Degradation of basement membranes by human matrix metalloproteinase 3 (stromelysin)

Pablo A. BEJARANO, Milton E. NOELKEN, Ko SUZUKI, Billy G. HUDSON and Hideaki NAGASE*
Department of Biochemistry, University of Kansas Medical Center, Kansas City, KS 66103, U.S.A.

Connective tissue cells synthesize and secrete a group of matrix metalloproteinases (MMPs), all of which are capable of degrading the extracellular-matrix components. One of them, MMP-3 (stromelysin) has been shown to degrade purified basement-membrane components, collagen IV and laminin [Okada, Y., Nagase, H. & Harris, E. D., Jr. (1986) *J. Biol. Chem.* **261**, 14245–14255]. Here we report that MMP-3 degrades collagen IV and laminin in intact basement membranes from bovine glomeruli (GBM) and bovine anterior-lens capsules (LBM). Degradation products were analysed by SDS/polyacrylamide-gel electrophoresis to determine the number and sizes of polypeptide fragments. Immunoblotting techniques were used to identify the origins of the fragments, i.e. collagen IV or laminin. The fragments of collagen IV were further mapped using specific antibodies that recognize the *N*-terminal (7 S) domain, the *C*-terminal (NC-1) domain, or the major triple-helical region between the terminal domains. Degradation of collagen IV was extensive; many fragments were found, from both GBM and LBM, in the M_r range 25000–380000. A large fragment of laminin ($M_r > 380000$) was found in the GBM digests without reduction, but it dissociated into 220000- M_r chains upon reduction. The results suggest that MMP-3 plays an important role in the catabolism of basement membranes.

INTRODUCTION

The basement membranes (BMs) located at the junction of epithelial cells and connective tissue play key roles in various biological processes, such as ultrafiltration of blood (Farquhar *et al.*, 1982), embryonic development, and maintenance of tissue architecture during remodelling and repair (Hay, 1984; Kefalides *et al.*, 1985). They also serve as a barrier to invasion and metastasis of malignant cancer cells (Schirmacher, 1985). The major components of BMs are type IV collagen and laminin. Collagen IV is considered to be the scaffold to which other BM constituents interact to form a functional extracellular matrix. The protomeric form of collagen IV is a triple-helical molecule comprised of two distinct α chains. In BM, the protomers form a supramolecular structure which is stabilized by end-to-end tetrameric association at the *N*-terminal (7 S) domain, dimeric association at the *C*-terminal non-collagenous (NC-1) domain and by lateral non-covalent associations (Timpl *et al.*, 1981). These interactions are further stabilized by intermolecular cross-links. Laminin, isolated from Engelbreth-Holmes-Schwarm (EHS) tumours is comprised of either two (Cooper *et al.*, 1981) or three (Palm *et al.*, 1985) similar B chains of M_r 230000 and one A chain of M_r 400000 (Timpl *et al.*, 1979). However, it is suggested that laminin of glomerular basement membrane (GBM) and anterior-lens-capsule basement membrane (LBM) may lack A chains (Mohan & Spiro, 1986). Other minor BM components characterized are entactin and proteoglycans (Carlin *et al.*, 1981; Kanwar & Farquhar, 1979).

Degradation of BMs occurs in many pathological conditions (Timpl & Dziadek, 1986) and increased levels

of fragments of collagen IV and laminin in biological fluids have been found. For example, serum and urine levels of laminin and collagen IV increase when rats become nephrotic (Jukkola *et al.*, 1985). Increased serum levels of collagen IV 7 S domain and pepsin-resistant laminin fragment P1 (Högemann *et al.*, 1986) have been reported in diabetic patients.

Certain proteinases secreted from connective-tissue cells (Sellers *et al.*, 1978; Vaes *et al.*, 1978; Meikle *et al.*, 1980; Murphy *et al.*, 1981; Okada *et al.*, 1986), neutrophils (Hibbs *et al.*, 1985), macrophages (Garbisa *et al.*, 1986) and tumour cells (Dabbous *et al.*, 1977), have been implicated in the degradation of BMs. Recently, we isolated a neutral metalloproteinase from the culture medium of rheumatoid synovial cells stimulated with the macrophage-conditioned medium that digests type IV collagen and laminin (Okada *et al.*, 1986). The enzyme referred to as matrix metalloproteinase 3 (MMP-3) also degrades proteoglycans, fibronectin, and gelatin (Okada *et al.*, 1986), and activates procollagenase (Ito & Nagase, 1988). MMP-3 is synthesized and secreted from connective-tissue cells as a set of zymogens of M_r 57000 and 59000 which can be activated to active species of M_r 45000 and 28000 by either proteinases or an organic mercurial compound (Okada *et al.*, 1988). The properties of MMP-3 are similar to those of rabbit stromelysin (Galloway *et al.*, 1983; Chin *et al.*, 1985), and the identity of the two in man has been established by cDNA sequencing (Whitham *et al.*, 1986; Saus *et al.*, 1988). The primary structure of MMP-3 has revealed that human proMMP-3 is equivalent to the previously described major protein product (M_r 53000) called 'transin' secreted from rat fibroblasts either treated with epidermal

Abbreviations used: BM, basement membrane; LBM, anterior-lens-capsule basement membrane; GBM, glomerular basement membrane; MMP, matrix metalloproteinase; SDS/PAGE, SDS/polyacrylamide-gel electrophoresis; APMA, 4-aminophenylmercuric acetate; EHS, Engelbreth-Holmes-Schwarm tumour.

* To whom correspondence should be sent.

growth factor or transformed by oncogenic viruses (Matrisian *et al.*, 1985, 1986). These observations suggest that MMP-3 plays a key role in the normal catabolism of basement membranes and in the degradation of basement membrane during tumour metastasis. This supposition is based largely on our previous findings that MMP-3 degrades the monomeric forms of purified collagen IV and laminin (Okada *et al.*, 1986). However, their susceptibility to MMP-3 may be distinctly different in intact basement membranes because collagen IV and laminin exist as complex supramolecular structures that are stabilized by a multiplicity of non-covalent and covalent interactions.

The purpose of the present study was to examine the action of purified human MMP-3 on collagen IV and laminin as they exist in two basement membranes (LBM and GBM) of avascular and vascular origins, respectively. Digestion products were analysed by SDS/polyacrylamide-gel electrophoresis (PAGE) and immunoblotting techniques. The results indicate that MMP-3 is a potent BM-degrading enzyme.

EXPERIMENTAL

Materials

Bovine kidneys were collected from a local slaughterhouse. Bovine lenses were obtained from Pel Freez Biologicals, Rogers, AR, U.S.A. Specific antibodies against each of the three main domains of bovine GBM type IV collagen were raised in rabbits, as described previously (Dean *et al.*, 1983; Butkowski *et al.*, 1985). Anti-7 S antibody was directed against the *N*-terminal cross-linking domain, a tetrameric structure comprising approximately 12% of the collagen molecule from the *N*-terminus. Anti-(NC-1) antibody was directed against the *C*-terminal 13% of the molecule. Anti-(type IV) antibody was raised against reduced and alkylated collagen type IV. This reacts primarily with the large central triple-helical region (about 1270 residues) between the 7 S and NC-1 domains, which we refer to as the triple-helical domain to distinguish it from the former two domains. Anti-(type IV) antibody also affords some redundancy in detecting the NC-1 domain as it partially reacts with the M_r 25000 monomeric and M_r 50000 dimeric NC-1 domains obtained after bacterial collagenase digestion of GBM. However, it does not react with the 7 S domain. Rabbit anti-(mouse EHS tumour laminin) serum was from GIBCO Laboratories, Grand Island, NY, U.S.A. Affi-Gel 10 was from Bio-Rad, Richmond, CA, U.S.A. Horseradish peroxidase-conjugated pig anti-(rabbit IgG) IgG, protein standards for SDS/PAGE, and bovine serum albumin were from Sigma Chemical Co., St. Louis, MO, U.S.A. Prestained protein standards were from Bethesda Research Laboratories, Bethesda, MD, U.S.A. The bicinchoninic acid protein-assay reagents were from Pierce Chemical Co., Rockford, IL, U.S.A.

Isolation of bovine GBM and LBM

All BM preparations were carried out at 0–4 °C in the presence of proteinase inhibitors (1 mM-phenylmethanesulphonylfluoride, 10 mM-EDTA, and 25 mM-6-aminohexanoic acid). Bovine GBM was prepared as described by Freytag *et al.* (1976). Briefly, kidney cortex was shaved into 0.15 M-NaCl containing proteinase inhibitors and was homogenized. Glomeruli separated from the

homogenate by a sieving technique (Spiro, 1967) were sonicated for 2 min at power setting 6 (Branson Sonifier, $\frac{1}{2}$ inch probe), and the suspension passed through a 200-mesh stainless-steel sieve to remove DNA. The membrane was sedimented by centrifugation, washed three times with 1 M-NaCl and once with distilled water, lyophilized and stored at –20 °C. LBM was prepared from bovine anterior-lens capsules according to Peczon *et al.* (1982), but without deoxycholate. The lenses were dissected from eyes, and the equatorial portions containing the zonular fibres trimmed off with a scalpel. The anterior capsules were sonicated in 1 M-NaCl for 3–6 min to remove epithelial cell debris.

Purification of human MMP-3

ProMMP-3 was purified from the culture medium of human rheumatoid synovial fibroblasts stimulated with rabbit macrophage-conditioned medium. The culture medium (100 ml) was directly applied to a column of anti-(MMP-3) IgG (93 mg) coupled to Affi-Gel 10 (25 ml). Bound proMMP-3 was eluted with 50 mM-Tris/HCl, pH 7.5, containing 0.2 M-NaCl, 5 mM-CaCl₂, 0.02% NaN₃ and 6 M-urea, and immediately dialysed against the above solution without urea. The yield of proMMP-3 was about 80%. The purified proMMP-3 was homogeneous on SDS/PAGE, exhibiting a typical doublet of M_r 57000 and M_r 59000. The activation of proMMP-3 was carried out with 1.5 mM-4-aminophenylmercuric acetate (APMA). One microgram of fully activated MMP-3 produced 0.87 µg of reduced carboxymethylated transferrin fragments soluble in 3.3% (w/v) trichloroacetic acid in 1 min at 37 °C.

Digestion of BM

Lyophilized GBM (5 mg) was suspended in 550 µl of 50 mM-Tris/HCl (pH 7.8)/0.15 M-NaCl/10 mM-CaCl₂/0.02% NaN₃ and incubated with 6 µg of proMMP-3, which was activated with 1.5 mM-APMA immediately before starting digestion. Digestions were carried out at 37 °C or 22 °C, and terminated by the addition of 15 mM-EDTA at times 0, 12, 24 and 48 h. The same procedures were carried out with LBM from half of an anterior-lens capsule washed previously several times in water and buffer.

Determination of protein and hydroxyproline

The amount of protein released from GBM by MMP-3 and the MMP-3 concentration were determined by the bicinchoninic acid protein-assay method of Smith *et al.* (1985) with bovine serum albumin as standard. The amount of hydroxyproline released from GBM by MMP-3 was measured according to the method of Huszar *et al.* (1980).

SDS/PAGE

The MMP-3 digestion products of the basement membrane (soluble material) were analysed by SDS/PAGE as described by Laemmli (1970) using linear gradients of 4–22% polyacrylamide under reducing or non-reducing conditions. Gels were stained with Coomassie Brilliant Blue R-250. To estimate the size of polypeptides, globular protein standards and calf-skin collagen type I were used after reduction and alkylation. For immunoblotting, separated proteins in unstained gels were electrophoretically transferred to nitrocellulose papers as described by Burnette (1981). Nitrocellulose was treated with antisera

Fig. 1. Degradation of bovine GBM by MMP-3

GBM (5 mg) was incubated with 6 μ g of MMP-3 in 550 μ l of 50 mM-Tris/HCl (pH 7.5)/0.15 M-NaCl/10 mM- CaCl_2 /0.02% NaN_3 /0.05% Brij 35 at 37 $^\circ\text{C}$ for various periods of time. The reaction was stopped by 30 μ l of 500 mM-EDTA and the insoluble membranes removed by centrifugation. Portions of the supernatant were measured for hydroxyproline and protein concentrations. The total hydroxyproline of the undigested GBM was measured after hydrolysing the membrane with 4 M-NaOH at 100 $^\circ\text{C}$ for 90 min.

and the antigen-antibody complexes were visualized by peroxidase-conjugated swine anti-(rabbit IgG) IgG as described by Butkowski *et al.* (1985), a modification of the metal-enhanced diaminobenzidine reaction method of De Blas & Cherwinski (1983). Prestained protein standards were used for the estimation of M_r .

RESULTS

Degradation of GBM and LBM by MMP-3

Incubation of GBM (5 mg) with 6 μ g of MMP-3 at 37 $^\circ\text{C}$ over a period of 48 h showed an effective degradation of GBM as measured by the release of hydroxyproline and protein (Fig. 1). Although the solubilization of the GBM components was not linear with the incubation time, the results demonstrate that 6 μ g of MMP-3 was capable of degrading about 1 mg of protein and releasing about 20 μ g of hydroxyproline from 5 mg of GBM (containing 340 μ g of hydroxyproline) during the first 18 h-incubation period under the specified conditions.

We then analysed the degradation products of GBM and LBM by SDS/PAGE. BMs were incubated with MMP-3 at 22 $^\circ\text{C}$ and 37 $^\circ\text{C}$ for various periods of time. After terminating the reaction with EDTA, the digests were subjected to SDS/PAGE with (Figs. 3 and 5) or without (Figs. 2 and 4) reduction. MMP-3 was shown to be effective at degrading GBM at both 22 $^\circ\text{C}$ and 37 $^\circ\text{C}$. Most species of the digestion products were observed after a 12 h incubation (Figs. 2a and 3a), and increased after 24 h and 48 h, indicating that the majority of these proteolytic fragments were the end-products of MMP-3 digestion. Enzymic action of MMP-3 on GBM was inhibited by 25 mM-EDTA. In contrast with GBM, the nature of the digestion products of LBM was temperature dependent. At 37 $^\circ\text{C}$ about 20 bands, with several major ones, were detected after a 24 h incubation, while only about four bands were observed at 22 $^\circ\text{C}$ even after a

48 h incubation. The most striking result from the digestion of LBM is the appearance of a band of M_r 24000, which represents the first major fragment produced by MMP-3 (Fig. 4). This fragment may be derived from a component of LBM that is highly susceptible to proteolysis. The presence of this fragment to some extent, even without MMP-3 digestion, suggests that it was also produced by endogenous proteinases. Other major bands were produced only after allowing the digestion to proceed beyond 12 h at 37 $^\circ\text{C}$.

The SDS/PAGE patterns of fragments derived from GBM were more complex than those from LBM. This difference is probably due to the greater structural complexity of GBM. For example, the collagen content of GBM is only about 40–45% by wt. (West *et al.*, 1980), whereas the collagen content of LBM is greater than 90% (Kefalides *et al.*, 1985). Other compositional differences between the two membranes have been reported (Mohan & Spiro, 1986; Butkowski *et al.*, 1987).

Identification of collagen type IV fragments in digests

The identification was carried out with 37 $^\circ\text{C}$ digests by the immunoblotting techniques using anti-(type IV collagen), anti-7 S and anti-(NC-1) antibodies.

GBM. By using anti-(type IV collagen), about 12 bands were observed under non-reducing conditions (Fig. 2b). Ten of these have apparent M_r in the range of 23000 to about 170000, and among them five bands showed strong positive staining with apparent M_r of about 23000, 40000, 68000, 110000 and 170000. Additional faint bands present at higher M_r barely penetrated the gel. The highest M_r band contains components derived from the N-terminal domain of collagen type IV, as shown by its reactivity with anti-7 S antibody (Fig. 2d). A band in the same location was found with Coomassie Blue staining (Fig. 2a). Upon reduction, nine major bands with M_r of 25000, 35000, 42000, 52000, 65000, 70000, 95000, 180000 and 200000 were detected with anti-(type IV) antibody (Fig. 3b), and several components readily detectable with anti-7 S antibody were found with apparent M_r of 33000, 64000, 90000, 100000, 110000, 150000, 190000, 220000, and other higher M_r components (Fig. 3d). The pattern detected by anti-7 S is similar to that observed for the 7 S domain of mouse-tumour-basement-membrane collagen type IV (M_r of 27000, 54000, 80000, 106000, 131000 and 153000) obtained after bacterial collagenase digestion (Risteli *et al.*, 1980). Thus MMP-3 releases from GBM a large disulphide-cross-linked fragment similar to the 7 S domain characterized for mouse-tumour collagen IV.

Immunoblotting analyses with anti-(NC-1) antiserum revealed three readily detectable bands with apparent M_r of 38000, 46000 and 75000 under non-reducing conditions (Fig. 2c). A similar pattern is obtained after reduction (Fig. 3c), although the intensity of staining was decreased. This decreased staining of the MMP-3 digest of GBM after reduction was not due to the degradation of the NC-1 domain by MMP-3, as was confirmed by the following experiments with NC-1 domain obtained after bacterial collagenase digestion of NC-1. (1) Reduction of the NC-1 domain disulphide bonds made it less reactive with anti-(NC-1) antibody, and (2) the NC-1 domain was not hydrolysed by MMP-3 to any appreciable extent even after incubation for 48 h at 37 $^\circ\text{C}$. A similar decrease

Fig. 2. SDS/PAGE and immunoblotting analyses of MMP-3 digests of bovine GBM without reduction

GBM (5 mg) was incubated at either 22 °C or 37 °C with 6 μ g of MMP-3 in a total volume of 550 μ l. After 0, 12, 24 or 48 h, the digestion was terminated by adding 30 μ l of 500 mM-EDTA. Solubilized GBM was separated from insoluble GBM by centrifugation. For Coomassie Blue staining (a), 25 μ l of the supernatants was used. The time of incubation (h) is indicated at the top of each lane. Protein M_r standards (st) were: calf-skin collagen (γ chain, 384 000; β chains, 266 000 and 244 000; α chains, 133 000 and 122 000); bovine serum albumin (66 000); ovalbumin (43 000); glyceraldehyde-3-phosphate dehydrogenase (36 000); carbonic anhydrase (29 000); trypsinogen (24 000); soyabean trypsin inhibitor (20 000); and α -lactalbumin (14 500). EDTA 48 refers to a control incubated for 48 h in which the MMP-3 was inhibited by EDTA, and NE 48 is a control incubated without enzyme for 48 h. Immunoblotting (b–e) was carried out as described in the Experimental section after electrophoresis of 12.5 μ l samples of digest at 37 °C. The antibodies used were: anti-(type IV) collagen (b; COL-IV); anti-(NC-1) (c; NC-1); anti-7 S (d; 7 S); and anti-laminin (e; LAM). The prestained protein markers were: myosin (203 000); phosphorylase b (102 000); bovine serum albumin (67 000); ovalbumin (42 000); α -chymotrypsinogen (25 000); β -lactoglobulin (18 000); and lysozyme (15 000).

Fig. 3. SDS/PAGE and immunoblotting analyses of MMP-3 digests of bovine GBM in the presence of 2-mercaptoethanol

See the legend to Fig. 2 for details.

in the immunoreactivity of the NC-1 domain after reduction was reported by Schuppan *et al.* (1986). The M_r values of the bands, 38 000, 46 000 and 75 000, are greater than the values of 25 000 and 45 000 found for NC-1 monomeric and dimeric domains produced by bac-

terial collagenase digestion (Butkowski *et al.*, 1985), indicating limited degradation of type IV collagen by MMP-3 at the triple-helical domain. The relative resistance of the triple-helical domain to MMP-3 was also demonstrated by anti-(type IV collagen) antibody (Fig. 3b).

Fig. 4. SDS/PAGE and immunoblotting analysis of MMP-3 digests of bovine LBM without reduction

LBM (one-half lens, about 1 mg of collagen IV content) was incubated with MMP-3 and the solubilized material analysed as described in the legend to Fig. 2.

Fig. 5. SDS/PAGE and immunoblotting analysis of MMP-3 digests of bovine LBM in the presence of 2-mercaptoethanol

See legends to Figs. 2 and 4 for details.

LBM. About 16 fragments were found to react with anti-(collagen IV) antibody in LBM digests under non-reducing conditions (Fig. 4b). Of these 14 have M_r in the range 13000–230000, and two components are of higher apparent M_r . The latter fragments contain the *N*-terminal (7 S) domain of collagen IV as shown by reactivity with anti-7 S antibody (Fig. 4d). A band in the same position was found with Coomassie Blue staining (Fig. 4a). Upon reduction several smaller, readily detectable fragments

were found with M_r of 33000, 65000, 100000, 110000, 200000 and 220000 (Fig. 5). In addition, there were materials with M_r greater than 220000. The overall patterns of immunoblots obtained with anti-7 S in LBM digests were very similar to those found with GBM digests.

Anti-(NC-1) antibody revealed five readily detectable bands (Fig. 4c). Two of these (M_r 29000 and 30000) have no counterparts in the GBM digest, and the other three

(M_r 42000, 54000 and 83000) have M_r of about 4000 to 8000 larger than the three NC-1 positive bands from GBM. These fragments were larger than those obtained after bacterial collagenase digestion. The LBM digest also showed decreased reactivity with anti-(NC-1) antibody after reduction of disulphide bonds (Fig. 5b).

The susceptibility of the triple-helical domain of LBM collagen type IV to MMP-3 is further illustrated by the immunoblot for collagen IV (Fig. 4b). There are several bands in the 23000–170000- M_r range that must originate from the triple-helical domain as they did not react with anti-7 S (Fig. 4d). As was the case with GBM, MMP-3 cleaved LBM collagen IV within the triple-helical domain to yield a 23000- M_r fragment (both with and without reduction) that did not react with either anti-7 S or anti-(NC-1). This indicates that MMP-3 attacks type IV collagen in LBM at several loci within the triple-helical domain.

Identification of laminin fragments in GBM digests

Immunoblotting of GBM digests with anti-laminin antibody demonstrated that MMP-3 digested laminin. The solubilized fragments were large and barely penetrated the polyacrylamide-gradient gel without reduction. Upon reduction, they dissociated into a major polypeptide of M_r 220000 and additional fragments of M_r 110000, 100000, 64000 and 50000 (Fig. 3e). Similar qualitative results were found for LBM, but the relative amounts of fragments were substantially lower than those of the GBM digests (results not shown). However, it could not be determined whether the 220000- M_r fragment was derived from the A chain or B chain of laminin by the antibody used in this study.

DISCUSSION

Connective-tissue cells are capable of synthesizing and secreting at least three distinct MMPs that digest various extracellular-matrix macromolecules. Among them collagenase (MMP-1) specifically digests interstitial collagen types I, II, III (Woolley *et al.*, 1978) and X (Schmid *et al.*, 1986), but not type IV collagen. A second enzyme (MMP-2) preferentially degrades gelatin (Seltzer *et al.*, 1981; Murphy *et al.*, 1985) and has a limited ability to degrade native type IV and type V collagens (Murphy *et al.*, 1985), whereas MMP-3 is capable of degrading many connective-tissue-matrix components, including type IV collagen and laminin (Galloway *et al.*, 1983; Chin *et al.*, 1985; Okada *et al.*, 1986). A GBM-degrading neutral metalloproteinase was reported in rat kidney glomeruli (Nguyen *et al.*, 1986), but its relationship to MMPs has yet to be established. In this report we have demonstrated that MMP-3 purified from human rheumatoid synovial cells effectively degrades native type IV collagen, laminin and other components of GBM and LBM, and the fragments are solubilized from the membrane preparations.

MMP-3 acted on type IV collagen of GBM and LBM at multiple sites. Several prominent fragments were identified in both GBM and LBM digests, but it was not possible to approximate the cleavage loci in the type IV molecules. Recent elucidation of the complete amino acid sequences of human $\alpha 1(IV)$ chain and $\alpha 2(IV)$ chain and the alignment of the two chains have demonstrated that there are 26 areas in the triple-helical domain where the triple helix is interrupted to various extents (Brazel

et al., 1988). Thus it is conceivable that these interrupted regions in the type IV collagen molecule may be the sites where MMP-3 attacks. The M_r of some major fragments (M_r of 25000, 42000, 52000, 70000 and 95000) detected by anti-(type IV) antibody agree closely with the expected sizes of fragments generated by possible hydrolysis at the pepsin-sensitive sites, interruption III, X and XIII [following the nomenclature of Brazel *et al.* (1988)], and near the NC-1 domain. However, the multiple fragments may have resulted from additional proteolytic attack of MMP-3 on the partially denatured type IV collagen chains after one or more initial cleavages of the native molecule. Indeed, the immunoblotting analyses of type IV collagen fragments at 22 °C revealed larger fragments than those observed at 37 °C (results not shown). Such temperature-dependent differences were especially prominent in LBM (Figs. 4 and 5). This is in contrast with the action of type IV collagenolytic metalloproteinases of M_r 70000 isolated from metastatic tumour cells (Liotta *et al.*, 1981; Salo *et al.*, 1983) and from human mononuclear phagocytes (Garbisa *et al.*, 1986). These enzymes degrade isolated type IV collagen near the 'kink' region, about one-quarter of the molecular length away from the N-terminus (Fessler *et al.*, 1984; Garbisa *et al.*, 1986). In contrast with collagen IV, degradation of laminin appears to be limited. Our previous experiments with isolated laminin showed that MMP-3 preferentially digests the A chain of laminin compared with the B chain (Okada *et al.*, 1986). Although A and B chains are not distinguishable by the antibody used for this study, the appearance of 220000- M_r species in GBM digests is consistent with our previous observation.

Although MMP-3 has been shown to be an effective enzyme for degrading BMs, the synthesis of MMP-3 and other MMPs by normal connective tissues is almost negligible. The production of these MMPs by connective-tissue cells is stimulated by treating the cultured cells with various agents, such as interleukin 1, phorbol myristate acetate, urate crystals, agents affecting the cytoskeleton (see Harris *et al.*, 1984, for review), several growth factors (Moscatelli *et al.*, 1986; Edwards *et al.*, 1987) and oncogenic viruses (Matrisian *et al.*, 1985). These observations suggest that the production of MMPs is controlled precisely during normal development and remodelling of tissues. When production is accelerated, MMPs may participate in pathogenesis of various connective-tissue diseases. Induction of MMP-3 synthesis by oncogene-transformed fibroblasts (Matrisian *et al.*, 1985) is indicative of its involvement in tumour cell invasion and metastasis. The present studies provide further evidence that MMP-3 plays a key role in basement-membrane degradation during development and remodelling of tissue and tumour metastasis.

This work was supported by National Institutes of Health Grants AR39189 and DK18381, grants from the Speas Foundation, and American Cancer Society Grant IN-1151.

REFERENCES

- Brazel, D., Pollner, R., Oberbäumer, I. & Kühn, K. (1988) *Eur. J. Biochem.* **172**, 35–42.
- Burnette, N. (1981) *Anal. Biochem.* **112**, 195–203.
- Butkowski, R. J., Wieslander, J., Wisdom, B. J., Barr, J. F., Noelken, M. E. & Hudson, B. G. (1985) *J. Biol. Chem.* **260**, 3739–3747.

- Butkowski, R. J., Langeveld, J. P. M., Wieslander, J., Hamilton, J. & Hudson, B. G. (1987) *J. Biol. Chem.* **262**, 7874–7877
- Carlin, B., Jaffe, R., Bender, B. & Chung, A. E. (1981) *J. Biol. Chem.* **256**, 5209–5214
- Chin, J. R., Murphy, G. & Werb, Z. (1985) *J. Biol. Chem.* **260**, 12367–12376
- Cooper, A. R., Kurkinen, M., Taylor, A. & Hogan, B. L. M. (1981) *Eur. J. Biochem.* **119**, 189–197
- Dabbous, M. K., Roberts, A. N. & Brinkley, B. (1977) *Cancer Res.* **37**, 3537–3544
- Dean, D. C., Barr, J. F., Freytag, J. W. & Hudson, B. G. (1983) *J. Biol. Chem.* **258**, 590–596
- De Blas, A. L. & Cherwinski, H. M. (1983) *Anal. Biochem.* **133**, 214–219
- Edwards, R. D., Murphy, G., Reynolds, J. J., Whitham, S. E., Docherty, A. J. P., Angel, P. & Heath, J. (1987) *EMBO J.* **6**, 1899–1904
- Farquhar, M. G., Courtoy, P. J., Lemkin, M. C. & Kanwar, Y. S. (1982) in *New Trends in Basement Membrane Research* (Kuehn, K., Schoene, H. & Timpl, R., eds.), pp. 9–29, Raven Press, New York
- Fessler, L. I., Duncan, K. G., Fessler, J. H., Salo, T. & Tryggvason, K. (1984) *J. Biol. Chem.* **259**, 9783–9789
- Freytag, J. W., Ohno, M. & Hudson, B. G. (1976) *Biochem. Biophys. Res. Commun.* **72**, 796–802
- Galloway, W. A., Murphy, G., Sandy, J. D., Gavrilovic, J., Cawston, T. E. & Reynolds, J. J. (1983) *Biochem. J.* **209**, 741–752
- Garbisa, S., Ballin, M., Daga-Gordini, D., Fastelli, G., Naturale, M., Negro, A., Semenzato, G. & Liotta, L. A. (1986) *J. Biol. Chem.* **261**, 2369–2375
- Harris, E. D., Jr., Welgus, H. G. & Krane, S. M. (1984) *Collagen Relat. Res.* **4**, 493–512
- Hay, E. D. (1984) in *The Role of Extracellular Matrix in Development* (Trelstad, R. L., ed.), pp. 1–32, Alan R. Liss, New York
- Hibbs, M. S., Hasty, K. A., Seyer, J. M., Kang, A. H. & Mainardi, C. L. (1985) *J. Biol. Chem.* **260**, 2493–2500
- Högemann, B., Voss, B., Altenwerth, F. J., Schneider, M., Rauterberg, J. & Gerlach, U. (1986) *Klin. Wochenschr.* **64**, 382–385
- Huszar, G., Maiocco, J. & Naftolin, F. (1980) *Anal. Biochem.* **105**, 424–429
- Ito, A. & Nagase, H. (1988) *Arch. Biochem. Biophys.*, in the press
- Jukkola, A., Risteli, J., Autio-Harmainen, H. & Risteli, L. (1985) *Biochem. J.* **226**, 243–250
- Kanwar, Y. S. & Farquhar, M. G. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1303–1307
- Kefalides, N. A., Howard, P. & Ohno, N. (1985) in *Basement Membranes* (Shibata, S., ed.), pp. 73–87, Elsevier Science Publishers, Amsterdam
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Liotta, L. A., Tryggvason, K., Garbisa, S., Gehron-Robey, P. & Abe, S. (1981) *Biochemistry* **20**, 100–104
- Matrisian, L. M., Glaichenhaus, N., Gesnel, M.-C. & Breathnach, R. (1985) *EMBO J.* **4**, 1435–1440
- Matrisian, L. M., Bowden, G. T., Krieg, P., Fürstenberger, G., Briand, J.-P., Leroy, P. & Breathnach, R. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9413–9417
- Meikle, M. C., Sellers, A. & Reynolds, J. J. (1980) *Calcif. Tissue Int.* **30**, 77–82
- Mohan, P. S. & Spiro, R. G. (1986) *J. Biol. Chem.* **261**, 4328–4336
- Moscattelli, D., Presta, M. & Rifkin, D. B. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2091–2095
- Murphy, G., Cawston, T. E., Galloway, W. A., Barnes, M. J., Bunning, R. A. D., Mercer, E., Reynolds, J. J. & Burgeson, R. E. (1981) *Biochem. J.* **199**, 807–811
- Murphy, G., McAlpine, C. G., Poll, C. T. & Reynolds, J. J. (1985) *Biochim. Biophys. Acta* **831**, 49–58
- Nguyen, H. H., Baricos, W. H. & Shah, S. V. (1986) *Biochem. Biophys. Res. Commun.* **141**, 898–903
- Okada, Y., Nagase, H. & Harris, E. D., Jr. (1986) *J. Biol. Chem.* **261**, 14245–14255
- Okada, Y., Harris, E. D., Jr. & Nagase, H. (1988) *Biochem. J.* **254**, 731–741
- Palm, S. L., McCarthy, J. B. & Furcht, L. T. (1985) *Biochemistry* **24**, 7753–7760
- Peczon, B. D., McCarthy, C. A. & Merritt, R. B. (1982) *Exp. Eye Res.* **35**, 643–651
- Risteli, J., Bächinger, H. P., Engel, J., Furthmayr, H. & Timpl, R. (1980) *Eur. J. Biochem.* **108**, 239–250
- Salo, T., Liotta, L. A. & Tryggvason, K. (1983) *J. Biol. Chem.* **258**, 3058–3063
- Saus, J., Quinones, S., Otani, Y., Nagase, H., Harris, E. D., Jr. & Kurkinen, M. (1988) *J. Biol. Chem.* **263**, 6742–6745
- Schirmacher, V. (1985) in *Advances in Cancer Research* (Klein, G. & Weinhouse, S., eds.), vol. 43, pp. 1–73, Academic Press, New York
- Schmid, T. M., Mayne, R., Jeffrey, J. J. & Linsenmayer, T. F. (1986) *J. Biol. Chem.* **261**, 4184–4189
- Schuppan, D., Besser, M., Schwarting, R. & Hahn, E. G. (1986) *J. Clin. Invest.* **78**, 241–248
- Sellers, A., Reynolds, J. J. & Meikle, M. C. (1978) *Biochem. J.* **171**, 493–496
- Seltzer, J. L., Adams, S. A., Grant, G. A. & Eisen, A. Z. (1981) *J. Biol. Chem.* **256**, 4662–4668
- Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J. & Klenk, D. C. (1985) *Anal. Biochem.* **150**, 76–85
- Spiro, R. G. (1967) *J. Biol. Chem.* **242**, 1915–1922
- Timpl, R. & Dziadek, M. (1986) in *International Review of Experimental Pathology* (Ritcher, G. W., ed.), vol. 29, pp. 1–112, Academic Press, London
- Timpl, R., Rohde, H., Gehron-Robey, P., Rennard, S. I., Foidart, J.-M. & Martin, G. R. (1979) *J. Biol. Chem.* **254**, 9933–9937
- Timpl, R., Wiedemann, H., Van Delden, V., Furthmayr, H. & Kühn, K. (1981) *Eur. J. Biochem.* **120**, 203–211
- Vaes, G., Eeckhout, Y., Lenaers-Claeys, G., Francois-Gillet, C. & Druetz, J.-E. (1978) *Biochem. J.* **172**, 261–274
- West, T. M., Fox, J. W., Jodlowski, M., Freytag, J. W. & Hudson, B. G. (1980) *J. Biol. Chem.* **255**, 10451–10459
- Whitham, S. E., Murphy, G., Angel, P., Rahmsdorf, H.-J., Smith, B. J., Lyons, A., Harris, T. J. R., Reynolds, J. J., Herrlich, P. & Docherty, A. J. P. (1986) *Biochem. J.* **240**, 913–916
- Woolley, D. E., Glanville, R. W., Roberts, D. R. & Evanson, J. M. (1978) *Biochem. J.* **169**, 265–276