Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2632–2636, April 1989
Biochemistry

Tissue cooperation in a proteolytic cascade activating human interstitial collagenase

CHENGSHI HE, SCOTT M. WILHELM, ALICE P. PENTLAND, BARRY L. MARMER, GREGORY A. GRANT, ARTHUR Z. EISEN, AND GREGORY I. GOLDBERG

Division of Dermatology, Washington University School of Medicine, Saint Louis, MO 63110

Communicated by William H. Daughaday, January 12, 1989

ABSTRACT **We present a cascade of proteolytic events catalyzed by the proteases secreted by cultured keratinocytes and fibroblasts that results in the activation of interstitial procollagenase. Cultured human skin fibroblasts constitutively secrete interstitial collagenase and stromelysin as proenzymes. In contrast, interstitial collagenase found in serum-free skin organ culture conditioned medium is activated. Cocultivation of the major cellular components of skin organ culture, dermal fibroblasts and epidermal keratinocytes, induces activation of interstitial procollagenase and prostromelysin in the presence of plasminogen. This activation occurs through a urokinase-dependent pathway where added keratinocytes secrete the plasminogen activator urokinase, which converts plasminogen into plasmin. Plasmin is capable of activating purified procollagenase and prostromelysin. Plasmin-dependent activation of procollagenase generates an enzyme species, by amino-terminal processing, identical to those generated by limited proteolysis with trypsin or treatment with organomercurial compounds. Catalytic amounts of activated stromelysin can in turn convert plasmin- or trypsin-activated collagenase into a fully active enzyme by removal of ≈15 amino acid residues from the carboxyl end of the enzyme. This results in a 5- to 8-fold increase in collagenase specific activity that is due to its proteolytic cleavage and not to the presence of the activator stromelysin. Stromelysin alone in both pro- and activated forms is not capable of efficient activation of human fibroblast interstitial procollagenase.**

Intensified tissue remodeling during morphogenesis, wound healing, and tumor invasion requires the presence of secreted metalloproteases capable of initiating the degradation of macromolecules of the extracellular matrix. Several enzymes of this class have been identified: fibroblast (1–3) and granulocyte (4, 5) collagenases, which degrade interstitial collagens, type IV collagenase, which degrades both basement membrane collagen and denatured collagen (gelatin) (6–8), and stromelysin, which degrades proteoglycans (9–13). We have determined the primary structure of fibroblast interstitial collagenase (14), stromelysin (12), and type IV collagenase (7), demonstrated a close structural relationship between these proteins (7), and compared their substrate specificities. Analysis of the genomic organization of the human fibroblast interstitial collagenase gene (15) in comparison with that of rabbit collagenase (16) and rat stromelysin (17) revealed that a close structural relationship on the protein level is reflected in the very similar genomic organization of at least two members of the secreted metalloprotease gene family. Interstitial collagenase (1, 18), stromelysin (12), and type IV collagenase (7) are constitutively secreted by cultured human skin fibroblasts in a proenzyme form and are subject to activation extracellularly. Both procollagenase and prostromelysin are secreted in two forms, one of which contains N-linked complex oligosaccharides (2, 12). *In vitro* all three enzymes can be activated by treatment with organomercurial compounds (19–21). Interstitial collagenase and stromelysin can be activated by limited proteolysis with trypsin (12, 17, 21), whereas type IV collagenase is a poor substrate for trypsin activation (7). The mechanism of activation of interstitial procollagenase has been studied in greater detail. We have shown (21) that trypsin-induced activation of procollagenase occurs as a result of an initial cleavage of the peptide bond between Arg-55 and Asn-56, generating a major intermediate of 46 kDa. Treatment of the proenzyme with organomercurials initially results in activation without loss of molecular mass (20, 21). The subsequent conversion of the intermediates into a 42-kDa active enzyme occurs through an intramolecular autoproteolytic reaction removing 81 amino acid residues from the amino terminus of the proenzyme. Trypsin activation of stromelysin similarly involves processing of its amino-terminal end with the removal of 84 amino acid residues (12). Although it has been suggested that several proteolytic enzymes may be involved in the biological activation process (22–27), the physiological pathway of procollagenase activation remains unclear. In this report we describe a proteolytic cascade leading to the complete activation of fibroblast interstitial procollagenase that is likely to serve as a major physiological activation pathway.

MATERIALS AND METHODS

Plasmin, plasminogen, aprotinin, and monoclonal anti-urokinase antibody were from American Diagnostica (Greenwich, CT). [Purified urokinase (Winkinase) was kindly provided by G. Murano (National Institutes of Health).]

Enzyme Purification. Serum-free fibroblast-conditioned medium was adjusted to 10 mM Tris·HCl (pH 7.5) and applied on a 1.6 × 15 cm zinc chelate Sepharose column equilibrated with the buffer containing 20 mM Tris·HCl (pH 7.5), 5 mM $CaCl_2$, and 150 mM NaCl. The column was washed with 1 vol of the same buffer and 1 vol of 20 mM Tris·HCl (pH 7.5) buffer containing 5 mM $CaCl_2$ and 25 mM glycine and developed in the same buffer with a 20–800 mM glycine gradient. Stromelysin was purified further as described (12). Interstitial collagenase eluted after 10–12 column vol of 800 mM glycine buffer. These fractions were dialyzed against L buffer (5 mM Tris·HCl, pH 7.5/0.1 mM $CaCl_2$/0.01% Brij) adjusted to 20 mM Tris·HCl (pH 7.5), 1 mM $CaCl_2$, 50 mM NaCl, and 0.01% Brij and applied to a 1 × 2.5 cm DEAE-Sepharose (Pharmacia) column connected to a 1 × 2.5 cm column of reactive green Sepharose (Sigma). The latter column was developed with a 0.05–2 M NaCl gradient in 20 mM Tris·HCl (pH 7.5) buffer containing 1 mM CaCl and 0.01% Brij. Both proenzymes were homogeneous by silver staining.

Cell Culture and Electrophoretic Transfer (Western) Blotting. Primary human skin fibroblasts (WUN 880080) and keratinocytes were cultured as described (28). Human skin

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: u-PA, urokinase plasminogen activator.

fibroblasts (5×10^4 cells) were plated in a 1-cm^2 well either with (10^5 cells) or without epidermal keratinocytes and incubated in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum for 48 hr. The cells were washed three times with Hanks' buffer and incubated in serum-free medium for 24 hr. Plasminogen was added to cultures in concentrations from 0.1 to 1.0 μg/ml. Monoclonal anti-urokinase plasminogen activator (u-PA) antibody or aprotinin was added to mixed cultures in the presence of plasminogen at a final concentration of 0.2–2.0 μg/ml or 1.0–10 μg/ml, respectively. Samples of 24-hr conditioned medium (200 μl) were dialyzed against L buffer, dried *in vacuo*, and subjected to immunoblot analysis as described (2, 12).

Proenzyme Activation and Enzyme Assays. Activation of procollagenase and prostromelysin with trypsin has been described (2, 12). Activation with plasmin was achieved in 200 μl of conditioned medium with plasmin concentrations ranging from 1 to 20 μg/ml as indicated. Reaction mixtures were incubated at 37°C for up to 1 hr, terminated by the addition of a 5-fold molar excess of aprotinin, and subjected to immunoblot analysis with the corresponding IgG.

Activation of purified procollagenase and prostromelysin with trypsin was performed at 37°C for 15 min using a 1:10 (wt/wt) ratio of trypsin to proenzyme (2, 12). Activation of purified procollagenase or prostromelysin with plasmin was performed at 37°C in 50 μl of 25 mM Tris·HCl (pH 7.5) buffer containing 25 mM NaCl, 5 mM $CaCl_2$, 1 μg of proenzyme, and plasmin at indicated concentrations (1–20 μg/ml). Reactions were terminated by the addition of a 5-fold molar excess of aprotinin; reaction mixtures were divided into two aliquots and used for electrophoretic analysis and activity measurements. The activity of collagenase and stromelysin was determined as described (1, 12).

Complete activation of collagenase with stromelysin was achieved by coactivation using an equimolar amount of procollagenase and prostromelysin, each at an initial concentration of 20 μg/ml with either trypsin or plasmin as described above. Alternatively, procollagenase and prostromelysin were activated separately and mixed at the indicated molar ratios and further incubated at 37°C for 1 hr. After complete activation collagenase was separated from stromelysin using an anti-stromelysin IgG Affi-Gel 10 column (29) or reactive green affinity column. The concentration of activated stromelysin was quantitated by immunodot assays on nitrocellulose (30).

Electroblotting and Sequencing. Samples were electroblotted onto Immobilon poly(vinylidene difluoride) membranes (31) from $NaDodSO_4$/polyacrylamide gels and loaded directly onto an Applied Biosystems 470A gas-phase sequencer (21).

RESULTS

Mixed Cultures of Human Dermal Fibroblasts and Epidermal Keratinocytes Induce Activation of Interstitial Collagenase and Stromelysin in the Presence of Plasminogen. Human skin fibroblasts in tissue culture constitutively secrete interstitial collagenase, stromelysin, and type IV collagenase in proenzyme form (1, 7, 12, 18). Earlier observations (22–25) suggested that interstitial collagenase can be activated by plasmin. However, the mechanism of the physiological pathway of collagenase activation has remained unclear.

As a basis for this investigation we used the observation that collagenase in human skin organ cultures is completely activated under "serum-free" incubation conditions (1, 2). Since epidermal keratinocytes together with dermal fibroblasts constitute the major cellular components of the skin organ culture system, we investigated the status of interstitial collagenase in the serum-free conditioned medium obtained from cocultivation of both types of cells. Under these conditions, the vast majority of collegenase is secreted by the fibroblasts in a proenzyme form (Fig. 1, lanes 1 and 2). Since cultures of dermal keratinocytes have been reported to secrete plasminogen activator of the urokinase type (u-PA) (32) we tested whether the addition of plasminogen to the combined cultures of fibroblasts and keratinocytes would have an effect on the activation of procollagenase. The results of these experiments are presented in Fig. 1 (lane 3) and show that all of the enzyme obtained under these conditions is activated. Keratinocyte-conditioned medium can be substituted for keratinocytes with similar effect (lane 4). The partial activation is most likely due to the continuous secretion of procollagenase by fibroblasts and the limited stability of u-PA during the 24-hr assay. The activation pathway is completely dependent upon the presence of u-PA since procollagenase activation is blocked in the presence of monoclonal anti-u-PA antibody (lane 6). Activation is also abolished in the presence of aprotinin (lane 5). The u-PA present in keratinocyte-conditioned medium can be replaced by a purified preparation of the enzyme (lane 7). Urokinase (lane 8) or plasminogen alone (lane 9) has no effect on the activation of procollagenase in fibroblast-conditioned medium. We concluded that the conversion of procollagenase to the activated enzyme form, observed previously in skin organ culture (1), occurs through a urokinase-plasminogen-dependent pathway.

Plasmin Is Capable of Activating Both Purified Procollagenase and Prostromelysin. We next addressed the question as to whether activation of purified procollagenase with plasmin yields an activated enzyme that is similar to that obtained after trypsin activation and also whether prostromelysin can be activated by plasmin. As shown in Fig. 2, purified procollagenase activated by trypsin (lane 5) or plasmin (lane 4) and the conditioned medium procollagenase activated in the presence of low plasmin concentrations, 1 μg/ml (lane 2), have the same molecular mass on $NaDodSO_4$/PAGE. In a separate experiment purified procollagenase activated by plasmin was separated on $NaDodSO_4$/PAGE, electroblotted onto a filter support (31), and subjected to amino acid sequence analysis. This experiment revealed that the amino-terminal sequence of the 42-kDa plasmin-activated enzyme is identical to that of the enzyme activated by trypsin (21). In agreement with this result, the specific activity of plasmin- or trypsin-activated enzymes was also identical (see below and Table 1, lines 2 and 3). Purified prostromelysin can also be activated by trypsin (lane 7) or plasmin (lane 8).

To answer the question whether prostromelysin and procollagenase can be activated simultaneously by plasmin in

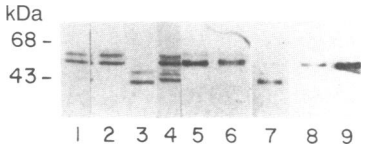

FIG. 1. Activation of procollagenase proceeds through a u-PA-plasmin-dependent pathway during cocultivation of dermal human skin fibroblasts (HSF) and epidermal keratinocytes. Samples of conditioned medium (CM) were treated as described in the text and subjected to immunoblot analysis with anti-collagenase antibody. Lane 1, serum-free CM obtained from HSF (5×10.4 cells per cm^2); lane 2, HSF cocultured with keratinocytes (HSFK, 5×10.4 cells per cm^2); lane 3, HSFK incubated in the presence of 1 μg of plasminogen per ml; lane 5, HSFK + plasminogen as in lane 3 + 10 μg of aprotinin per ml; lane 6, HSFK + plasminogen as in lane 3 + 2 μg of monoclonal anti-urokinase antibody per ml. Keratinocytes and HSFK were substituted by 200 μl of keratinocyte CM with the addition of plasminogen (lane 4) or HSF and incubated in the presence of 30 units of u-PA and plasminogen (lane 7). Lane 8, urokinase alone; lane 9, plasminogen alone.

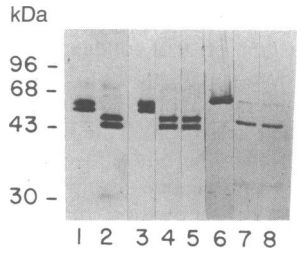

FIG. 2. Plasmin activates both purified interstitial procollagenase and prostromelysin. Procollagenase secreted by human skin fibroblasts in culture (lane 1) was activated by addition of 1 μg of plasmin per ml to fibroblast CM for 1 hr at 37°C (lane 2). One-half microgram of purified procollagenase (lane 3) was activated with 0.5 μg of plasmin under the same conditions (lane 4) or 0.05 μg of trypsin for 15 min at 37°C (lane 5). Purified prostromelysin (lane 6) was activated with plasmin (lane 7) or with trypsin under identical conditions at 37°C (lane 8). The activation reaction was terminated by the addition of a 5-fold molar excess of aprotinin (plasmin) or soybean trypsin inhibitor (trypsin). Samples were subjected to immunoblot analysis using anti-collagenase antibody.

conditioned medium, samples of fibroblast-conditioned medium were treated with different concentrations of plasmin. The results presented in Fig. 3 show that both enzymes were activated by plasmin in the conditioned medium. However, conversion of procollagenase into the lower molecular mass activated enzyme species occurred faster under the same conditions. This result is not due to the presence of inhibitors in the conditioned medium, since the purified enzymes behave in a similar fashion (data not shown). Moreover, at the time when both procollagenase and prostromelysin are activated in the medium, the molecular mass of activated collagenase decreased by an additional 2 kDa (Fig. 3*A*, lane 8).

Activated Stromelysin Completes the Activation of Trypsin- or Plasmin-Activated Collagenase, Resulting in a Marked Increase in Collagenase Specific Activity. Since lower molecular mass species of interstitial collagenase appeared in plasmin-treated fibroblast-conditioned medium at a time when a significant portion of secreted prostromelysin was activated, we investigated the interaction of stromelysin with collagenase using purified enzymes. We previously reported (12) that purified stromelysin in either pro- or activated forms cannot efficiently activate procollagenase. The confirmation of this observation is shown in Fig. 4 *A* and *B*, lanes 3, and Table 1, lines 4 and 5. No significant proteolysis of procollagenase was observed upon treatment with trypsin- or plasmin-activated stromelysin (Fig. 4 *A* and *B*, lanes 3). This is in good agreement with the low levels of activity obtained when procollagenase was treated with either trypsin- or

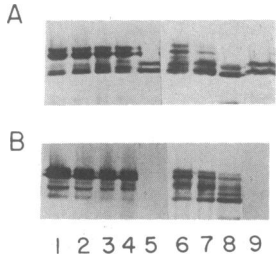

FIG. 3. Comparison of plasmin-catalyzed activation of procollagenase and prostromelysin in fibroblast-conditioned medium (FCM). Samples of FCM were treated as described in the text, divided into two aliquots, and subjected to immunoblot analysis with anti-collagenase (*A*) or anti-stromelysin (*B*) antibody. FCM (lane 1) was treated with 1 μg of plasmin per ml (lanes 2–4) or 20 μg of plasmin per ml (lanes 6–8) at 37°C for 5 min (lanes 2 and 6), 20 min (lanes 3 and 7), and 1 hr (lanes 4 and 8). A sample of trypsin-activated procollagenase is displayed in *A* (lanes 5 and 9) for comparison.

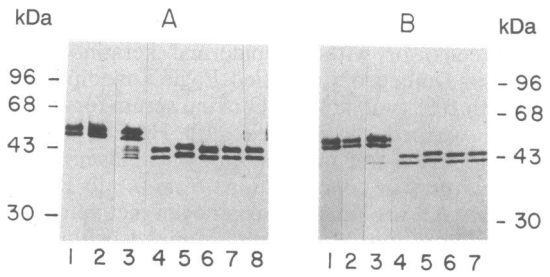

FIG. 4. Activated stromelysin can complete the activation of plasmin- or trypsin-activated collagenase. Purified procollagenase (lanes 1 in *A* and *B*) and prostromelysin were activated by trypsin (*A*) or plasmin (*B*), the reaction was terminated as in Fig. 2, and the samples were subjected to immunoblot analysis with anti-collagenase antibody. Inhibition of trypsin activation of procollagenase by soybean trypsin inhibitor is shown in lane 2 in *A* and inhibition of plasmin activation of procollagenase by aprotinin is shown in lane 2 in *B*. Inhibitors were added at time zero. Lanes 3 in *A* and *B*, purified procollagenase incubated with activated stromelysin. Lanes 4, purified procollagenase coactivated with purified prostromelysin and trypsin (*A*) or with prostromelysin and plasmin (*B*). Trypsin- and plasmin-activated procollagenase (lanes 5 in *A* and *B*) or trypsin- and plasmin-activated collagenase and stromelysin were coincubated at a 1:1 molar ratio (lanes 6 in *A* and *B*) or a 10:1 molar ratio (lanes 7 in *A* and *B*) for 1 hr at 37°C. Lane 8 in *A*, collagenase activated by stromelysin as in lane 7 after removal of stromelysin using an antibody affinity column.

plasmin-activated stromelysin (Table 1, lines 4 and 5). Coactivation of both proenzymes with trypsin leads to the appearance of lower molecular mass collagenase species (Fig. 4*A*, lane 4) concomitant with a 5- to 8-fold increase in the specific activity of the enzyme (Table 1, line 6). Similar results were obtained using plasmin as an activating agent (Fig. 4*B*, lane 4, and Table 1, line 7). In these experiments, coactivation was performed at an equimolar ratio of procollagenase to prostromelysin. Two possible mechanisms can explain these findings. (*i*) Activated stromelysin can catalyze the conversion of trypsin- or plasmin-activated collagenase

Table 1. Collagenolytic activity of fibroblast interstitial collagenase

Sample	cpm/hr	Specific activity, units/mg of enzyme protein
ProCL	30	66
TCL	140	373
PCL	172	458
ProCL + TSL	270	720
ProCL + PSL	251	670
ProCL + PSL (1:1) + T	1104	2943
ProCL + ProSL (1:1) + P	842	2233
TCL + TSL (1:1)	1033	2750
TCL + TSL (10:1)	1130	3013
TCL + TSL (100:1)	806	2150
TCL + TSL (200:1)	715	1906
TCL + TSL (500:1)	676	1803
TCL + TSL (1000:1)	358	953
TCL + TSL (10:1)	941	2517
TCL + TSL (10:1)	772	2058
PCL + PSL (1:1)	856	2282

Purified procollagenase (ProCL) or prostromelysin (ProSL) was activated by trypsin (TCL or TSL) or plasmin (PCL or PSL), and the activity was measured at 37°C for 1 hr using the [^{14}C]collagen fibril assay. The molar ratio of collagenase to stromelysin used in each reaction is indicated in parentheses. After the reaction was completed, stromelysin was separated from activated collagenase by using either an antibody affinity (14) or a reactive green (15) column. Coactivation was achieved by treatment of a procollagenase and prostromelysin mixture with trypsin (6) or plasmin (7).

into lower molecular mass species with higher specific activity. (*ii*) Activated stromelysin forms a complex with collagenase that in turn degrades collagen more efficiently. To ascertain the mechanism of activation we activated both enzymes with trypsin separately. The activated enzymes were then mixed at different ratios and the conversion of collagenase into its activated form was followed by $NaDodSO_4$/PAGE (Fig. 4 *A* and *B*, lanes 5–8) and enzymatic activity (Table 1, lines 8–13). In addition, the activation reaction was performed at a 10:1 ratio of collagenase to stromelysin and the resultant lower molecular mass collagenase was separated from the activating stromelysin using either an anti-stromelysin IgG Affi-Gel 10 column (Fig. 4*A*, lane 8; Table 1, line 14) or a reactive green affinity column (Table 1, line 15). Removal of stromelysin did not affect either the molecular mass or the specific activity of the activated enzyme. We conclude that the complete activation of collagenase to a 40-kDa form is catalyzed by activated stromelysin, resulting in an additional 2-kDa loss in molecular mass from the 42-kDa collagenase species.

To ascertain the mechanism of complete activation of collagenase by stromelysin we determined the amino-terminal sequence of the 40-kDa stromelysin-activated collagenase by electroblotting methods (21, 31). This sequence (data not shown) was identical to the amino-terminal sequence of collagenase activated by trypsin, plasmin, or organomercurial compounds. We conclude that the observed 2-kDa reduction in molecular mass of stromelysin-activated collagenase results from proteolytic cleavage of ≈15 amino acid residues from the carboxyl end of the molecule.

DISCUSSION

The activity of secreted metalloproteases capable of initiating the degradation of extracellular matrix macromolecules is controlled extracellularly by at least two known factors: (*i*) tissue inhibitor of metalloproteases, a universal and specific inhibitor of this class of metalloproteases (2, 33, 34); and (*ii*) all extracellular matrix metalloproteases known to date are secreted as zymogens, which are then subject to extracellular activation.

Although *in vitro* activation of the purified enzymes has been studied in detail (see above), the physiologically relevant pathways of procollagenase and prostromelysin activation remain a subject of speculation. Several proteases have been implicated in the activation of interstitial fibroblast procollagenase. One of the earlier observations by Werb *et al.* (23) pointed to the possible role of plasmin. The presence of a specific activator of procollagenase has also been suggested (26). The molecular mass of this activator was estimated to be similar or identical to that of stromelysin. Several laboratories have cloned human and rat stromelysin (11–13, 17) and characterized it enzymatically (9, 10, 12). Based on some similarities between the putative activator and stromelysin, Brinckerhoff *et al.* (35) postulated that the two were identical.

We have previously reported that purified stromelysin cannot efficiently activate procollagenase (12). Murphy *et al.* (27) and subsequently Ito and Nagase (36) have shown that stromelysin can fulfill the role of a collagenase activator in the presence of trypsin, although the mechanism of action of stromelysin was unclear. The effect of stromelysin in the study by Murphy *et al.* (27) was apparent only under "coactivation" conditions and only at the high (2:1) molar ratio of stromelysin to collagenase. For these reasons the formation of a collagenase–stromelysin complex or an altered pathway of trypsin activation in the presence of an excess of stromelysin, which results in an increase in enzyme activity, could not be excluded. Activation of procollagenase by stromelysin yielded an enzyme with an activity similar to that obtained by trypsin alone. Our data show that stromelysin can partially activate some procollagenase preparations but that mostly intermediate proenzyme conversion products are formed. Activation by stromelysin does not result in the accumulation of the active enzyme species characteristically produced by either trypsin or plasmin activation (Fig. 4, lanes 3, and Table 1). Since collagenase activation is very sensitive to the conformational integrity of the enzyme, this partial activation by stromelysin alone may be an *in vitro* artifact due to the presence of a small enzyme fraction susceptible to the proteolytic cleavage by stromelysin. Based on this observation we conclude that stromelysin alone is unable to convert procollagenase into the fully activated form.

In earlier experiments (1) we observed that activation of procollagenase occurred in human skin organ culture and that serum inhibited the activation reaction. We therefore investigated the possible pathway of the collagenase activation in this system. The following considerations contributed to the experimental design. Dermal fibroblasts and epidermal keratinocytes constitute the major cellular components of skin in organ culture. The amount of collagenase secreted by keratinocytes in these cultures is low in comparison to that secreted by fibroblasts (1). Keratinocytes secrete plasminogen activator of the urokinase type (32) and interleukin 1 (37). The latter stimulates the expression of both interstitial procollagenase and prostromelysin by fibroblasts (38–41). The results clearly show that procollagenase is completely activated when mixed cultures of fibroblasts and keratinocytes are grown in the presence of plasminogen. The pathway is u-PA and plasminogen dependent. Activation can be completely abolished by the addition of either an anticatalytic monoclonal anti-u-PA antibody or aprotinin. Purified plasmin activated both procollagenase and prostromelysin, although the activation of the latter occurred more efficiently at higher plasmin concentrations. Plasmin activation of procollagenase results in complete conversion of the enzyme to the 42-kDa active enzyme form that is identical to that obtained by trypsin activation (21) both in structure (same amino-terminal sequence) and in activity. Plasmin or trypsin activation of procollagenase in the conditioned medium results in an additional reduction in molecular mass of 2 kDa. The further reduction in molecular mass is due to the presence of simultaneously activated stromelysin. Plasmin- or trypsin-activated stromelysin cleaves the carboxyl terminus of plasmin- or trypsin-activated collagenase, removing ≈15 amino acid residues with a concomitant 5- to 8-fold rise in specific activity. Catalytic amounts of stromelysin are sufficient (see Table 1) to complete the activation. Moreover, the effect is due to a true conversion of collagenase since the removal of the stromelysin after activation does not affect the specific activity of the 40-kDa activated enzyme.

To address the question of whether the pathway for the activation of collagenase in mixed cultures of keratinocytes and fibroblasts is the same pathway responsible for collagenase activation in tissue, we have attempted to block the activation of collagenase in skin organ culture with monoclonal antibody against u-PA and by α_2-antiplasmin. Partial inhibition of collagenase activation was obtained in these experiments (data not shown), which supports our conclusions. The lack of full effectiveness of these inhibitors in organ culture is most likely due to the fact that the site of enzyme activation may not be easily accessible to externally added macromolecules in organ culture.

The proteolytic cascade presented in Fig. 5 constitutes a pathway of procollagenase activation involving the cooperative action of proteases secreted by the resident cells of dermal and epidermal tissues. Activation of procollagenase occurs in two steps. The initial step involves processing of the amino terminus of procollagenase, generating a 42-kDa enzyme species of intermediate enzyme activity. The next

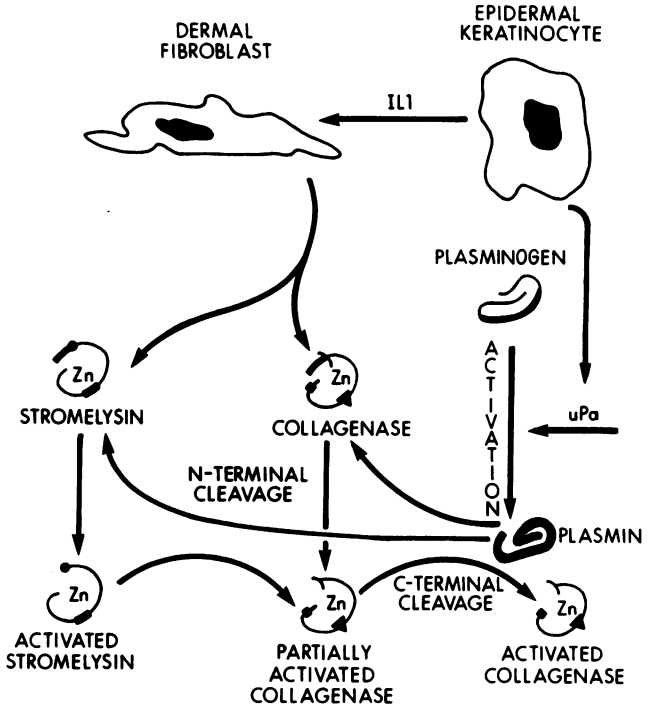

FIG. 5. Proteolytic cascade of activation of human fibroblast interstitial collagenase. Epidermal keratinocytes secrete cytokines (interleukin 1, IL1) that stimulate fibroblast production of procollagenase and prostromelysin. Keratinocytes also produce urokinase-type plasminogen activator. The initial step in the activation of procollagenase involves the removal by plasmin of 81 residues from the amino-terminal portion of the molecule, leading to the formation of a partially activated 42-kDa species. Prostromelsyin is also activated by plasmin, resulting in a 45-kDa active enzyme species. In the presence of active stromelysin, collagenase is further processed at the carboxyl-terminal end of the molecule, resulting in the fully activated enzyme.

reaction occurs in the presence of activated stromelysin and involves processing of the carboxyl terminus of the enzyme obtained as a result of the first step in the activation process.

These experiments do not rule out the possibility of other pathways for collagenase activation. The pathway of activation for a third major extracellular matrix metalloprotease, type IV collagenase (7), present in these cultures remains to be elucidated. Finally, the involvement of u-PA, plasminogen, stromelysin, and tissue inhibitor of methalloproteases, in the regulation of collagenase activity in tissues brings to focus the outstanding question of extracellular compartmentalization of the components involved in the activation process and its effects on the interaction between them.

We thank Rosemarie Brannan and Ginger Roberts for preparation of this manuscript. This study was supported by National Institutes of Health Grants AM 12129, AR 39472, and TO-AM07284 and by the Monsanto Company/Washington University Research Agreement.

1. Stricklin, G. P., Bauer, E. A., Jeffrey, J. J. & Eisen, A. Z. (1977) *Biochemistry* **16**, 1607–1615.
2. Wilhelm, S. M., Eisen, A. Z., Teter, M., Clark, S. D., Kronberger, A. & Goldberg, G. I. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3756–3760.
3. Harris, E. D., Jr., Welgus, H. G. & Krane, S. M. (1984) *Collagen Relat. Res.* **4**, 493–510.
4. Hibbs, M., Hasty, K. A., Seyer, J. M., Kang, A. H. & Mainardi, C. M. (1985) *J. Biol. Chem.* **260**, 2493–2500.
5. Hasty, K. A., Jeffrey, J. J., Hibbs, M. S. & Welgus, H. G. (1987) *J. Biol. Chem.* **262**, 10048–10052.
6. Liotta, L. A., Rao, C. N. & Weaver, U. M. (1986) *Annu. Rev. Biochem.* **55**, 1037–1057.
7. Collier, I. E., Wilhelm, S. M., Eisen, A. Z., Marmer, B. L., Grant, G. A., Seltzer, J. L., Kronberger, A., He, C., Bauer, E. A. & Goldberg, G. I. (1988) *J. Biol. Chem.* **263**, 6579–6587.
8. Seltzer, J. L., Adams, S. A., Grant, G. A. & Eisen, A. Z. (1981) *J. Biol. Chem.* **256**, 4662–4668.
9. Chin, J. R., Murphy, G. & Werb, Z. (1985) *J. Biol. Chem.* **260**, 12367–12376.
10. Okada, Y., Nagase, H. & Harris, E. D., Jr. (1986) *J. Biol. Chem.* **261**, 14245–14255.
11. 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.
12. Wilhelm, S. M., Collier, I. E., Kronberger, A., Eisen, A. Z., Marmer, B. L., Grant, G. A., Bauer, E. A. & Goldberg, G. I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6725–6729.
13. Saus, J., Quinones, S., Yashihide, O., Nagase, H., Harris, E. D., Jr. & Kurkinen, M. (1988) *J. Biol. Chem.* **263**, 6742–6745.
14. Goldberg, G. I., Wilhelm, S. M., Kronberger, A., Bauer, E. A., Grant, G. A. & Eisen, A. Z. (1986) *J. Biol. Chem.* **261**, 6600–6605.
15. Collier, I. E., Smith, J., Kronberger, A., Wilhelm, S. M., Bauer, E. A., Eisen, A. Z. & Goldberg, G. I. (1988) *J. Biol. Chem.* **263**, 10711–10713.
16. Fini, M. E., Plucinska, I. M., Mayer, A. S., Gross, R. H. & Brinckerhoff, C. E. (1987) *Biochemistry* **26**, 6156–6165.
17. Matrisian, L. M., Glaichenhaus, N., Gesnel, M.-C. & Breathnach, R. (1985) *EMBO J.* **4**, 1435–1440.
18. Stricklin, G. P., Eisen, A. Z., Bauer, E. A. & Jeffrey, J. J. (1978) *Biochemistry* **17**, 2331–2337.
19. Sellers, A., Cartwright, E., Murphy, G. & Reynolds, J. J. (1977) *Biochem. J.* **163**, 303–307.
20. Stricklin, G. P., Jeffrey, J. J., Roswit, W. T. & Eisen, A. Z. (1983) *Biochemistry* **22**, 61–68.
21. Grant, G. A., Eisen, A. Z., Marmer, B. L., Roswit, W. T. & Goldberg, G. I. (1987) *J. Biol. Chem.* **262**, 5886–5889.
22. Eechout, Y. & Vaes, G. (1974) *Archiv. Int. Physiol. Biochim.* **82**, 786.
23. Werb, Z., Mainardi, C. L., Vater, C. A. & Harris, E. D., Jr. (1977) *N. Engl. J. Med.* **296**, 1017–1023.
24. Paranjpe, M., Engel, L., Young, N. & Liotta, L. A. (1980) *Life Sci.* **26**, 1223–1231.
25. Ishibashi, M., Ito, A., Sakyo, K. & Mori, Y. (1987) *Biochem. J.* **244**, 527–534.
26. Vater, C. A., Nagase, H. & Harris, E. D., Jr. (1983) *J. Biol. Chem.* **258**, 9374–9382.
27. Murphy, G., Cockett, M. I., Stephens, P. E., Smith, B. J. & Doherty, A. J. P. (1987) *Biochem. J.* **248**, 265–268.
28. Pentland, A. & Needleman, P. (1986) *J. Clin. Invest.* **77**, 246–251.
29. Herron, G. S., Banda, M. J., Clark, E. J., Gavrilovic, J. & Werb, Z. (1986) *J. Biol. Chem.* **261**, 2814–2818.
30. Blake, M. S., Johnston, K. H., Russell-Jones, G. H. & Gotschlich, E. C. (1984) *Anal. Biochem.* **136**, 175–179.
31. Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035–10038.
32. Morioka, S., Jensen, P. J. & Lazarus, G. S. (1985) *Exp. Cell Res.* **161**, 364–372.
33. Welgus, H. G., Stricklin, G. P., Eisen, A. Z., Bauer, E. A., Cooney, R. V. & Jeffrey, J. J. (1979) *J. Biol. Chem.* **254**, 1938–1943.
34. Welgus, H. G. & Stricklin, G. P. (1983) *J. Biol. Chem.* **258**, 14162–14165.
35. Fini, M. E., Karmilowicz, M. J., Ruby, P. L., Beeman, A. M., Borges, K. A. & Brinckerhoff, C. E. (1987) *Arthritis Rheum.* **30**, 1254–1264.
36. Ito, A. & Nagase, H. (1988) *Arch. Biochem. Biophys.* **267**, 211–216.
37. Kupper, T. S., Flood, P. M., Chua, A. O., McGuire, J. S. & Gubler, U. (1987) *J. Clin. Invest.* **80**, 430–436.
38. Postlethwaite, A. E., Lackman, L. B., Mainardi, C. & Kang, A. H. (1983) *J. Exp. Med.* **157**, 801–806.
39. Dayer, J.-M., de Rochemonteix, B., Burrius, B., Demczuk, S. & Dinarello, C. A. (1986) *J. Clin. Invest.* **77**, 645–648.
40. Goslen, J. B., Eisen, A. Z. & Bauer, E. A. (1985) *J. Invest. Dermatol.* **85**, 161–164.
41. Dayer, J. M., Beutler, B. & Arami, A. (1985) *J. Exp. Med.* **162**, 2163–2168.