A chymotrypsin-like proteinase was purified 2400-fold from human skin. The procedure involves extraction of the proteinase from skin in 2 M KCl, precipitation with protamine chloride, fractionation by gel filtration chromatography, and fractionation by chromatography using a CH-Sepharose-n-tryptophan methyl affinity column. The properties of this proteinase were compared to the rat mast cell proteinase I and human cathepsin G. Differences were observed in the rates at which the proteinases were inhibited by diisopropyl fluorophosphate, the sensitivity of the proteinases to protein proteolytic inhibitors, the relative hydrolytic rates of the proteinases for a series of substrates, and the kinetic constants of the proteinases for synthetic substrates. The human skin proteinase did not react with antiserum to the rat skin proteinase and did not elute in the same position as the rat skin proteinase on gel filtration columns. These data demonstrate that the human skin proteinase is distinct from the other proteinases. Extracts of involved skin from patients with cutaneous mastocytosis had 15-fold higher levels of chymotryptic activity than extracts of uninvolved skin or skin from normal controls. The enzymatic properties of the material extracted from the biopsied skin were similar to those of the proteinase from normal skin, suggesting that the human skin chymotrypsin-like proteinase is a mast cell constituent.

High ionic strength extracts from rat (1, 2) and human (1, 3) skin contain high levels of chymotrypsic activity. Seppä and Järvinen (2, 4) have shown that the high salt extract from rat skin has one predominant chymotrypsin-like proteinase which is located exclusively in the cytoplasmic granules of dermal mast cells. Immunologically, the proteinase is identical to rat mast cell proteinase I (4, 5). Mast cells are a constituent of the skin of humans and other mammals (6), but except for the rat, no other mast cell chymotrypsic proteinase has been isolated and characterized. Chymotrypsin-like proteinases have been solubilized from mastocytoma tissue of dogs and mice (7), and human skin proteinase is a mast cell constituent.

The purpose of this study was to purify the human skin chymotrypsic-like proteinase and to determine whether it is similar or identical to other cellular chymotrypsin-like proteinases. The proteinases studied include the rat skin proteinase, rat mast cell proteinase I (15), and human cathepsin G (16-19), the chymotrypsin-like proteinase of human polymorphonuclear leukocytes. Chymotrypsic activity in patients with cutaneous mastocytosis, a condition characterized by increased numbers of mast cells within the skin, was also examined to determine whether the skin chymotrypsin-like proteinase is a mast cell constituent.

**EXPERIMENTAL PROCEDURES**

**Materials**—Proteins for molecular weight standards, Bz-Tyr-OEt, Ac-Tyr-OEt, casein, phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, lima bean trypsin inhibitor, hen ovoinhibitor (type IV-O), turkey ovomucoid (type II-T), trypsin inhibitor, and Ac-AAPV-Lys-Phe-amidase III were obtained from either Sigma or Calbiochem. Bovine pancreatic trypsin inhibitor was purchased from Worthington. Unlabeled DFP was from Aldrich. [1,3-14C]DFP (100 mCi/mmol) and [3H]acetic anhydride (100 mCi/mmol) were from New England Nuclear, and [3H]DFP (6.5 Ci/mmol) was from Amersham. Type XR-5 x-ray film was supplied by Eastman Kodak and the autoradiographic image enhancer, Alphaphor, was obtained from National Diagnostics. Pattern D immunodiffusion plates were from Hyland Diagnostics.

**Purification of the Human Skin Chymotrypsic-like Proteinase**—Chymotryptic and trypsinic activities were quantitated by measuring the hydrolysis of 10 mM Ac-Tyr-OEt and 10 mM N-benzyloxycarbonyl-L-arginine methyl ester according to the method of Hestrin (20). Elastolytic activity was assayed by measuring the hydrolysis of 1.5 mM N-succinyl-t-alanyl-t-alanyl-t-alanyl-p-nitroanilide at 410 nm. Carboxypeptidase D activity was measured by quantitating the hydrolysis of radioactive hemoglobin at pH 4.7, and neutral proteolytic activity was determined by measuring the hydrolysis of radioactive casein as described by Levine et al. (21). Protein concentration was measured by the method of McGuire et al. (22). Skin was obtained from breast reductions and leg amputations. Specimens were cleaned of subcutaneous tissue by scraping with a scalpel, washed with cold saline, blotted, weighed, and then stored at -20°C. Preparations used about 700 g of skin which was minced with scissors, and then extracted by the two-step method.
Chymotrypsin-like Proteinase from Human Skin

Feiki and Hopsu-Havu (3). Skin (1 g of tissue/10 ml of solution) was frozen and thawed five times in 0.1 M potassium phosphate, pH 7.0 (low salt buffer), collected by filtration through a 50-mm bone, rinsed and extracted by stirring overnight in 2.0 M KCl, 0.01 M sodium phosphate, pH 7.0 (high salt buffer), at a concentration of 1 g of tissue/5 ml of solution. The 2.0 M KCl extract was clarified by centrifugation at 10,000 rpm for 10 min in a Beckman JA-10 rotor. Further purification of the human skin, cathepsin-like proteinase was achieved by the precipi-
tation of the enzyme from the high salt extract. Dialysis of the extract against low salt buffer resulted in the precipitation of 30% of the activity and the addition of 0.025% protamine chloride resulted in the precipitation of the remainder of the activity. Precipitated material was washed with 0.5 M KCl and dissolved in 1.2 M KCl, 0.01 M sodium phosphate, pH 7.0, at 4 °C. Elution of proteinases was monitored by following the hydrolysis of Bz-Tyr-OEt. Fractions containing 1 to 3 g of solution were collected and the flow rate was about 10 g of solution/h. Prior to the analyses the column was calibrated with the following proteins: bovine serum albumin (66,000), hemoglobin (64,500), ovalbumin (43,000), and cytochrome c (14,000). DFP DNA was used to measure the coefficient of friction (V), of each protein and proteinase was determined from the relationship:

\[ V = V_0 - V_0/V - V_0 \]

where \( V \) is the elution volume of each protein.

Inhibition of Proteinases by DFP—Inhibition rates were measured at 37 °C, in a solution containing DFP, 10% propylene glycol, 1.5 mM Bz-Tyr-OEt. The time required for each inhibition study was less than 0.1 μM. After the addition of DFP, 0.1 ml aliquots were removed from incubations at the indicated times, diluted with 0.9 ml of substrate solution and assayed spectrophotometrically as described previously with Bz-Tyr-OEt. Each inhibition study was less than 0.1 ml, and the length of time required to inactivate 50% (\( t_{1/2} \)) of the proteinase. Inhibition rates were standardized by measuring the rate of chymotrypsin inactivation. A 0.02 mM DFP solution inhibited chymotrypsin 50% in 5 min at 37 °C.

Radioactive Labeling of Proteinases—Samples from gel filtration columns were concentrated using Aqueaide III to obtain solutions of high activity. Either \( [3H]DFP \) (6.5 Ci/mmol) or \( [14C]DFP \) (100 Ci/mmol) was incubated with proteinase samples at 37 °C in 0.1 M solution containing 2 M KCl, 0.01 M Tris-HCl, pH 8.0, and 30–50% propylene glycol. The concentration of DFP was between 0.6 and 0.3 mM, and incubations were between 1.5–2.5 h. Reactions were stopped by the addition of 0.05 ml of 0.2 M unlabeled DFP. Unbound DFP was removed by exhaustive dialysis against 0.9% NaCl, 0.01 M sodium phosphate, pH 7.0, followed by precipitation of protein in 6% perchloric acid. To ensure quantitative precipitation, 0.01 ml of carrier proteins (25% bovine serum albumin, ovalbumin, and lysozyme) was added to the dialyzed proteinase solution prior to the addition of perchloric acid. Precipitated material was collected by low speed centrifugation and solubilized in 100–250 μl of a solution of 2.5% SDS, 5% dithiothreitol. The amount of bound DFP was then quantitated by scintillation counting. The precipitation step was also required to remove an apparent contaminant which affected the resolution of protein bands on SDS gels. We suspect contamination occurred during the concentration of samples in Aqueaide III.

SDS-Polyacrylamide Gel Electrophoresis—Radioactive samples solubilized from perichorial acid precipitates were neutralized with 2.0 M NaOH, heated to 100 °C for 5 min, and dialyzed against a solution of 2.5% SDS, 10 mM dithiothreitol, 0.02 M Tris-HCl, pH 8.0. The proteinase was then analyzed by SDS-polyacrylamide slab gel electrophoresis as described by Anderson et al. (29). The running gel contained 17.5% acrylamide and 0.8% bisacrylamide. Proteins used for calibration standards were phosphorylase (100,000), bovine serum albumin (97,000), catalase (60,000), ovalbumin (43,000), bovine pancreatic trypsin inhibitor (25,000), soybean trypsin inhibitor (21,500), and lysozyme (14,000). Gels were stained with Coomassie brilliant blue, destained in a solution of 14% methanol, 7% acetic acid, impregnated with fluor (National Diagnostics Auto-
fluor) and dried under vacuum at 25 °C. Dried gels were then placed at −70 °C for 1–3 days and left at −20 °C for 1–3 days. The gel was placed in 0.1 M Tris-HCl, pH 6.8, and the proteinase was eluted from the gel. SDS-polyacrylamide gel analyses of unlabeled protein inhibitors were performed in the high ionic strength condi-
tions described for enzymatic assays and residual activity was measured by a 10-min incubation period by following the hydrolysis of Bz-Tyr-OEt. Inhibitor concentrations reported are those in the incubations prior to measurement of residual activity, and they represent the sum of bound and unbound inhibitor. Dilution due to the assay procedure was 1.4-fold. Molarity of inhibitors were calculated from the molar extinction coefficients of Bz-Tyr-OEt, bovine pancreatic trypsin inhibitor (26,000), bovine pancreatic trypsin inhibitor (25,000), corn trypsin inhibitor (6,500), lima bean trypsin inhibitor (10,000), and ovo inhibitor (46,500).

Analytical Gel Filtration Chromatography—Apparent molecular weights of the proteinases were determined on Sephadex G-150 (1.5 cm x 90 cm) in 1.2 M KCl, 0.01 M sodium phosphate, pH 7.0, at 4 °C. Elution of proteinases was monitored by following the hydrolysis of Ac-Tyr-OEt. Fractions containing 1 to 3 g of solution were collected and the flow rate was about 10 g of solution/h. Prior to the analyses the column was calibrated with the following proteins: bovine serum albumin (66,000), hemoglobin (64,500), ovalbumin (43,000), and cytochrome c (14,000). DFP DNA was used to measure the coefficient of friction (V), of each protein and proteinase was determined from the relationship:

\[ V = V_0 - V_0/V - V_0 \]

where \( V \) is the elution volume of each protein.
Proteinase were performed in tubes by the method of Weber and Osborn (30).

**Molar Specific Activities**—Specific activities based on the molarity of each proteinase were determined by correlating hydrolytic activity of a sample with the molarity of the proteinase. Standardizations for skin proteinases were performed on material purified by the method of Schmidt and Havemann (16). Hydrolytic activity was measured using 0.5 mM Bz-Tyr-OEt as substrate, and the concentration of proteinase was determined by radioactive DFP labeling as described above. The length of incubation time was such that >98% of the proteinase was inhibited. Moles of proteinase were calculated from bound radioactivity using the specific activities for each isotope. The efficiency for quantitating \(^3\)H by scintillation counting was 38%, and the efficiency for quantitating \(^14\)C was 90%. In a 1-ml assay performed under the conditions described in the previous section, 52 pmol of the rat skin proteinase, 13.4 pmol of the human skin proteinase, and 80 pmol of cathepsin G each produced a hydrolysis rate of 0.1 absorbance unit (256 nm)/min.

**Double Immunodiffusion Studies**—Immunodiffusion plates were equilibrated with 2 M KCl, 0.01 M sodium phosphate, pH 7.0, prior to use. Phenylmethylsulfonyl fluoride-inhibited proteinase samples (6 \(\mu\)l) in high salt buffer were placed in each well and allowed to diffuse against antiserum for 48-48 h at 25 °C. The gel was then washed with saline, washed with distilled water, dried onto a glass slide, and stained for 10 min with 0.1% Amido black dissolved in a solution of 5% methanol, 10% acetic acid. Unbound dye was removed by washing for 15 min in the above methanol/acetic acid solution. Antiserum to rat skin chymotrypsin-like proteinase was a generous gift from Dr. Hekki Seppa, University of Oulu, Finland. Lyophilized antiserum was equilibrated with 2 M KCl, 0.01 M sodium phosphate, pH 7.0. Antiserum only reacted with the rat skin proteinase over the concentration range of 0.1-0.9 \(\mu\)M (for these studies rat skin proteinase was purified to homogeneity) and not with the human skin proteinase (0.6 \(\mu\)M) obtained from proteinase-prepurified material or that obtained (0.4 \(\mu\)M) from dialysis-precipitated material.

**Studies of Patients with Cutaneous Mast Cell Disease**—Four patients with cutaneous mastocytosis (one child, three adults) were studied after informed consent was obtained. All patients were diagnosed clinically (by G. S. L.), and after Xylocaine anesthetic 6-mm punch biopsies from involved and uninvolved skin were obtained. Sections of each biopsy were processed for histological examination including staining with toluidine blue. Involved skin demonstrated classic mast cell infiltration, whereas uninvolved skin was histologically normal. Biopsies from 30 normal patients served as controls. For proteinase analysis biopsies were blotted, weighed, and then extracted by the method of Fraki and Hopau-Havu (3) as described previously. Chymotryptic activity and protein measurements in the extracts were performed by previously described methods.

**RESULTS**

**Purification of the Human Skin Chymotrypsin-like Proteinase**

The purification method is described in Table I. Dialysis of the high salt skin extract against low ionic strength buffer results in the separation of chymotryptic activity into two fractions: a low salt insoluble fraction and low salt soluble fraction that precipitates in the presence of protamine. The data to be presented subsequently indicate that the proteinases in both fractions are the same catalytic entity. Gel filtration of the precipitated material in both fractions results in the elution of a single peak of hydrolytic activity at a position corresponding to \(M_w=28,000\). Skin chymotrypsin-like proteinases bind strongly to a CH-Sepharose-D-tryptophan methyl ester affinity column (Fig. 1) and can be eluted with free D-tryptophan methyl ester. The proteinase at this stage of the purification is 25–60% pure based on the comparison of the specific activity of preparations with that determined by labeling with radioactive DFP (see Table III). Removal of minor high and low molecular weight contaminants leading to preparations routinely 60–80% pure is accomplished by further fractionation on a Sephadex G-100 column. Human skin chymotrypsin-like proteinase preparations had no detectable tryptic, elastolytic, or cathepsin D-like activity. The hydrolysis of casein, a nonspecific proteinase substrate, was inhibited over 95% by DFP, indicating that the preparations were not contaminated with nonsensitive class proteinases capable of hydrolyzing casein.

Chymotrypsin-like proteinases obtained from Sephacryl S-200 chromatography were labeled with radioactive DFP and analyzed by SDS-slab gel electrophoresis as shown in Fig. 2a. One broad band with \(M_w=30,000\) is the major radioactive component observed in proteinase preparations obtained from both the dialysis (DP)- and protamine (PP)-precipitated fractions. Human polymorphonuclear leukocyte cathepsin G and the rat skin chymotrypsin-like proteinase purified as described under "Experimental Procedures" are shown in the tracks G and R, respectively. SDS gel analyses of the proteinases in the most highly purified preparations were performed by the method of Weber and Osborn (30) as shown in Fig. 2b. The Weber and Osborn method was employed as an alternative method of analysis to determine whether the diffuse banding pattern was the result of the discontinuous buffer system used in slab gel electrophoresis. Gels stained with Coomassie brilliant blue show broad bands at \(M_w=30,000\), consistent with that observed for radioactively labeled proteinases. The high molecular weight bands are contaminants, since they were not labeled with DFP.

The diffuse banding pattern is a quality that has been observed in most SDS-polyacrylamide gel analyses of the human skin proteinases; the reason for this behavior has not been established. Kinetic and inhibition studies indicate there is only one catalytic entity in the preparations, however. Lineweaver-Burk plots to determine kinetic constants were

**TABLE I**

| Fractionation step | Increase in specific activity | % recovery* |
|--------------------|-------------------------------|------------|
| 1. 2.0 M extract   | 1                             | 100        |
| 2. Precipitation   |                               |            |
| A. Dialysis        | 7.5                           | 31         |
| B. 0.025% Protamine| 5.0                           | 60         |
| 3. Sephacryl S-200 |                               |            |
| A. Dialysis precipitated | 30                             | 23         |
| B. Protamine precipitated | 20                             | 45         |
| 4. CH-Sepharose-D-tryptophan methyl ester column | | |
| A. Dialysis precipitate | 2400                           | 13         |
| B. Protamine precipitate | 2400                           | 25         |

*Recoveries reported are relative to the amount of proteinase in high salt extract.
of the inhibition were linear until greater than 75% of the studies and the inhibition rate of the proteinase by DFP followed pseudo-first order kinetics (Fig. 3). Logarithmic plots linear, having correlation coefficients greater than 0.99 in most cases of the inhibition with nonserine class proteinases.

Further differences between the three proteinases are shown in Table III, where kinetic constants and inhibition properties are compared. The human skin proteinase hydrolyzed the synthetic substrates Bz-Tyr-OEt and Ac-Tyr-OEt more efficiently than either the rat skin proteinase or cathepsin G as evidenced by the higher $k_{cat}/K_m$ ratios and the higher specific activity for Bz-Tyr-OEt. The $K_m$ values obtained for the human, rat, and cathepsin G proteinases were 1.1, 1.0, and 3.4 mM, respectively, when Bz-Tyr-OEt was the substrate, and 1.8, 2.0, and not measured when Ac-Tyr-OEt was the substrate. The human skin proteinase is the least efficient in hydrolyzing casein.

Inhibition Properties—In Fig. 4, the inhibition rates of the three chymotrypsin-like proteinases by DFP are compared and the $t_{1/2}$ values are reported in Table III. The human skin proteinase is inhibited 3-fold more slowly by DFP than the rat skin proteinase and cathepsin G. Also, the human skin proteinases purified from the dialysis- and protamine-precipitated material have identical inhibition rates (Fig. 4). The inhibition of the proteinases by a series of proteinolytic inhibitors is compared in Table III. The patterns of inhibition obtained for the rat skin proteinase and the human skin proteinase were similar. Both differed markedly from cathepsin G, which was sensitive to all the inhibitors. Bovine pancreatic chymotrypsin was inhibited greater than 90% by all the proteinolytic inhibitors.

Comparison of Physical Properties—The differences in the banding patterns of the human skin proteinase with the rat skin proteinase and cathepsin G on SDS gels suggests structural differences between the proteases. Analysis of the

![Figure 2](http://www.jbc.org/)  
**Figure 2.** SDS-polyacrylamide slab gel electrophoresis of proteinases labeled with radioactive DFP (a) and SDS-polyacrylamide gels of the most highly purified preparations of the human skin proteinases (b). a, composite of several autoradiographs in which bands were aligned at their approximate positions relative to standards. Tracks DP and PP are of human skin chymotrypsin-like proteinases purified from dialysis- and protamine-precipitated material, respectively. Track G represents cathepsin G and track R represents rat skin proteinase. Carbonic anhydrase ($M_r = 30,000$), and $\alpha$-chymotrypsinogen ($M_r = 25,700$) had the same migration rate. b, about 7 $\mu$g of protein were analyzed on each gel and the bands were stained with Coomassie brilliant blue. Gel S represents the migration of standard proteins. Gels DP and PP were from different analyses, but in both cases the major band migrated as a polypeptide chain with $M_r = 30,000$.

**Table II**

| Substrate    | Conc  | Human—PP | Human—DP | Rat skin | Cathepsin G |
|--------------|-------|----------|----------|----------|-------------|
| Bz-Tyr-OEt   | 0.5 mM| 1.0      | 1.0      | 1.0      | 1.0         |
| Ac-Tyr-OEt   | 1.2 mM| 5.8      | 5.9 (±0.5) | 2.0 (±0.3) | 0.6         |
| Casein       | 2.2 mg/ml | 0.1 (±0.1) | 0.1 (±0.1) | 1.5 (±0.2) | 1.4         |

* PP, protamine-precipitated fraction; DP, dialysis-precipitated fraction.
* Parentheses represent the range of two determinations which were the average of measurements at two different substrate concentrations.

**Figure 3.** Inhibition of the human skin chymotrypsin-like proteinase by DFP. Incubations with 0.4 mM DFP (■) and 0.8 mM DFP (●) were performed at 37 °C. Further conditions and the method for measuring residual enzymatic activity are described under "Experimental Procedures." Proteinase concentration was 35 nM in the former study and 90 nM in the latter study.
human and rat skin proteinases by gel filtration chromatography also suggests a marked difference in molecular weight and/or shape (Fig. 5). Schmidt and Havemann, (16) have shown that cathepsin G migrates as a broad band under similar chromatographic conditions indicating it may be undergoing a self-aggregation process. Cross-reaction was not observed between the rat and human skin proteinases by double immunodiffusion analysis using antiserum to the rat skin proteinase (data not presented). Cross-reaction was also

![Table III](https://www.jbc.org/)

| Enzymatic properties                  | Human skin | Cathepsin G | Rat skin |
|---------------------------------------|------------|-------------|----------|
| Specific activity (10⁻³) (units/μmol) | 7.7        | 1.3         | 2.0      |
| kcat/Km ratio (s⁻¹)                   |            |             |          |
| Bz-Tyr-ΟΕτ                            | 3.4 × 10⁵  | 0.5 × 10⁵   | 1.0 × 10⁶ |
| Ac-Tyr-ΟΕτ                            | 8.0 × 10⁵  | Poor hydrolysis | 0.8 × 10⁶ |
| Casein hydrolysis (μg casein/min/μmol) | 700        | 1750        | 2800     |

Inhibition Properties

| t₁/₂ 0.4 mM DFP (min) | 28 | 8 | 10 |
|-----------------------|----|---|----|
| Protein proteolytic inhibitors (% inhibition) | | | |
| Lima bean trypsin inhibition (4.0 μM) | 90 | 90 | 90 |
| Soybean trypsin inhibitor (15 μM) | 50 | 90 | 70 |
| Bovine pancreatic trypsin inhibitor (10 μM) | 0 | 85 | 0 |
| Hen ovoinhibitor (6.0 μM) | 0 | 55 | 0 |
| Turkey ovomucoid (0.15 mg/ml) | 0 | 13 | 0 |

* Measured using 0.5 mM Bz-Tyr-ΟΕτ to measure activity, and radioactive DFP labeling to determine concentration. Specific activity of the human proteinase is the mean of nine determinations (S.D. = 1.0). The specific activity of cathepsin G is the mean of two determinations (range 0.0). Specific activity of the rat skin proteinase is the mean of five determinations (S.D. = 0.2).

* Range of error between determinations at different enzyme concentrations was less than 10%.

* Assay performed in 60 μl of high salt solution containing 2.2 μg/ml of casein radioactively labeled with [³H]acetic anhydride.

![Fig. 4](https://www.jbc.org/)

**Inhibition of rat and human skin chymotrypsin-like proteinases and cathepsin G by DFP.** All reactions were performed at 37°C in 0.4 mM DFP. Further incubation conditions and the method of measuring residual activity are described under "Experimental Procedures." The activity of controls (data not shown) did not change with the time of incubation. ●, human skin proteinase precipitated by protamine; ○, human skin proteinase precipitated by dialysis; ■, rat skin proteinase; Δ, cathepsin G.

![Fig. 5](https://www.jbc.org/)

**Gel filtration chromatography of the rat and human skin chymotrypsin-like proteinases.** ●, elution of proteins used for calibration. In order of increasing Ka is bovine serum albumin, hemoglobin, ovalbumin, and lysozyme. ○ marks the elution of the human skin proteinase, and the error bar indicates S.D. for three determinations. Human preparations analyzed include proteinase precipitated by dialysis, proteinase precipitated by protamine, and a sample representing both proteinases obtained by the concentration of the high salt extract. ▲ marks the elution of the rat proteinase and the error bar indicates the range for two determinations. Rat skin proteinase was the first and last sample analyzed on the column, showing the stability of the calibration profile.

**Table IV**

| Enzymatic and inhibition properties of chymotrypsin-like proteinases from human skin, human mastocytoma tissue, and rat skin |
|---------------------------------------------------------------|
| Chymotrypsin-like proteinases                                  |
| Human skin mastocytoma extract                                |
| Human skin                                                     |
| Rat skin                                                       |
| Ratio: casein/Ac-Tyr-ΟΕτ hydrolysis*                           |
| t₁/₂ inhibition (min)*                                         |
| 0.80 mM DFP                                                    | 17.5 (±1.0) | 19.0 (±0.0) | 7.0 |
| 0.40 mM DFP                                                    | 37.5        | 54.0        | 12.0 |

* Values in parentheses are the S.D. of the ratios obtained for three patients and for three normal skin samples. Casein hydrolysis was measured at a substrate concentration of 2.2 μg/ml and Ac-Tyr-ΟΕτ hydrolysis was measured at a substrate concentration of 1.0 mM.

* Data obtained from one mastocytoma patient and values in parentheses are the range of duplicates. Hydrolytic activity was measured using the substrate Ac-Tyr-ΟΕτ.

**Localization of the Chymotrypsin-like Proteinase in Skin**

Cutaneous mastocytosis is an uncommon condition manifested by the presence of increased numbers of mast cells within the skin. Biopsies from four patients having this condition were examined. The hydrolysis rate of Ac-Tyr-ΟΕτ per gram of tissue in the high salt extracts from this skin were 15-fold higher (patients = 4, mean = 480 activity units, range = 190–800) than that found in extracts of uninvolved skin or skin from normal patients (mean = 32, range 0–42). The kinetic properties of the "proteinase" solubilized from three patients having the highest hydrolytic activity were nearly identical to the chymotrypsin-like proteinase from normal human skin and different from those of the rat skin proteinase (Table IV). Chymotryptic activity in the mastocytoma extracts was not inhibitable by bovine pancreatic trypsin inhibitor. This observation coupled with the slow inhibition rate of

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1. C. Reilly and J. Travis, personal communication.
proteinase by DFP demonstrates that the proteinase in the mastocytoma extracts is not polymorphonuclear leukocyte cathepsin G. These data suggest that the normal human skin chymotrypsin-like proteinase is a mast cell constituent. There appeared to be some correlation between the degree of mast cell infiltration established histologically and proteinase concentration in our specimens; however, such correlations are difficult to establish in a small number of biopsies.

Consistent with the above observation, most of the chymotryptic activity of skin was localized to the dermal layer of skin where mast cells are located. This was accomplished by separation of freshly obtained skin into dermal and epidermal components using 2 M KCl as described by Levine et al. (17), followed by the extraction of each layer as described under "Experimental Procedures." About 90% of the hydrolytic activity for Ac-Tyr-OEt found in the whole skin control was present in the 2 M KCl extract of the dermal component.

DISCUSSION

The 2 M KCl extract of human skin contains one major chymotrypsin-like proteinase which was purified 2400-fold. The proteinase appears to be a constituent of mast cell granules as evidenced by the elevated level of the proteinase in extracts of skin obtained from patients with cutaneous mastocytoma. The enzymatic and physical properties of the human skin proteinase were only 25% as active as the rat skin proteinase which was purified 2400-fold. The concentration of the human skin chymotrypsin-like proteinase in hydrolyzing denatured casein, a nonspecific substrate, is about 0.2-2 molecules of the human skin proteinase/mast cell. This value is 5-50-fold less than reported for the rat mast cell proteinase I (37). Schwartz et al. (13, 38) have found that isolated human lung mast cells contain a trypsin-like proteinase which is present at a concentration of approximately 10^6 molecules (M, 35,000)/mast cell. Chymotrypsin-like proteolytic activity was reported in their mast cell preparations, but the catalytic properties of this proteinase were not examined. Degranulation of lung mast cells by treatment with rabbit IgG anti-human IgE led to the solubilization of trypsin-like activity but not chymotrypsin-like activity, suggesting that the chymotrypsin-like proteinase of human mast cells may not be analogous to rat mast cell proteinase I.

The human skin chymotrypsin-like proteinase and cathepsin G are serine proteinases produced in different tissues of the same species. Cathepsin G is a constituent of human polymorphonuclear leukocytes and the human skin chymotrypsin-like proteinase appears to be a constituent of mast cells. The differences in catalytic and inhibition properties presented in this study demonstrate that both proteinases are different genetic products. The relationship between chymotrypsin-like proteinases of skin and polymorphonuclear leukocytes has not been reported for any other species.

The physical and kinetic dissimilarities between the rat and human skin proteinases may reflect the evolutionary divergence of these skin proteinases from a common ancestral gene product and may suggest that these enzymes have evolved different biological functions within the mast cell. Ultimately, the evolutionary relationship between these chymotrypsin-like proteinases and cathepsin G will be ascertained from their respective amino acid sequences and their biological roles.

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