Susceptibility of Soluble and Matrix Fibronectins to Degradation by Tissue Proteinases, Mast Cell Chymase and Cathepsin G*

(Received for publication, February 26, 1980, and in revised form, May 12, 1980)

Tapio Vartio
From the Department of Virology and Department of Pathology, University of Helsinki, SF-00290 Helsinki 29, Finland
Heikki Seppä‡
From the Department of Anatomy, University of Oulu, SF-90220 Oulu 22, Finland
Antti Vaheri
From the Department of Virology, University of Helsinki, SF-00290 Helsinki 29, Finland

Fibronectin is a glycoprotein present in soluble form in plasma and other body fluids and in insoluble form in extracellular matrix. We have studied the effect of two neutral tissue proteinases, rat mast cell chymase and the closely related human cathepsin G, on soluble and matrix forms of fibronectin and compared them to other enzymes and substrates.

Chymase was purified to apparent homogeneity from rat skin extracts, and cathepsin G from human neutrophil leukocytes. Both enzymes digested plasma fibronectin (disulfide-bonded dimer of 220,000-dalton subunits) in a characteristic pattern when analyzed in NaDodSO4-polyacrylamide gels (enzyme/substrate ratio 1:500 w/w). The fragments included sequentially produced 200,000-, 70,000-, and 40,000-dalton polypeptides binding to gelatin-agarose. The digests retained antigenic activity to antibodies against native plasma fibronectin, as judged by double immunodiffusion and radioimmunoassay. In digestion of human plasma, either native, PhCH3SO4F-, or acid-treated, cleavage of α2-macroglobulin and fibronectin were among the most apparent changes. No degradation of purified laminin, another basement membrane glycoprotein, by the enzymes was detected. Of the metabolically labeled proteins secreted into the culture medium by human fibroblasts or human HT-1080 sarcoma cells, fibronectin and an unidentified 160,000-dalton polypeptide secreted by the latter were digested effectively by these proteinases. Loss of propeptides from interstitial procollagens and degradation of type IV procollagen occurred more slowly in labeled culture media. Digestion of insoluble fibronectin was detected when fibroblast cultures or their isolated pericellular matrices were treated for 2 h with the enzymes at a concentration of 0.1 μg/ml. These results suggest that fibronectin either in soluble or matrix form is a sensitive and rather selective substrate to neutral tissue proteinases. Destruction of fibronectin may be critically involved after mast cell activation and in inflammatory conditions in vivo.

Fibronectin is a high molecular weight glycoprotein (for references see 1-3) found in basement membranes, intimal connective tissue matrix (4) and in soluble form in plasma (5) and other body fluids (6-8). According to immunohistological studies the protein is particularly abundant in vascular walls and in the submucosa (4). The soluble form of fibronectin has been characterized biochemically; it is known to be composed of two disulfide-bonded polypeptides of apparent molecular weight 220,000 (9-12). The insoluble tissue fibronectin has been only partially characterized (13).

Several types of adherent cells, such as fibroblasts (14), endothelial cells (15-17), and hepatocytes (18), are known to synthesize and secrete large amounts of fibronectin in cell culture. Most of the protein synthesized by these cells is secreted in soluble form into the culture medium. In addition, normal cells, unlike malignantly transformed cells in general, deposit fibronectin in a pericellular matrix (2, 19). Recent studies have implicated a role for fibronectin in cell adhesion (20-22), in cell motility in vitro (23), and in cell migration in vivo (24). In vivo, the pericellular matrix is apparently degraded in certain physiological and pathological conditions. These include situations in which cells migrate or invade, as well as inflammatory conditions where defense cells and humoral defense factors enter the tissues.

The present studies on degradation of fibronectin by purified tissue proteinases were initiated by experiments with rat mast cell chymase, an enzyme responsible for a major part of the neutral proteolytic activity in many tissues (25-28). During the progress of the studies, cathepsin G (29), a human enzyme closely resembling chymase, purified from neutrophil leukocytes, became available and the experiments were repeated using cathepsin G.

The mast cells are connective tissue cells widely distributed throughout the body and are typically positioned at the mucosal and cutaneous surfaces and near small blood vessels in deeper tissues, and have recognition units for IgE and anaphylactic complement fragments (30). Activation of the mast cell leads to a rapid release of the granular contents including heparin, histamine, and the neutral proteinase chymase as a major protein component. This enzyme, conveniently isolated from rat skin mast cells, is a small-sized serine protease, M = 27,500, which in its enzymatic properties resembles pancreatic α-chymotrypsin in several respects (26, 31). It hydrol-
ytes the esters of tyrosine, acetyltyrosine, and acetylphenylalanine, and is effectively inhibited by serine proteinase inhibitors such as diisopropylphosphorofluoridate, and phenylmethylsulfonyl fluoride (PhCH$_2$S02F). The activity of the chymase in the mast cell granule is partially masked by its binding to macromolecular proteoglycan heparkin with which it is released by immunologic activation of the isolated rat mast cell (31). The active site of soluble chymase is inhibited by 5-hydroxytryptamine but not by histamine (26), indicating that the expression of the proteolytic activity of mast cell granule chymase is regulated by two additional mediator components of the granule.

Cathepsin G (29) has been shown to be one of the major neutral proteinases in neutrophil leukocytes and in the spleen. In neutrophils it is located in the azurophilic granules. It is a serine proteinase and resembles chymase in several respects. Cathepsin G is a basic protein with a molecular weight of 28,000, is able to form a complex with heparin, and is totally inhibited by diisopropylphosphorofluoridate and PhCH$_2$S02F (92, 93). It seems likely that cathepsin G and chymase are the same enzyme (29); however, no direct protein-chemical or immunochemical evidence has been presented to prove this view.

The present results indicate that both soluble and insoluble fibronecins are sensitive and in many situations relatively selective substrates of chymase and cathepsin G.

**MATERIALS AND METHODS**

**Enzymes—**The mast cell chymase of rat skin was isolated as described earlier (26). The purified enzyme, 60 µg/ml, was stored in 0.33 mM potassium phosphate, pH 6.8, at -70°C, and had an activity similar to that of chymase obtained from rats of a modified method based on that of Baugh and Travis (35). The purified enzyme was stored lyophilized, had an activity of 50 µmol/min of acetylated tyrosine ethyl ester, and 470 azocasein units/mg. Ten micrograms of protein gave a homogenous band in sodium dodecyl sulfate (NaDodSO$_4$) polyacrylamide gel electrophoreses, with an apparent molecular weight of 27,500. Inactivated enzyme was prepared by adding PhCH$_2$S02F to a final concentration of 5 mM, incubating for 18 h at +4°C and extensive dialysis against the storage buffer, 330 mM potassium phosphate, pH 6.8.

Cathepsin G, a gift from Drs. Jeremy Saklatvala and Phyllis M. Starkey, Strangeways Research Laboratory, Cambridge, United Kingdom, had been purified from neutrophil leukocytes obtained from a patient with chronic myeloid leukemia by a modified method based on that of Baugh and Travis (35). The purified enzyme was stored lyophilized, had an activity of 350 azocasein units/mg, and gave a single band with apparent molecular weight of 28,000 in NaDodSO$_4$-polyacrylamide gel electrophoreses. For the experiments cathepsin G was dissolved in a concentration of 500 µg/ml in 50 mM Tris-HC1, pH 7.5, containing 0.05% Brij 35.

Human plasmin (15 casein units/mg, Kabı, Stockholm, Sweden) had been prepared by activation with solid-phase activator from affinity-purified human plasminogen. Bovine pancreatic trypsin (type III, twice crystallized, 12,000 α-N-benzoyl-L-arginine ethyl ester units/mg) was obtained from Sigma.

**Purified Plasma Fibronecin—**Fibronecin was purified from human plasma by a two-step affinity chromatography procedure in nondenaturing conditions, using gelatin-Sepharose and arginine-Sepharose as described (36). The purified protein in 0.1 M NaCl in 0.05 M Tris-HCl, pH 7.5, in the present experiments was homogenous by the following criteria. Twenty micrograms of chemically reduced protein showed on NaDodSO$_4$-polyacrylamide gel electrophoreses a single polypeptide band with a molecular weight of 220,000. Two-dimensional immunoelectrophoresis against polyclonal antibodies to human plasma proteins gave only one immunoprecipitate arc identical with that obtained with anti-fibronecin antibodies.

**NaDodSO$_4$-Polyacrylamide Gel Electrophoreses—**Polyacrylamide gel electrophoreses in the presence of NaDodSO$_4$, was performed according to the method of Laemmlli (37), using vertical slab gels. The acrylamide concentration was 3.3% in the spacer gel and 5, 6, or 8% in the separating gel, as indicated. Unless otherwise specified, the samples were reduced with 10% β-mercaptoethanol in Laemmlli’s sample buffer. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 (38). Gels containing radioactively labeled material were fluorographed by scintillation autoradiography (39), using Kodak RP X-Omat film. The molecular weight markers were chemically reduced purified human immunoglobulin G (M, 170,000), phosphorylase a (M, 92,000), human serum albumin (M, 68,000), ovalbumin (M, 43,000), and ribonuclease a (M, 13,700). The proteins were labeled with [14C]formaldehyde by the method of Rice and Means (40). In experiments with unlabelled proteins, commercially obtained low molecular weight markers (Pharmacia) were used.

**Digestion of Purified Proteins—**Digestion of plasma fibronecin, 500 to 1500 µg/ml in 0.1 M NaCl in 0.05 M Tris-HCl buffer, pH 7.5, with rat mast cell chymase or cathepsin G was performed at an enzyme/substrate ratio of 1:500 at 37°C. Samples were withdrawn at given time intervals, and digestion was terminated by the addition of Laemmlli’s sample buffer (37) or of PhCH$_2$S02F to give a final concentration of 5 mM for gelatin-binding, immunodiffusion, or radioimmunoassay experiments. Control samples were incubated without enzyme or with PhCH$_2$S02F-treated enzyme during the whole experiment at 37°C. Samples in which the digestion was terminated by PhCH$_2$S02F were diluted with 0.05 M Tris-HCl, pH 7.5, 0.1 M NaCl, to 500 µl and 100 µl of 50% gelatin-Sepharose or plain Sepharose 4B was then added. After slow rotation in an end-over-mixer overnight at +4°C, the material bound to gelatin-Sepharose was centrifuged, washed, dissolved in Laemmlli’s sample buffer, and analyzed by electrophoresis in polyacrylamide slab gels.

Digestion with plasmin was performed under identical conditions at 37°C. Purified human IgG (gift from Orion Diagnostica, Helsinki) and human serum albumin (crystallized, Sigma) was incubated together with fibronecin and enzyme. The proteins were mixed at a molar ratio (440 µg/ml of fibronecin, 150 µg/ml of IgG, and 68 µg/ml of albumin), and enzyme was added at an enzyme/total substrate ratio of 1:500. The reaction mixture was incubated at 37°C, and aliquots were then analyzed by gelatin electrophoresis. Purified human plasminogen (41; gift from Dr. T. Timpl, Max-Planck Institut fur Biochemie, Martinsried, Germany) was also studied at an enzyme/substrate ratio of 1:500.

**Digestion of Human Plasma—**Normal human plasma, collected using citrate as an anticoagulant, was used either in native form or after treatment by PhCH$_2$S02F or HCl. PhCH$_2$S02F was added to citrated human plasma to give a final concentration of 5 mM. After incubation for 2 h at room temperature, it was dialyzed five times against 0.1 M NaCl in 0.05 M Tris-HCl, pH 7.5, to remove unbound PhCH$_2$S02F. To another aliquot of plasma, 2 n HCl was added to bring the pH to 3. After incubation for 1 h at room temperature, the pH was raised to neutral with 2 n NaOH. The aliquots of native, PhCH$_2$S02F-, or acid-treated plasma were digested at an enzyme/substrate ratio of 1:200.

**Digestion of Cell Cultures and Isolated Media—**Human embryonic fibroblasts (established locally) and HT-1080 sarcoma cells (ATCC, CCL 121) were grown at +37°C in a humidified 5% CO$_2$ atmosphere in Eagle’s basal medium modified for diploid cells and supplemented with 10% fetal calf serum, 100 units/ml of penicillin and 50 µg/ml of streptomycin. They were subcultured (1:2) twice weekly, using a solution of 0.25% trypsin and 0.02% EDTA in Hanks’ buffered saline to disperse the cells. For experiments with enzyme, the medium was changed to contain 0.2% bovine serum albumin and no serum. The radioactive precursor [15-H]proline (15 Ci/mmol, Radiochemical Centre, Amersham) was added to a concentration of 10 µCi/ml, and the cultures were incubated overnight. Enzyme (100 ng/ml or control buffer was then added, and the cultures were incubated at 37°C for the indicated periods of time. After the incubation the culture media were collected, and the digestion was terminated by the addition of PhCH$_2$S02F to a concentration of 5 mM. Proteins in the medium were precipitated with ammonium sulfate (176,000 g/ml) for analysis by gel electrophoresis. The cell lysates were washed three times with NaCl/P, and dissolved in Laemmlli’s sample buffer.

In another set of experiments conditioned labeled medium was collected, clarified by centrifugation at 100 x g, and this isolated medium was digested as above.

**The Effects of the Enzymes on the Pericellular Matrix—**Pericellular cell-free matrix was prepared, using the deoxycholate procedure (42), from confluent fibroblast cultures on 20-cm$^2$ plates labeled for 3
days with [3H]proline. The matrices remained attached to the surfaces of the plates, and were overlayed with 2 ml of NaCl/P; with or without enzyme at 100 ng/ml. After incubation at 37°C for time periods of 6, 20, 60, and 180 min, NaCl/P, was collected from the plates. The proteins were precipitated with ammonium sulfate, and the resulting precipitates and the remaining matrices were dissolved in Laemmli's sample buffer for analysis by gel electrophoresis.

Immunofluorescence Staining—For immunofluorescence analysis human embryonic skin fibroblasts were grown on glass coverslips, washed with serum-free medium, and incubated in serum-free medium containing 0.2% bovine serum albumin with or without enzymes. The cell layers were then fixed by p-formaldehyde and acetone and stained for fibronectin as described previously (48) using rabbit anti-fibronectin serum diluted 1:200 and fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG sheep immunoglobulin. The anti-fibronectin was specific by the following criteria. Immunofluorescence obtained by preimmune serum or antisera preincubated with purified plasma fibronectin gave minimal or no staining. The anti-fibronectin serum gave a single precipitation arc against normal human plasma, extract of human fibroblasts, and the purified antigen in immunodiffusion analysis (43), and it gave a single polypeptide band in immunoprecipitation with radiolabeled fibroblast extracts (44).

Other Methods—Double diffusion precipitation tests were made on 1% agarose plates, according to Ouchterlony (45), using anti-human plasma fibronectin.

Fibronectin radioimmunoassay, as described in detail elsewhere (46), was based on radioiodination (47; The Radiochemical Centre) of purified plasma fibronectin by a modification of the chloramine-T method (47) and use of rabbit anti-fibronectin and sheep anti-rabbit γ-globulin antisera in a double antibody assay. All dilutions were made in NaCl/P, containing 0.05% bovine serum albumin.

The protein concentrations were assayed according to Lowry et al. (34), using bovine serum albumin as standard.

RESULTS

Digestion of the Purified Fibronectin by Chymase and Cathepsin G—Fibronectin was digested by rat skin mast cell chymase or human neutrophil cathepsin G at an enzyme/substrate ratio of 1:500. Fig. 1 shows a NaDodSO4-polyacrylamide slab gel electrophoresis pattern of fibronectin digested by chymase for various periods of time, as indicated in the figure legend. After a few minutes’ digestion, a 70,000-dalton peptide was the main product. In prolonged digestion it disappeared and, concomitantly as it seemed, a 40,000-dalton band appeared. After digestion for 24 h, there were only three major polypeptide bands: 105,000, 92,000, and 40,000 daltons,

![Fig. 1. Digestion of purified plasma fibronectin by chymase.](image)

Fibronectin was digested by chymase at an enzyme/substrate ratio of 1:500 at 37°C, and the digestion products were analyzed by NaDodSO4-polyacrylamide gel electrophoresis, using 6% slab gels. Track 1 shows the starting material and Tracks 2 to 11 show the digestion products at the time periods indicated. Molecular masses are indicated in kilodaltons. FN = fibronectin.

which apparently were the final products of the digestion.

Similar electrophoretic analysis under nonreducing conditions indicated that these polypeptides were monomeric. When fibronectin was digested with chymase pretreated with PhCH&02F, no digestion occurred. The two closely spaced subunits of fibronectin, designated previously as A and B chains (apparent Mr, 220,000 and 215,000, respectively; Ref. 12) were not resolved when large amounts were applied to the gel. In experiments in which smaller amounts were used we observed that, similar to previous observations on plasminolysis (12), both chymase and cathepsin G preferentially digested the A chain (data not shown).

To discover which of the digestion products bind to gelatin, aliquots withdrawn from the reaction mixture were treated with PhCH&02F to terminate the action of chymase, and gelatin-Sepharose was added. Analysis in NaDodSO4-polyacrylamide gels showed that initially only intact fibronectin and a large fragment slightly smaller than it bound to gelatin-Sepharose (Fig. 2), and these were replaced by the 70,000-dalton polypeptide and finally by the 40,000-dalton digestion product. No peptides bound to plain Sepharose.

The gelatin-binding properties of the cleavage products were also studied in experiments in which purified fibronectin was first bound to gelatin-Sepharose and then exposed to digestion by chymase. Again a polypeptide with Mr = 40,000 was recovered as a terminal digestion product from the gelatin-bound material when analyzed in NaDodSO4-polyacrylamide gel (data not shown).

Experiments similar to those described above for chymase were done with cathepsin G. Fig. 3 shows a NaDodSO4-polyacrylamide gel. The kinetics of digestion, cleavage pattern, and gelatin-binding fragments resembled those obtained by chymase.

Samples in which the digestion of purified fibronectin with chymase or cathepsin G was terminated by PhCH&02F were tested for antigenic activity. In double diffusion analysis against anti-fibronectin serum all samples, including those containing the 24-h cleavage products, gave a single precipitation arc, indicating immunological identity with intact fibronectin. Analysis by radioimmunoassay indicated that the final cleavage products retained 20 to 25% of the antigenic activity present in intact fibronectin (data not shown).

Comparison to Other Enzymes and Substrate Proteins—To compare the effects of the neutral tissue proteinases and plasmin, fibronectin was digested separately by chymase and...
Fibronectin disappeared from the reaction mixture about 5 times more rapidly than when digested by plasmin (data not shown). These experiments showed that when digested by chymase, intact fibronectin was degraded. Since fibronectin is a component of basement membranes, its susceptibility to chymase was compared to that of laminin, another noncollagenous glycoprotein of basement membranes. Both chymase and cathepsin G failed to digest laminin within 24 h (Fig. 4). Digestion of native or PhCH$_5$SO$_2$F-treated (to inhibit possible trace activity of plasmin) plasma showed that an early cleavage of $\alpha_2$-macroglobulin and of fibronectin were among the most prominent changes detected (Fig. 5). When plasma was pretreated with acid to prevent the effects of macromolecular proteinase inhibitors, the digestion patterns of $\alpha_2$-macroglobulin and fibronectin were identical with those of native and PhCH$_5$SO$_2$F-treated plasma. It also appeared that many proteins, in the molecular weight range of 80,000 to 200,000, had disappeared, probably due to the acid treatment. When samples of native, PhCH$_5$SO$_2$F- or acid-treated plasma were incubated with PhCH$_5$SO$_2$F-treated chymase, no digestion was detected.

**Effects on Proteins in Fibroblast and Sarcoma Cell Cultures**—Chymase or cathepsin G was added to isolated metabolically labeled media from cultures of normal human embryonic skin fibroblasts and of HT-1080 human sarcoma cells, recently shown to produce type IV (basement membrane) procollagen as the major collagenous protein (49). In fibroblast medium (Fig. 6, Track A), fibronectin (identified by immunoprecipitation and binding to gelatin-Sepharose) was rapidly degraded by the enzymes, and the interstitial procollagen chains pro-$\alpha$-I(1) and pro-$\alpha$-I(1) were converted to $\alpha$-sized chains. No further cleavage of the latter was observed. In view of the difficulty in quantitation, it was not possible to estimate whether fibronectin was more susceptible to the enzymes than the interstitial procollagens. The Type IV procollagen chains, pro-$\alpha$(IV) and pro-$\alpha$-I(IV), were apparently less susceptible to the enzyme than fibronectin (Fig. 6, Track B). The 190,000-dalton polypeptide, previously characterized as a noncollagenous disulfide-bonded protein (49), was also rapidly degraded.

When the enzymes were added directly to the cell cultures, the changes in the media were as above and in fibroblast layers the 220,000-dalton polypeptide in the position corresponding to fibronectin decreased (data not shown).

**Effects on Pericellular Matrix of Cultured Fibroblasts**—In cultures of normal fibroblasts, deposition of fibronectin occurs into fibrillar pericellular structures, as visualized by immunofluorescence. Fig. 7 shows the loss of pericellular matrix fibronectin in subconfluent cultures of human fibroblasts treated by chymase. The intracellular fibronectin, shown in other studies (50) to be located in the rough endoplasmic reticulum, appeared unaltered. Both chymase and cathepsin G as well as trypsin when used at 100 ng/ml removed matrix.
fibronectin within 2 h, while plasmin even at 1000 ng/ml removed only a part.

To study further the effects of the tissue proteinases on matrix proteins, the pericellular cell-free matrix attached to the culture plate was isolated from metabolically labeled fibronectin within 2 h, while plasmin even at 1000 ng/ml removed only a part.

To study further the effects of the tissue proteinases on matrix proteins, the pericellular cell-free matrix attached to the culture plate was isolated from metabolically labeled

![Fig. 6. Digestion of separated fibroblast and sarcoma cell culture media by cathepsin G. Media from $[^3H]proline$-labeled cultures of human embryonic skin fibroblasts (A) and HT-1080 sarcoma cells (B) were separated and treated by cathepsin G (100 ng/ml) for the time periods indicated (Tracks 2 to 5). Track 1 shows the starting material. The analysis was by 5% NaDodSO$_4$-polyacrylamide gels. Molecular masses are indicated in kilodaltons. FN = fibronectin; pro-$\alpha_1$(IV), pro-$\alpha_2$(IV), pro-$\alpha_1$(I), and pro-$\alpha_2$(I) = type IV and I procollagen $\alpha_1$ and $\alpha_2$ chains; $\alpha_1$(I) and $\alpha_2$(I) = type I collagen $\alpha_1$ and $\alpha_2$ chains.](image)

![Fig. 7. Fibronectin immunofluorescence of subconfluent fibroblast cultures with and without chymase treatment. A, fibroblast stained for fibronectin without pretreatment with chymase. B, fibroblast cultures after a treatment of 1 $\mu$g of chymase/ml for 2 h. Fixation was with p-formaldehyde and acetone to reveal both the fibrillar pericellular staining and the intracellular granular staining. × 800.](image)

![Fig. 8. Treatment of isolated pericellular matrix of cultured human fibroblasts by chymase. Pericellular matrices from $[^3H]proline$-labeled fibroblast cultures were isolated as described under "Materials and Methods." They were overlaid with NaCl/P$i$ with (+) or without (−) chymase (100 ng/ml). The incubation periods were as indicated. Top, the isolated matrices. Bottom, the NaCl/P$i$ supernatants of the same matrices. The analysis was in 5% NaDodSO$_4$-polyacrylamide gels. Molecular masses are indicated in kilodaltons. FN = fibronectin; pro-$\alpha_1$(I) and pro-$\alpha_2$(I) = type I procollagen $\alpha_1$ and $\alpha_2$ chains.](image)

DISCUSSION

The present results show that fibronectin, both in soluble and in matrix forms, is highly susceptible to degradation by the neutral tissue proteinases chymase and cathepsin G. This conclusion is based on the use of pure enzymes and the substrate and the inhibition of both enzymes by PhCH$_2$SO$_2$F. Comparison of fibronectin to other substrates, both plasma and matrix proteins, indicated that it was in several experimental conditions a relatively selective substrate for these enzymes. Furthermore, there was no obvious difference in the susceptibility of plasma fibronectin and the soluble and matrix forms of fibroblast fibronectin to chymase and cathepsin G. Although fibronectin is known to be sensitive to various proteinases (see Ref. 2 and discussion below), its susceptibility.
to chymase and cathepsin G is of particular interest since these enzymes occur in large amounts in tissues. The enzymes also degraded propeptides in interstitial procollagens, converting them to α-sized chains. However, this is unlikely to have relevance since in vivo at least type I procollagens are rapidly processed by specific enzymes to collagens (51). Of the basement membrane proteins studied, fibronectin was clearly more susceptible to the enzymes than type IV procollagen and no degradation of laminin was observed. Under the conditions used, plasmin degraded intact fibronectin apparently more slowly than chymase or cathepsin G, as judged by time course experiments. Plasmin degrades fibronectin in a characteristic pattern, resembling that of tissue proteinases, as described earlier in detail (12, 52). Since tissue proteinases and plasmin are expected to function in different environmental conditions, the relevance of the in vitro comparison of these enzymes is difficult to assess.

Binding to collagen, and to denatured collagen (gelatin) in particular, is a specific property of fibronectin (53–55). The present study shows that the major initial product of digestion by chymase and cathepsin G, the 70,000-dalton polypeptide, and the 40,000-dalton polypeptide apparently derived from it, carry collagen-binding domains of fibronectin. The latter gelatin-binding fragment was also obtained when fibronectin was first bound to gelatin-Sepharose and then treated with the enzymes. More recently we found that, when more enzyme was used per substrate, the 40,000-dalton fragment was converted to a 30,000-dalton polypeptide which bound to gelatin. In analogous studies with other enzymes, gelatin-binding activity has been located in a 72,000-dalton fragment in cathepsin D-treated plasma fibronectin (56), a 40,000-dalton chymotryptic fragment (57, 58), 70,000- and 30,000-dalton tryptic fragments (59), and 50,000- and 30,000-dalton fragments produced by subtilisin (60). Of the above enzymes, chymase and cathepsin G are the ones that are more likely to act on tissue fibronectin, because they are present in mammalian tissues and are evidently also excreted extracellularly. Hence, in studies on the possible biological role of the collagen-binding fragments, these enzymes should be preferred.

In a previous study, McDonald et al. (61) reported that fibronectin is a sensitive target of proteases released from polymorphonuclear leukocyte granules. These effects are probably due to cathepsin G and possibly also to elastase, both of which are major neutral proteases in neutrophil leukocytes and are known to be located in their azurophilic granules (29).

Chymase and cathepsin G, as discussed in the introduction, resemble each other in both molecular and enzymatic properties. In the present study these two enzymes, even though derived from different species, rat and human, had rather similar effects on fibronectin. Both chymase and cathepsin G are especially basic proteins. Fibronectin is known to bind positively charged molecules such as polyamines (62). An ionic interaction with a cationic enzyme and negatively charged binding sites in fibronectin could mediate the selective susceptibility of it to the action of proteinases. The effects of chymase in vivo are apparently regulated by heparin (25), which by its electrical properties is bound to chymase when it is released from the mast cell granules.

The appearance and distribution of fibronectin in vivo make it a plausible substrate for proteolysis in various physiological and pathological conditions. Unlike other matrix proteins, no mechanical role has been attributed to fibronectin. On the contrary, there is evidence that it has a temporary role during the early stages of connective tissue formation. It is deposited early in developing tissues, is abundant in loose connective tissue, and disappears during maturation of connective tissue matrices (24, 63–65).

We can only speculate on the physiological role of the tissue proteinases in degradation of fibronectin in vivo. Activation of both mast cells and neutrophil leukocytes occurs in inflammatory conditions, that of mast cells in particular in immediate hypersensitivity reactions, in which degradation of the basement membrane may contribute to the increase in vascular permeability. Conceivably, in migration of neutrophils from the circulation to the tissues, penetration of the fibronectin-containing matrix in the basement membranes and the interstitium involves release of neutral proteinases.

Acknowledgments—We thank Dr. Jeremy Saklatvala for purified cathepsin G, Dr. Rupert Timpl for purified laminin, Drs. Phyllis M. Starkey and Alan J. Barrett for valuable discussions on the tissue proteinases, and Ms. Anja Virtanen for expert technical assistance.

REFERENCES
1. Mosesson, M. W. (1973) Thromb. Haemostasis 38, 742–750
2. Vaheri, A., and Mosher, D. F. (1978) Biochim. Biophys. Acta 516, 1–25
3. Yamada, K. M., and Olden, K. (1978) Nature 275, 179–184
4. Stenman, S., and Vaheri, A. (1978) J. Exp. Med. 147, 1054–1064
5. Mosesson, M. W., and Umfleet, R. A. (1970) J. Biol. Chem. 245, 5728–5736
6. Chen, A. B., Mosesson, W. M., and Solash, G. I. (1976) Am. J. Obstet. Gynecol. 125, 986–991
7. Kuusela, P., Vaheri, A., Palo, J., and Ruoslahti, E. (1978) J. Lab. Clin. Med. 92, 595–601
8. Vartio, T., Vaheri, A., von Essen, R., Isomäki, H., and Stenman, S. (1980) submitted for publication
9. Mosesson, M. W., Chen, A. B., and Hiseby, R. M. (1975) Biochim. Biophys. Acta 386, 509–524
10. Mosher, D. F. (1975) J. Biol. Chem. 250, 6614–6621
11. Wagner, D. D., and Hynes, R. O. (1979) J. Biol. Chem. 254, 6746–6754
12. Kurkinen, M., Vartio, T., and Vaheri, A. (1980) Biochim. Biophys. Acta 624, 490–498
13. Bray, B. A. (1978) J. Clin. Invest. 62, 745–752
14. Ruoslahti, E., and Vaheri, A. (1974) Nature 248, 789–791
15. Birdwell, C. R., Gospodarowicz, D., and Nicolson, G. L. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3273–3277
16. Jaffe, E. A., and Mosher, D. F. (1978) J. Exp. Med. 147, 1793–1797
17. Macarak, E. J., Kirby, E., Kirk, T., and Kefalides, N. A. (1978) Proc. Natl. Acad. Sci. U. S. A. 73, 2621–2625
18. Voss, B., Alam, S., Rauterberg, J., Ulrich, K., Gieselmann, V., and von Figura, K. (1979) Biochem. Biophys. Res. Commun. 90, 1348–1354
19. Hynes, R. O. (1976) Biochim. Biophys. Acta 458, 73–107
20. Yamada, K. M., Yamada, S. S., and Pastan, I. (1978) Proc. Natl. Acad. Sci. U. S. A. 73, 1217–1221
21. Ali, I. U., Mautner, V., Lanza, R., and Hynes, R. O. (1977) Cell 11, 115–126
22. Höök, M., Rubin, K., Oldberg, Å., Obrink, B., and Vaheri, A. (1977) Biochem. Biophys. Res. Commun. 79, 726–733
23. Ali, I. U., and Hynes, R. O. (1978) Cell 14, 439–436
24. Kurkinen, M., Aitala, K., Vaheri, A., Stenman, S., and Saxén, L. (1979) Dec. Biol. 69, 589–600
25. Pastan, I., and Alnqvist, S. (1966). J. Biol. Chem. 241, 5900–5904
26. Seppa, H. F. J., and Järvinen, M. (1978) J. Invest. Dermatol. 70, 84–99
27. Woodbury, R. G., Everitt, M., Sanada, Y., Katunuma, N., Lagunoff, D., and Neurath, H. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5311–5313
28. Haas, R., Heinrich, P. C., and Sasse, D. (1979) FEBs Lett. 103, 168–171
29. Starkey, P. M. (1977) in Proteins in Mammalian Cells and Tissues (Barrett, A. J., ed) pp. 57–89, Elsevier/North-Holland Biomedical Press, Amsterdam
30. Austen, K. F. (1978) J. Immunol. 121, 793–805
31. Yurt, R., and Austen, K. F. (1977) J. Exp. Med. 146, 1405–1419
32. Starkey, P. M., and Barrett, A. J. (1976) Biochem. J. 155, 255–263
33. Starkey, P. M., and Barrett, A. J. (1976) Biochem. J. 155, 273–278
Fibronectin and Tissue Proteinases

34. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
35. Baugh, R. J., and Travis, J. (1976) Biochemistry 15, 836-841
36. Vuento, M., and Vaheri, A. (1979) Biochem. J. 183, 331-337
37. Laemmli, U. K. (1970) Nature (Lond.) 227, 680-685
38. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) Biochemistry 10, 2606-2617
39. Laskey, R. A., and Mills, A. D. (1975) Eur. J. Biochem. 56, 335-341
40. Rice, R. H., and Means, G. E. (1971) J. Biol. Chem. 246, 831-832
41. Timpl, R., Rohde, H., Robey, P. G., Rennard, S. I., Foidart, J.-M., and Martin, G. R. (1979) J. Biol. Chem. 254, 9933-9937
42. Hedman, K., Kurkinen, M., Alitalo, K., Vaheri, A., Johansson, S., and Håkkinen, M. (1979) J. Cell Biol. 81, 83-91
43. Stenman, S., Wartiovaara, J., and Vaheri, A. (1977) J. Cell Biol. 74, 453-467
44. Hedman, K., Vaheri, A., and Wartiovaara, J. (1978) J. Cell Biol. 76, 748-760
45. Ouchterlony, O. (1958) Prog. Allergy V, 1-78
46. Mosher, D. F., and Vaheri, A. (1978) Exp. Cell Res. 122, 323-334
47. Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963) Biochem. J. 89, 114-123
48. Vaheri, A., Kurkinen, M., Lehto, V.-P., Linder, E., and Timpl, R. (1979) Proc. Natl. Acad. Sci. U. S. A. 75, 4944-4948
49. Alitalo, K., Vaheri, A., Krieg, T., and Timpl, R. (1980) Eur. J. Biochem. 109, 247-255
50. Hedman, K. (1980) J. Cell Biol., in press.
51. Bornstein, P., and Traub, W. (1979) in The Proteins (Neurath, H., and Hill, R. L., eds) Vol. IV, pp. 411-632, Academic Press, New York
52. Jilek, F., and Hormann, H. (1977) Hoppe-Seyler's Z. Physiol. Chem. 358, 1165-1168
53. Engvall, E., and Ruoslahti, E. (1977) Int. J. Cancer 20, 1-5
54. Dessau, W., Adelmann, B. C., Timpl, R., and Martin, G. R. (1978) Biochem. J. 169, 55-59
55. Kleinman, H. K., Martin, G. R., and Fishman, P. H. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3367-3371
56. Balian, G., Click, E. M., Crouch, E., Davidson, J. M., and Bornstein, P. (1979) J. Biol. Chem. 254, 1429-1432
57. Hahn, L.-H. E., and Yamada, K. M. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1160-1163
58. Hahn, L.-H. E., and Yamada, K. M. (1979) Cell 18, 1043-1051
59. Ruoslahti, E., Hayman, E. G., Kuusela, P., Shively, J. E., and Engvall, E. (1979) J. Biol. Chem. 254, 6054-6059
60. Gold, L. I., Garcia-Pardo, A., Frangione, B., Franklin, E. C., and Pearlstein, E. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4803-4807
61. McDonald, J. A., Baum, B. J., Rosenberg, D. M., Kelman, J. A., Brin, S. C., and Crystal, H. G. (1979) Lab. Invest. 40, 350-357
62. Vuento, M., and Vaheri, A. (1978) Biochem. J. 175, 333-336
63. Linder, E., Vaheri, A., Ruoslahti, E., and Wartiovaara, J. (1975) J. Exp. Med. 142, 41-49
64. Wartiovaara, J., Leivo, I., and Vaheri, A. (1979) Dev. Biol. 69, 247-257
65. Thesleff, I., Stenman, S., Vaheri, A., and Timpl, R. (1979) Dev. Biol. 70, 116-126