Biochimica et Biophysica Acta, 452 (1976) 458—467

BBA 67983

PROTEINASES OF HUMAN EPIDERMIS; A POSSIBLE MECHANISM FOR POLYMORPHONUCLEAR LEUKOCYTE CHEMOTAXIS

NORMAN LEVINE, VICTOR B. HATCHER and GERALD S. LAZARUS *

Departments of Medicine and Biochemistry of the Albert Einstein College of Medicine, the Division of Dermatology, Montefiore Hospital and Medical Center, Bronx, N. Y. and the Division of Dermatology, Duke University Medical Center, Durham, N. C. (U.S.A.)

(Received May 10th, 1976)

Summary

Three neutral proteinases (EC 3.4.—.—) and cathepsin D have been identified in human epidermis utilizing a highly sensitive radioactive method. The proteinases were extracted in 1.0 M KCl and 0.1% Triton X-100 and separated by Sephadex G-75 chromatography. The neutral proteinase peaks were all inhibited by diisopropyl fluorophosphate and thus were serine proteinases. Incubation of the enzyme fractions with [^{3}H]diisopropyl fluorophosphate followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis demonstrated that the two larger molecular weight proteinases were enzyme mixtures. The small molecular weight [^{3}H]diisopropyl fluorophosphate proteinase migrated as a single band. Injection of the small molecular weight neutral proteinase into rabbit skin produced a polymorphonuclear leukocyte infiltration and edema. The reaction was not observed with the diisopropyl fluorophosphate-inhibited enzyme fraction. The release of neutral proteinases may be one of the signal events in the epidermal inflammatory response.

Introduction

Numerous catabolic events occur in epidermis. The production of the stratum corneum is associated with almost complete resorption of cellular organelles and processes such as epidermal phagocytosis undoubtedly involve tissue proteinases. Neutral proteinases (EC 3.4.—.—.) were first discovered in skin by Beloff and Peters [1] and subsequently, several investigators have studied these

* Senior Investigator of the Arthritis Foundation. Present address: Department of Medicine, Division of Dermatology, Duke University Medical Center. Reprint requests should be addressed to Dr. Gerald S. Lazarus.

Abbreviation: iPr_2P-F, diisopropyl fluorophosphate.

enzymes in various species [2,3,4]. Most recently, Lazarus and Barrett have described a small molecular weight neutral proteinase from rabbit skin which was capable of degrading structural protein and inducing chemotaxis of polymorphonuclear leukocytes [5].

This study details a new, highly sensitive radioactive proteinase assay which is based on the production of small trichloracetic acid soluble ^{3}H-labelled peptides from high molecular weight proteins. Utilizing this assay we have characterized some of the proteinases of separated human epidermis. We report that extracts of human epidermis contain cathepsin D and a serine proteinase which is capable of inducing polymorphonuclear leukocyte chemotaxis. Such an enzyme might be the mechanism by which epithelial injury induces polymorphonuclear leukocyte infiltration.

Methods

A. Colorimetric assay for trypsin

Trypsin (Sigma Chemicals, St. Louis, Mo., activity 12 000 BAEE units/mg) was dissolved in a 0.2 M Tris · HCl buffer, pH 8.1, containing 0.005 M $CaCl_2$. Enzyme preparations (100 μl) were added to solutions containing 100 μl of casein (8% w/v) and 100 μl of the appropriate buffer. After incubation at 40°C for 1 h, 4 ml of trichloracetic acid (4.75% w/v) was added to the reaction buffer which was then vigorously mixed, incubated at 25°C for 5 min and filtered through a Whatman No. 3 filter paper. Enzyme blanks consisted of assay mixtures to which enzyme was added after the addition of trichloracetic acid. The absorbance of the filtrate was read at 280 nm in a Gilford Spectrophotometer (Gilford Instruments, Oberlin, Ohio).

B. Colorimetric assay for cathepsin D

The colorimetric assay was performed according to the method of Barrett [6].

C. ^{3}H-acetylation of substrate proteins

Casein nach Hammarsten (E.M. Chemicals, Elmsford, N.Y., 8% w/v) was suspended in distilled water, adjusted to pH 7.0 with 1.0 M NaOH and incubated at 65°C for 30 min. The casein solution (2 ml, 50 mg/ml) was dialyzed against 0.1 M sodium phosphate, pH 7.4, containing 0.14 M NaCl, 0.02 M KCl and 0.05 M sodium acetate. [^{3}H]Acetic anhydride (New England Nuclear, Boston, Mass., 5.0 mCi, 400 Ci/μmol) was added to the casein solution with vigorous mixing. After incubation at 4°C for 1 h, the acetylated sample was dialyzed exhaustively against the buffer at 4°C. Hemoglobin solution (2.0 ml, 50 mg/ml), prepared according to the method of Barrett [7], was conjugated with 10 mCi of [^{3}H]acetic anhydride (400 Ci/μmol) in a manner identical to that for casein. The acetylated hemoglobin was dialyzed against the reaction buffer at 4°C.

D. Radioactive assay for trypsin, cathepsin D and neutral proteinase

The reaction mixture, 20 μl of trypsin dissolved in 0.2 M Tris · HCl buffer, pH 8.1, 20 μl of 0.2 M Tris · HCl, pH 8.1, containing 0.005 M $CaCl_2$ and 0.25

M KCl and 20 μl of [^{3}H]acetyl casein, was incubated at 40°C for 1 h. At the conclusion of the incubation, 50 μl of unlabelled casein (30 mg/ml) dissolved in water, followed by 100 μl of trichloracetic acid (6% w/v) was added. After incubation in an ice bath for 30 min the samples were centrifuged at 15 000 × *g* for 10 min and a 100 μl aliquot of the supernatant was counted in 10 ml of Bray's solution [8]. The radioactive assay for cathepsin D was similar to that described for trypsin except that the [^{3}H]hemoglobin stock solution was dissolved in 1.0 M formate buffer, pH 3.4 and the reaction buffer was 1.0 M formate buffer, pH 3.4. The [^{3}H]casein for the neutral proteinase assays was dissolved in 0.05 M sodium phosphate pH 7.4 and the reaction buffer was 0.5 M sodium phosphate buffer pH 7.4.

E. Chromatography of the reaction products from the neutral proteinase assays on Biogel P-2

A standard radioactive assay using 100 ng of trypsin and the appropriate control was performed. The trichloracetic soluble peptides were chromatographed on a column of Biogel P-2 (Bio-Rad, Richmond, Calif.) (95 cm, 1.5 cm 15 ml/h, 4°C) equilibrated with 0.1 M sodium phosphate buffer, pH 8.1. Fractions (1.2 ml) were collected and aliquots (1.0 ml) were counted in 10 ml of Bray's solution in a scintillation spectrometer.

F. Extraction and separation of proteolytic enzymes from human epidermis

Human skin was obtained from amputation limbs after surgery (approved by human research committee and informed consent obtained). The entire epidermis and upper dermis was removed using a Castroviejo keratome (Storz, St. Louis) set a 0.4 mm depth. Skin slices were incubated in KBr (2 M for 30 min at 37°C) and the epidermis was mechanically separated from the underlying dermis at the basement membrane. The purity of epidermal preparations was frequently checked by histological examination of the epidermal pellet. The dermis was discarded and the epidermal sheets were exhaustively washed in phosphate-buffered saline at 4°C. The epidermis was then homogenized in ice-cold extraction buffer (1 gm/ml) containing KCl (1 M), Triton X-100 (0.1%) and sodium azide (0.1%) in 0.05 M sodium phosphate buffer, pH 7.5 using a Polytron homogenizer (Brinkmann, Lucerne, Switz.). The temperature of the mixture did not exceed 12°C during the homogenization. The mixture was then stirred overnight at 4°C, centrifuged at 15 000 × *g* for 60 min in a Sorval RC-5 centrifuge (Dupont-Sorvall, Norwalk, Conn.) and the supernatant collected. Chromatography of the supernatant containing the epidermal proteinase was performed on a column of Sephadex G-75 superfine gel (Pharmacia, Upsala, Sweden) (100 cm × 1.5 cm, 2 ml/h, 4°C) equilibrated with the extraction buffer. Aliquots (3 ml) were collected and each fraction was assayed for protein [9], cathepsin D and neutral proteolytic activity as described above.

G. Characterization of neutral proteinase in epidermis

The pH optimum of the separated proteinases was determined with the following 0.5 M buffers: pH 2.5, glycine buffer, pH 3.0—3.5, sodium formate buffer, pH 4.5—5.5, sodium acetate buffer, pH 6—8.5, sodium phosphate buffer, pH 9.0—9.5, Tris · HCl buffer. The following proteinase inhibitors were in-

cubated with each of the separated enzyme fractions at the final concentrations indicated: Diisopropyl fluorophosphate (iPr_2P-F) (5 mM), pepstatin (0.5 mg/ml), disodium EDTA (2 mM), and *N*-ethyl maleimide (MalNEt) (2 mM). In addition, the small molecular weight proteinase was incubated with the following: α_2-macroglobulin (48 μg/60 ml), α_1-antitrypsin (44 μg/60 μl), $\bar{C}1$ inactivator (50 μg/60 μl), *N*-α-*p*-tosyl-L-lysinechloromethylketone (TosLysCH_2Cl) (5 mM), Kunitz pancreatic trypsin inhibitor (10 μg/60 μl), L-1-*p*-tosylamido-2 phenethyl-chloromethylketone (Tos-Phe-CH_2Cl) (5 mM), ε-amino caproic acid (20 μg/100 μl), Trasylol 1000 KI units, Ac$(Ala)_3$-Pro-CH_2Cl (0.3 mM), Ac$(Ala)_4$-CH_2Cl (0.3 mM).

Serine proteinases which are inhibited by iPr_2P-F, were further characterized by incubating appropriate preparations with [^{3}H]iPr_2P-F followed by SDS polyacrylamide gel electrophoresis [10]. 0.5 ml of each enzyme peak was incubated with 20 μl of [^{3}H]iPr_2P-F (1 Ci/mol), (New England Nuclear, Boston, Mass.) at 20°C for 24 h. Unreacted [^{3}H]iPr_2P-F was removed by exhaustive dialysis against urea (8 M), sodium lauryl sulfate (1%), and β-mercaptoethanol (1%) in sodium phosphate buffer (1.0 M) pH 7.0 until the dialysate contained no radioactivity. The amount of radioactivity in each sample was determined and 200 μl of each labelled enzyme peak was separated by SDS polyacrylamide gel electrophoresis. The gels were cut into 80 equal slices and then solubilized in 50 μl of H_2O_2 (30%) at 60°C for 16 h. After the addition of 250 μl of Protosol (NEN, Boston), 10 ml of toluene scintillant was added and the radioactivity was determined in a Packard Tricarb scintillation counter.

H. Demonstration of in vivo chemotaxis after injection of epidermal proteinase

The small molecular weight proteinase fraction was concentrated approximately 10—15 times in Aquacide III (Calbiochem, San Diego) and was dialyzed against normal saline. Aliquots (0.1 ml) were injected intradermally into the backs of shaved albino rabbits. An aliquot (0.1 ml) of the normal saline dialysis solution and an aliquot of the enzyme fraction that was previously inhibited by iPr_2P-F and extensively dialyzed were also injected intradermally into the rabbits as controls. Skin punch biopsies (4 mm) were taken from the test areas at 2 h and 20 h after injection; the samples were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. Specimens were examined with a Zeiss photomicroscope.

Results

A. Acetylation of substrate proteins

The ^{3}H-acetylated hemoglobin used in the radioactive assay had a specific activity of 460 cpm/pmol based on a molecular weight of 68 000 (20 μl of Hb contained 10 μg [^{3}H]hemoglobin, 147 pmol, 68 000 cpm, 0.12 μCi) while the ^{3}H-acetylated casein had a specific activity of 2970 cpm/pmol based on a molecular weight of 375 000 (20 μl of casein contained 10 μg ^{3}H-acetylated casein, 26.7 pmol, 78 000 cpm, 0.14 μCi). The efficiency of conjugation was 14% for hemoglobin and 8% for casein.

B. Radioactive assay for proteinase

The radioactive assay for trypsin detected enzyme activity in the 1—2 ng

range which was at least 1000 times more sensitive than the colorimetric method. The relationship of activity with time was evaluated by incubating 10 ng of trypsin with [^{3}H]casein and terminating the reaction at 10 min intervals for 80 min. A linear relation between time and activity was obtained up to 60 min. Enzyme activity was independent of substrate concentration under assay conditions; incubation of trypsin (5 ng) with final concentrations of casein from 0.25 mg/ml to 1.875 mg/ml revealed no change in enzymatic activity.

Reaction products were examined by Biogel P-2 chromatography (Fig. 1). The column was calibrated by applying [^{3}H]casein and [^{3}H]acetic anhydride. The [^{3}H]casein eluted at 24—28 ml and the free [^{3}H]acetic anhydride eluted at 55—57 ml. Chromatography of the control reaction mixture where enzyme was added after the addition of trichloracetic acid revealed only an inconsequential peak at the void colume. Chromatography of the trichloracetic acid soluble peptides from a mixture containing [^{3}H]casein and trypsin resulted in a peak at the void volume representing peptides 1800 daltons or greater and a second more diffuse peak at a volume of 26—40 ml. No radioactivity was noted at the elution position of free acetic anhydride. Consequently, production of trichloracetic acid soluble counts reflected degradation of substrate to small molecular weight peptides and not deacetylation of the substrate.

C. Human epidermal proteinase

Chromatography of human epidermal extracts revealed cathepsin D and three neutral proteinase activities (Fig. 2). A peak of hemoglobinolytic activity,

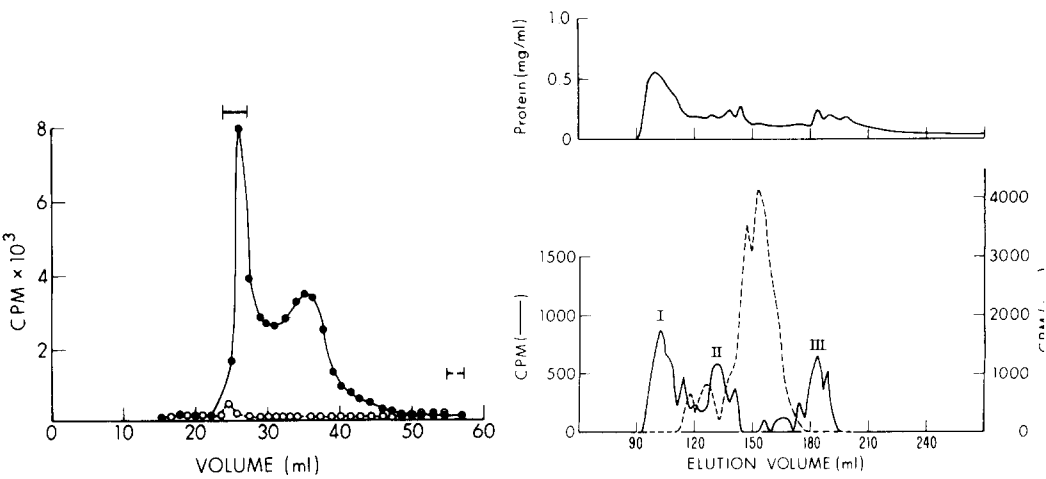

Fig. 1. Chromatography of the products of the radioactive neutral proteinase assay on a column of Biogel P-2. Elution pattern of radioactively labelled trichloroacetic acid soluble peptides after incubation with trypsin (●———●). Elution pattern of comparable amounts of [^{3}H]casein to which trypsin was added after the addition of trichloroacetic acid (○- - - - -○). Elution position of casein (———). Elution position of acetic anhydride (⊢⊣).

Fig. 2. Sephadex G-75 Chromatography of human epidermal neutral proteinases (———) and acid proteinase (- - - - -). Three distinct peaks of neutral proteolytic activity (I-III) and a single large peak of acidic proteolytic activity. The protein concentration of each tube is shown in the upper panel.

operative at pH 3.5 and totally inhibited by pepstatin, was eluted from the column at an apparent molecular weight of approximately 40 000. This enzyme is the proteinase cathepsin D which has been shown to be capable of both intracellular and extracellular protein digestion [12].

Three distinct peaks of neutral proteolytic activity eluted from the column (Fig. 2). Peak I eluted at the void volume and represents all proteinases of $M_r \geqslant$ 75 000 or greater. The pH optimum of caseinolytic activity was 7.5—8.0 and there was a 20% inhibition of its proteolytic activity by diisopropyl fluorophosphate. Essentially no inhibition could be achieved with disodium ethylenediamine tetracetic acid, MalNEt or pepstatin. The small molecular weight neutral proteinase peak III, M_r = 30 000 had a pH optimum of 7.5—8.0 and could be 100% inhibited by diisopropyl fluorophosphate, soybean inhibitor, α_2-macroglobulin, α_1-antitrypsin and $\bar{C}1$ inactivator (a gift of Dr. Peter Harpel). It was inhibited by TosLysCH$_2$Cl but it was unaffected by TosPheCH$_2$Cl, Kunitz pancreatic trypsin inhibitor, ϵ-amino caproic acid, Trasylol, MalNEt, disodium ethylenediamine tetracetic acid, Ac(Ala)$_3$-Pro-CH$_2$Cl and Ac(Ala)$_4$-CH$_2$Cl.

The three neutral proteinase fractions were inhibited with [^{3}H]iPr$_2$*P*-F followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The results are shown in Fig. 3. Neutral proteinase peak I contained 4 labelled proteins and peak II contained two distinct labelled proteins. The small molecular weight neutral proteinase, peak III, had a single labelled band of M_r = 30 000. Since peak III contained a proteinase which was totally inhibited by diisopropyl fluorophosphate and there was a single labelled band on polyacrylamide gel electrophoresis it seems reasonable to assume that there was a single serine proteinase in this peak.

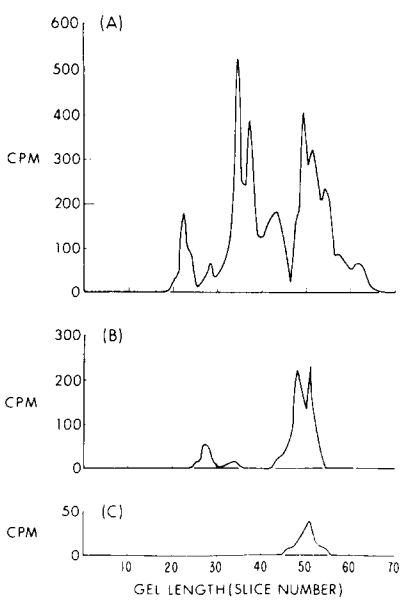

Fig. 3. SDS polyacrylamide gel electrophoresis of human epidermal neutral proteinases. Peak I (A) and peak II (B) have several iPr$_2$*P*-F-reacting components. Peak III (C) contains a single iPr$_2$*P*-F-reacting component.

D. Demonstration of chemotaxis after injection of neutral proteinase peak III

A concentrated solution of neutral proteinase peak III and the same solution pre-incubated with diisopropyl fluorophosphate were selected for intradermal injection into rabbits. Fig. 3 demonstrates the histologic pattern obtained 20 h after the injection of enzyme and appropriate controls. Panel A is a low power view of the skin after being injected with the peak III proteinase. There is edema leading to dermal/epidermal separation and significant polymorphonuclear leukocyte infiltration in the upper dermis with a perivascular prominence of the infiltrate. Panel B is a high power view showing the perivascular distribution of the leukocyte infiltrate. Panels C and D are sections taken from the skin injected with diisopropyl fluorophosphate treated peak III and a normal saline control solution. In neither of these controls was there evidence of polymorph infiltration. This experiment, repeated on three different occasions, demonstrates that the small molecular weight neutral proteinase in human epidermis is

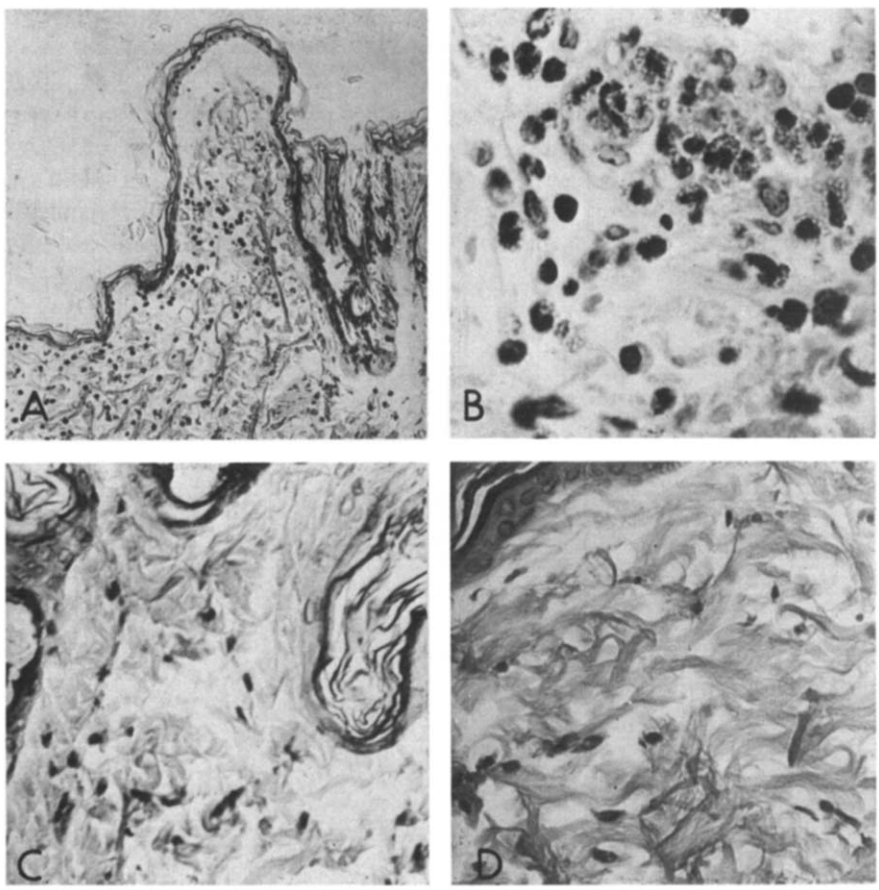

Fig. 4. Photomicrographs of inflammatory response obtained after injection of human epidermal neutral proteinase into rabbit skin. (A) Section of a skin biopsy 20 h after intradermal injection of neutral proteinase (b) High power view of neutral proteinase-injected skin demonstrating infiltration by granulocytes (c) Section of a skin biopsy 20 h after intradermal injection of iPr_2P-F-inactivated neutral proteinase (d) Section of a skin biopsy 20 h after intradermal injection of saline control.

capable of eliciting polymorphonuclear leukocyte chemotaxis in vivo and that the response is dependent upon enzymatic activity. Similar results were obtained when biopsies taken 2 hours after injection were examined.

Discussion

A sensitive radioactive assay has allowed us to isolate and characterize cathepsin D and several neutral proteinases from human epidermis. The assay is based on the production of small trichloracetic acid soluble ^{3}H-labelled peptides from high molecular weight proteins. It is a versatile assay in that different protein substrates can be labelled with [^{3}H]acetic anhydride and activity can be studies over a wide pH range. Our method uses a one hour incubation since this is a convenient time and there is a linear relation between the enzyme activity and product. An unlabelled protein substrate is added to the reaction mixture before the addition of trichloracetic acid to saturate the enzyme and to effect total precipitation of the labelled substrate. Incubation of the reaction mixture in an ice bath after the addition of TCA enhanced precipitation of the proteins.

Other methods have been developed to measure proteolytic activity. Several fluorometric methods for proteinases have been described which measure the production of trichloracetic acid soluble fluoresceinated products [13—15]. Bosmann [16] and Hille et al. [17] have described sensitive radioactive method for determining proteolytic activity which we have modified; we have also characterized the nature of the trichloracetic acid soluble radioactive products.

Epidermis was separated from the underlying dermis by incubating the skin in 2 M KBr. This method was first described by Felsher [18] and since has been used by others [19—21]. Histologic examination of our preparations revealed that our epidermal preparations contained negligible amounts of dermal connective tissue. The mechanism of action of KBr is unclear but it may invoke a dissolution of the hemidesmosomes attaching the basal cells to the basal lamina.

Our studies reveal that there is a significant amount of cathepsin D in human epidermis. Cathepsin D has been identified in epidermis of other species. Poole et al. [22] have demonstrated cathepsin D in chick epidermal cell cultures and Lazarus and Poole [23] using immunocytochemical techniques have localized this enzyme to the basal and granular layer of rabbit epidermis. Removal of cathepsin D from a crude extract of rabbit skin by immunoprecipitation with a monospecific antibody revealed that this lysosomal proteinase was responsible for most of the hemoglobinolytic activity operative below pH 5 in skin extracts [24]. Autolysis experiments using immunoinhibition of cathepsin D demonstrated that this enzyme was also responsible for degradation of structural skin-protein at acid pH [24]. Cathepsin D levels increase dramatically during the times of remodelling in chick skin [25,26] and in experimental inflammation in rabbits induced by turpentine [27]. Our demonstration of cathepsin D in human epidermis might offer an enzymatic mechanism for certain catabolic processes in human epithelium.

Our studies reveal three distinct peaks of neutral proteinase activity in human epidermis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of [^{3}H] iPr_2P-F labelled peaks contain multiple enzyme subunits while the third

low molecular weight peak contains a single serine proteinase of M_r = 30 000. This enzyme is capable of protein degradation in vitro and it induces chemitaxis of polymorphonuclear leukocytes when injected into rabbits. Similar studies with a very highly purified enzyme from whole human skin corroborates our findings with the epidermal enzyme (Hatcher, Lazarus and Levine, unpublished data). The chemotactic activity of our proteinase is dependent upon enzymatic activity since preincubation with diisopropyl fluorophosphate inhibits leukocyte infiltration in rabbit skin. The exact mechanism by which this enzyme induces chemotaxis is unknown but preliminary studies using Boyden chambers suggests that serum is necessary to induce leukocyte migration (Lazarus, Hatcher, Snyderman and Gigli, unpublished data).

The enzyme is probably an intrinsic constituent of the epidermis since it has been found in cultures of newborn mouse epithelial cells (Burk et al., unpublished data). The subcellular localization of this small molecular weight proteinase in epidermis is unknown but studies of the localization of this proteinase in human fibroblast culture reveals that this enzyme is significantly enriched in the lysosomal fraction (Lazarus, Levine, Hatcher et al., submitted for publication).

Hill and Ward [28] demonstrated that extracts of various rat tissues, especially heart, were chemotactic for polymorphonuclear leukocytes in serum filled Boyden chambers. These investigators suggested that the chemotactic activity was a result of activation of C3 by proteolytic enzymes in their preparations. Goldstein and Weissman [29] have shown that leukocyte lysosomes are capable of activating C5. Our studies demonstrate that normal human epithelium, a tissue devoid of inflammatory cells, contains a proteinase capable of inducing chemotaxis. This observation permits formulation of an hypothesis to explain how epidermal cell injury instigates an inflammatory response. Damage to epidermal cells could result in release of neutral proteinase possibly from lysosomes, which through proteolysis of serum constituents might generate chemotactic factors. Infiltration by polymorphonuclear leukocytes coupled with release of endogenous proteinases might then result in tissue catabolism. Identification of this enzyme in other cell types may imply that this mechanism is applicable to other forms of tissue injury as well.

Acknowledgement

Ms. Alyce Miller and Ms. Deborah Cleary provided expert assistance in the preparation of this manuscript. Dr. Peter Harpel, Cornell Medical Center, kindly provided purified α_2-macroglobulin, α_1-antitrypsin and $\overline{C1}$ inactivator. This work was supported by a grant from the National Institutes of Arthritis, Metabolic and Digestive Diseases, 7 RO1 AM17370-02 and a grant from the Syntex Corporation to Dr. Levine.

References

1 Beloff, A. and Peters, R.A. (1945) J. Physiol. 103, 461—476
2 Fraki, J.E. and Hopsu-Havu, V.K. (1972) Arch. Derm. Forsch. 242, 329—342
3 Fraki, J.E. and Hopsu-Havu, V.K. (1972) Arch. Derm. Forsch. 243, 52—56

4 Lazarus, G.S. and Hatcher, V.B. (1975) in Lysosomes (Dingle. J.T. and Dean, R.T., eds.), Vol. 4, pp. 111—143, North Holland, Amsterdam
5 Lazarus, G.S. and Barrett, A.J. (1974) Biochim. Biophys. Acta 350, 1—12
6 Barrett, A.J. (1972) in Lysosomes (Dingle, J.T., ed.), pp. 46—145, North Holland, Amsterdam
7 Barrett, A.J. (1970) Biochem. J. 131, 809—821
8 Bray, G. (1960) Analyt. Biochem. 1, 279—285
9 Lowry, O.H., Rosebrough, N., Farr, A.D. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
10 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
11 Dingle, J.T., Poole, A.R., Lazarus, G.S. and Barrett, A.J. (1973) J. Exp. Med. 137, 1124—41
12 Dingle, J.T., Barrett, A.J. and Weston, P.D. (1971) Biochem. J. 123, 1—13
13 Schwabe, C. (1973) Analyt. Biochem. 53, 484—490
14 Hirsch, H. and Parks, M. (1973) J. Histochem. 21, 453
15 DeLumen, B.O. and Tappel. A.L. (1970) Analyt. Biochem. 36, 22—29
16 Bosmann, H.B. (1974) Nature 249, 144—145
17 Hille, M.B. and Barrett, A.J. (1970) Exp. Cell Res. 61, 470—472
18 Felsher, S. (1946) Proc. Soc. Exp. Biol. Med. 62, 213—215
19 Felsher, S. (1947) J. Invest. Dermatol. 8, 35—47
20 Sato, T. and Kawada, A. (1972) J. Invest. Dermatol. 58, 392—395
21 Wolf, J.G. and Hubler, W.R. (1975) Clin. Res. 23, 91H 9 Abstr.
22 Poole, A.R., Dingle, J.T., Barrett, A.J. (1972) J. Histochem. Cytochem. 20, 261—265
23 Lazarus, G.S. and Poole, A.R. (1975) Arch. Dermatol. 111, 1150—1153
24 Lazarus, G.S. and Dingle, J.T. (1974) J. Invest. Dermatol. 62, 61—66
25 Woessner, J.F. (1970) in Chemistry and Molecular Biology of the Intracellular Matrix (Balaza, L.A., ed.), pp. 1663—1669, Academic Press, New York
26 Woessner, J.F. (1971) in Tissue Proteinases (Barrett, A.J. and Dingle, J.T., eds.), pp. 291—311, North-Holland, Amsterdam
27 Lazarus, G.S. (1974) J. Invest. Dermatol. 62, 367—371
28 Hill, J.H. and Ward, P.A. (1969) J. Exp. Med. 130, 505—518
29 Goldstein, I.M. and Weissman, G. (1974) J. Immunol. 113, 1583—88