 3% of the gradient volume were collected and assessed for NaCl concentration, protein content, and TAMe-esterase activity. Fractions containing about 70% of the enzyme activity were combined and dialyzed against 10 mM 2-(N-morpholino)ethanesulfonic acid, pH 6.2, 2 mM CaCl_2 until the NaCl concentration was less than 40 mM.

The dialyzed A-25 eluate was applied to heparin-agarose equilibrated in 10 mM 2-(N-morpholino)ethanesulfonic acid, pH 6.2, 2 mM CaCl_2 (~1 mg of protein/ml of bed volume). The resin was washed with 5 column volumes of equilibration buffer. TAMe-esterase activity was eluted with a linear 0 to 2 M NaCl gradient (20 column volumes) in equilibration buffer. Gradient fraction volumes equivalent to 2 to 3% of the gradient volume were collected and assessed for protein concentration, enzyme activity, and NaCl concentration and stored individually at −70 °C to maintain stable enzyme activity.

**Analytical Procedures**—Nondenatured preparations of tryptase were subjected to electrophoresis on polyacrylamide gels at room temperature. Samples for electrophoresis in acid, alkaline, 3% (w/v) sucrose and 5% (w/v) bromphenol blue, in the presence of 4 mM dithiothreitol and heated at 8.3°C for 3 min (9), at pH 4.3 in the presence of 4 mM urea. Electrophoresis was carried out in a walled-jacket disc gel electrophoresis unit from Buchler Instruments at 20 °C by application of 1 mA/tube until the dye front entered the gel. The dye front was sliced with a Bio-Rad Model Gel Slicer and scanned spectrophotometrically with a Gilford 2520 Gel Scanner at 600 nm.

**RESULTS**

*Purification of Human Lung Mast Cell Tryptase*—The mean level of TAMe-esterase activity per 10^6 mast cells in preparations of 20 to 80% pure human pulmonary mast cells was 1.1 ± 0.6 units (mean ± S.D., n = 10). The mean level of TAMe-esterase activity per 10^6 cells in preparations of 75 to 95% monocytes was 0.002 ± 0.003 units and in preparations of 80 to 95% eosinophils and 90 to 95% neutrophils was less than 0.003 units. Thus, quantities of TAMe-esterase activity in mast cells are more than 100-fold higher than those in human peripheral blood leukocytes.

**Table I**

| Protein        | Activity | Specific Activity | Yield |
|----------------|----------|------------------|-------|
|                | µg units | units/mg protein | %     |
| Sonicate       | 5,750    | 49               | 8.5   | 100 |
| Dowex 1-X2     | 5,100    | 52               | 10    | 106 |
| A-25           | 1,020    | 19               | 19    | 39  |
| Heparin-agarose|          |                  |       |     |
| Minor peak     | (Fractions 13–16) | 88               | 1.1   | 13  | 2   |
| Major peak     | (Fractions 27–37) | 210              | 16.3  | 78  | 33  |
| (Fractions 31–34) | 113             | 11               | 97    | 22  |
The purification of tryptase from a sonicated suspension of 40 \times 10^6 mast cells of 40% purity containing 49 units of TAME-esterase activity is summarized in Table I. The sonicate was applied to a column (1.1 cm \times 2.5 cm) containing Dowex 1-X2, and 52 units of enzyme activity were recovered in the effluent fractions along with about 90% of the protein. Dialyzed effluent fractions were subjected to chromatography on a column (1.1 cm \times 5.3 cm) of A-25. TAME-esterase activity was completely adsorbed to the resin at low ionic strength and eluted at 0.14 to 0.18 M NaCl in a single peak containing 26 units (Fig. 1). Fractions 25–31, containing 19 units, were combined, dialyzed, and subjected to chromatography on a column (1.1 cm \times 1.1 cm) of heparin-agarose. Enzyme activity was completely adsorbed and was eluted by the salt gradient in two peaks at about 0.08 and 0.27 M NaCl. The first peak, containing 1.1 units of enzyme, was observed in 1 of 2 other purifications and again represented less than 10% of the total enzyme activity. The second peak contained 16.3 units of enzyme activity and accounted for 86% of the 19 units applied to the column. In fractions 27–37, the dominant peak of enzyme activity co-eluted with the protein (Fig. 2); specific activities in the peak fractions (31–34) were not appreciably different from each other and averaged 97 \pm 10 units/mg (\pm S.D.). In fractions 14 and 15 with the highest activities of the minor peak, specific activities were 15 and 12 units/mg, respectively. As summarized in Table I, the final purification of the dominant TAME-esterase activity, termed mast cell tryp-, was about 11-fold and the final recovery (fractions 13–16 and 27–37) is 35%. Had all activity recovered from the A-25 column been subjected to heparin-agarose chromatography, a theoretical yield of about 48% should have been obtained.

Two additional tryptase purifications were performed from one-half of a preparation of 20 \times 10^6 mast cells (33% purity, 19 units). Actual final recoveries of activity were 10 and 14%, and when corrections were made for the enzyme discarded from the A-25 fractions, theoretical recoveries of 21 and 36% were obtained. Because of the small amounts and concentrations of protein in most of the final tryptase fractions (<16 \mu g/ml) of these two additional preparations, only a maximal estimate of protein could be made by the method of Lowry et al. (5). Values for the specific activity of purified tryptase were estimated to be greater than 61 units/mg and were analyzed for purity by gel electrophoresis (see below).

Subunit Structure of Mast Cell Tryptase—Purified tryptase obtained from the enzyme peak eluting with 0.75 M NaCl from the heparin-agarose column was assessed for purity by electrophoresis in polyacrylamide gels. Up to 6 \mu g of protein were subjected to electrophoresis in 6% polyacrylamide gels under nondenaturing conditions with the acid and alkaline pH systems without revealing a stained band of protein. To facilitate entry of tryptase into the gel, electrophoresis was performed in the presence of 4 M urea at pH 4.3 using 7.5% polyacrylamide as shown in Fig. 3. One band of protein was observed at an Rf of 0.4, indicating that the tryptase was apparently homogeneous.

Electrophoresis of purified tryptase was also performed under denaturing conditions in the presence of SDS in gels of 12.5% polyacrylamide. With both unreduced (Fig. 4A) and reduced (Fig. 4B) enzyme from all three consecutive tryptase preparations, two major bands of protein with distinct electrophoretic mobilities were resolved. The molecular weights of the two subunits were 37,000 \pm 600 (mean \pm S.D., n = 6) for the slower migrating band and 35,000 \pm 500 (mean \pm S.D., n = 6) for the one with greater electrophoretic mobility under nonreducing conditions. The molecular weights were not appreciably different with reduction. Molar ratios of the two subunits were 1:1 as indicated by the relative peak heights of the two subunits.

Electrophoresis under denaturing conditions was also performed with protein from the minor peak of enzyme activity eluted from heparin-agarose with 0.08 M NaCl. Prominent protein bands at positions of 37,000 M, and 35,000 M, were detected with a molar ratio of about 1:3. Additional bands at higher and lower molecular weight positions were also observed and indicated the presence of contaminant proteins.

In order to determine which tryptase subunits have active sites, two experiments were performed in which a 10-fold molar excess of [H]DFF was incubated for 1 h at room temperature with purified tryptase by which time greater than 90% inhibition of enzyme activity had occurred. The mixtures were then dialyzed against 19 changes of 1 liter of 10-fold diluted SDS electrode buffer over 36 h, lyophilized

![Fig. 3. Polyacrylamide gel electrophoresis at pH 4.3 in the presence of 4 M urea of human mast cell tryptase (fraction 30, Fig. 2) containing 8 \mu g of protein and 0.5 units of enzyme activity.](image-url)
Purification of Human Lung Mast Cell Tryptase

treated human mast cell tryptase. Shown in Fig. 2 and consisting of protein at 35,000 lower molecular weight subunit which was not apparent before the DFP experiment. A minor band of protein was seen in Fig. 2 at 45,000 molecular weight standards are marked.

**Amino Acid Analysis**—The average amino acid composition of tryptase from two 24-h timed hydrolysates is shown in Table II. The number of amino acid residues per mol of tryptase was calculated to be 1,163 based on the molecular weight of each amino acid and on a molecular weight of 144,000 for the holoenzyme. The amount of protein applied in each of two analyses (10.2 μg) was essentially completely recovered in the combined amino acid residues (10.3 and 10.8 μg). Because of limiting quantities of sample, separate analyses for determination of cysteine and tryptophan were not performed; thus, the value of 3 mol of cysteine/mol of tryptase is a minimum value. For the same reason, analysis of 48- and 72-h timed hydrolysates also were not performed.

**DISCUSSION**

Characterization of the dominant neutral protease (chymase) of purified rat serosal mast cells has been facilitated by the availability of this cell type (12-14), but characterization of the neutral protease of human mast cells required the development of techniques for the dispersion and purification of mast cells from solid tissue (6), and even then has been limited by the supply of human lung obtained during pulmonary surgery. The enzyme responsible for the TAME-esterase activity of human pulmonary mast cells, referred to as tryptase (1), was purified to homogeneity by sequential chromatography on Dowex 1-X2, A-25 (Fig. 1) and heparin-agarose (Fig. 2). From 40 × 10⁶ mast cells of 40% purity the yield was 35% and the specific activity was 97 units/mg of protein (Table I). Criteria for the purity of tryptase included a constant ratio of enzyme activity to protein in the peak fractions of enzyme eluted from the heparin-agarose column (Fig. 2, fractions 31-34) and a single protein band on a polyacrylamide gel after electrophoresis of tryptase at pH 4.3 in the presence of 4 M urea (Fig. 3).

Tryptase has been localized to mast cell secretory granules by its release from pulmonary mast cells after IgE-dependent activation in a fixed ratio to histamine of 0.78 (1). Appreciable amounts of TAME-esterase activity in contaminant cells of the pulmonary tissue were not apparent, because mast cell purity had no significant effect on the fraction of TAME-esterase activity available for release. The tryptase activity extracted from mast cells is accounted for by one predominant enzyme as indicated by the elution of one peak of activity from a G-100 column (1), by the resolution of one predominant peak of activity after separation from heparin and chromatography on columns of A-25 (Fig. 1) and heparin-agarose (Fig. 2), and by the presence of one band of protein after electrophoresis of purified tryptase into a polyacrylamide gel at pH 4.3 in the presence of 4 M urea (Fig. 3). Holoenzyme degradation was observed by SDS-gel analysis if the purified enzyme was preincubated at room temperature and probably accounts for the minor peak of TAME-esterase activity eluting from heparin-agarose at 0.08 M NaCl. Thus, tryptase activity resides predominantly in mast cells and is accounted for by a single enzyme. Based on the specific activity of purified tryptase for TAME-esterase activity, levels per 10⁶ mast cells of about 1.1 ± 0.6 units, 6 to 19 μg of tryptase are present in 10⁶ mast cells. If one assumes equal contributions of protein from the different cell types in the 40% pure mast cell preparation, the tryptase which was purified 11-fold accounts for about 23% of the mast cell protein. This situation is analogous to that with the rat mast cell, where chymase, the predominant neutral protease of that cell, accounts for about 24 μg of protein/10⁶ mast cells or 21% of the cell protein (12). TAME-esterase levels in mast cells are more than 100-fold higher than those in human neutrophils, eosinophils, and monocytes. The high neutral protease level in mast cells also contrasts to the neutral protease levels in 10⁶ human neutrophils which contain less than 1 μg of elastase and cathepsin G (15, 16).

Analysis of the subunit structure of purified tryptase by SDS polyacrylamide gel electrophoresis revealed equal amounts of two protein bands with apparent molecular weights of 45,000 and 47,000. SDS-polyacrylamide gel electrophoresis revealed equal amounts of two protein bands with apparent molecular weights of 45,000 and 47,000.
weights of 37,000 and 35,000 with and without reduction and alkylation (Fig. 4). Because of its apparent molecular weight of 120,000 to 240,000 by gel filtration (1), the holoenzyme is considered to have a tetrameric subunit structure composed of two 37,000 and two 35,000 M₀ subunits. When the tryptase was incubated with [³H]DFP and analyzed by SDS-polyacrylamide gel electrophoresis, each of the distinct subunits contained ³H (Fig. 5), the ratio of larger to smaller molecular weight subunits being 1.5 and 0.96 in two experiments. This variability in part may reflect cross-contamination in the gel slices of one subunit with the other, but clearly indicates the capacity of each subunit to bind DFP. Molar amounts of DFP bound to a mole of holoenzyme averaged 4.9 based on spectrophotometric calibration of Coomassie brilliant blue-stained protein bands and averaged 1.5 based on the Lowry et al. (5) assay of protein placed into the enzyme DFP incubation mixture. The marked difference in the molar ratios of DFP to tryptase is due to the different values of protein obtained by different techniques at separate stages of the experiment, and may reflect both preferential binding of Coomassie brilliant blue to the bovine serum albumin rather than to the tryptase in the gels and incomplete recovery of protein after preparation of the DFP-treated tryptase for SDS-polyacrylamide gel electrophoresis. However, based on the correspondence of protein and ³H in the gels, the 35,000 and 37,000 M₀ subunits each bind DFP, indicating that both subunits of the tetrameric tryptase have active site capability.

In preliminary amino acid composition analysis of purified tryptase, 17 amino acids were detected (Table II). The value for cysteine is a minimum and tryptophan was not assessed. Of the 1,163 amino acid residues detected/mol of tryptase, 251 residues were acidic, 103 residues were basic, 415 residues had nonpolar aliphatic side chains, 99 residues contained aromatic rings, 277 residues consisted of serine, glycine, and threonine, and 18 residues contained sulfur.

Tryptase bound to heparin-agarose so as to require 0.75 M NaCl for elution, indicating the likely association of tryptase with heparin proteoglycan at the presumably lower ionic strengths in the mast cell secretory granule, which also contains histamine and several acid hydrolases (1). In rat mast cells, the neutral proteases chymase and carboxypeptidase A also bind tightly to heparin proteoglycan and remain cell-associated after their secretion in vitro, and presumably remain concentrated at their site of release in vivo (17). Human mast cell heparin proteoglycan (60,000 M₀) (7) appears to be smaller than that of the rat (750,000 M₀) (18), whereas the tryptase is larger than the rat chymase (25,000 M₀) (13). The contribution of tryptase-heparin complexes to the crystalline structures unique to human mast cell secretory granules (6, 19), as compared to the amorphous rat granules (12, 20-22), has been suggested (1) but not proven. The dominant neutral protease of rat skin has recently been defined as rat mast cell chymase (23). Mast cell concentrations in normal human skin are 7,000 to 12,000 cells/mm³ (24, 25) and, if their content of tryptase is similar to that of pulmonary mast cells, there would be appreciable quantities of tryptase (0.15 µg/mm³) in this tissue. A trypsinic activity has been reported in human skin which may be the mast cell tryptase, because it also has an apparent molecular weight of 120,000 and is not inhibited by α-trypsin inhibitor (26). The high levels of tryptase found in human mast cell secretory granules, and the localization of mast cells in connective tissue around blood vessels and airways and at mucosal surfaces, suggests a purposeful role for the tryptase at these sites.

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