Reciprocated Matrix Metalloproteinase Activation: A Process Performed by Interstitial Collagenase and Progelatinase A

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ABSTRACT: Gelatinase A, a member of the matrix metalloproteinase (MMP) family, is secreted possessing an 80 amino acid N-terminal propeptide that must be removed in order to generate the active enzyme. Purified progelatinase A was activated to 38% of maximum by a 6 h incubation at 37 °C with equimolar concentrations of trypsin-activated interstitial collagenase (another MMP). The increase in activity was accompanied by cleavage of the M_r 72 000 progelatinase A to the M_r 66 000 active enzyme that has Y^{81} as its N-terminus. At low concentrations, progelatinase A was processed via an inactive intermediate, suggesting that its activation is a biphasic process. This was confirmed by the action of collagenase on proE^{375}→A (a mutant of progelatinase A that cannot become active) because, in this instance, only an M_r 68 000 species with L^{38} as the N-terminus was produced. The remaining propeptide amino acids to Y^{81} could be readily removed by added active gelatinase A, indicating that collagenase works by generating an intermediate that is susceptible to autolytic activation. Although relatively slow, the rate of activation could be increased approximately 10-fold by the addition of 100 μg/mL heparin. This binds to the C-terminal domain of collagenase and progelatinase A and presumably acts as a template that positions the reactants close to one another. Collagenase activated by trypsin retains 8 or 14 amino acids of its propeptide. The activated gelatinase A was able to remove these by cleaving the Q^{80}−F^{81} peptide bond, an event that has been shown to significantly increase the activity of collagenase against fibrillar collagen [Suzuki, K., Enghild, J. J., Morodomi, T., Salvesen, G., & Nagase, H. (1990) *Biochemistry 29*, 10261−10270]. The fact that the complete degradation of native collagen requires the activities of both a collagenase and a gelatinase provides a functional basis for this reciprocated mechanism of activation.

The matrix metalloproteinases (MMPs)[1] are a family of enzymes that between them degrade the proteinaceous components of the extracellular matrix (Woessner, 1991). The means by which their activities are regulated requires investigation because the MMPs are thought to contribute to the progression of diseases such as the arthritides and cancer (Harris, 1989; Liotta et al., 1991). The MMPs are secreted by connective tissue cells as inactive precursors that gain activity through losing an approximately 80 amino acid N-terminal propeptide, and it is possible that alterations to the normal rates of activation are at least partly responsible for any pathological matrix breakdown.

The proenzymes are latent because a conserved cysteine residue within the propeptide occupies the fourth coordination site of the catalytic zinc, and a functional active site requires that this position be taken up by a water molecule (Van Wart & Birkedal-Hansen, 1990; Holz et al., 1992). All the MMPs can be activated by their incubation with organomercurials such as 4-aminophenyl)mercuric acetate (APMA), which most likely work by destroying the cysteine−zinc interaction (Springman et al., 1990). A second function of the propeptide is to protect the peptide bond that defines the end of this domain from intermolecular autolytic cleavage (Nagase et al., 1990; Crabbe et al., 1992). Activated MMP removes the propeptide from its latent counterpart at a rate that is almost undetectable unless the conformation of the propeptide is somehow disrupted. In the case of gelatinase A, however, the rate of autolytic activation can also be increased by the addition of polyanionic molecules such as heparin (Crabbe et al., 1993). This binds to the noncatalytic C-terminal domain of gelatinase A and acts as template that approximates the active enzyme to the proenzyme.

In vivo it is probable that activation of the MMPs is caused by proteinases able to remove the first 30−40 amino acids of the propeptide (Nagase et al., 1991). Cleavage occurs within a "bait" region, the sequence of which dictates the proteinases able to activate the MMP in question. Thus, the prostromelysin 1 bait region has a sequence that allows its cleavage by either plasmin, neutrophil elastase, or cathepsin G (Nagase et al., 1990; Okada & Nakanishi, 1989) while the corresponding region within interstitial procollagenase is cleaved by proteinases that display trypsin's site of action specificity (Suzuki et al., 1990). The loss of part of the

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[1] Abbreviations: MMPs, matrix metalloproteinases; APMA, (4-aminophenyl)mercuric acetate; MT-MMP, membrane-type matrix metalloproteinase; (Δ418−631)progelatinase A, deletion mutant of human progelatinase A lacking amino acids 418−631 (C-terminal domain); (Δ243−450)procollagenase, deletion mutant of human interstitial procollagenase lacking amino acids 243−450 (C-terminal domain); proE^{375}→A, active site mutant of human progelatinase A; TIMP-1 and -2, tissue inhibitor of metalloproteinases 1 and 2; TPCK, *N*-tosyl-L-phenylanaline chloromethyl ketone; PMSF, phenylmethanesulfonyl fluoride; SBTI, soybean trypsin inhibitor; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; McaPLGLDpaAR, (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-[3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl]-Ala-Arg-NH_2.

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propeptide presumably causes a conformational disruption because it allows the rapid autolytic removal of the remaining amino acids. As the MMPs are often coordinately expressed, it is no surprise that in some instances they can also activate each other. The full collagenolytic activity of both interstitial and neutrophil collagenase, for example, is only achieved if the trypsin cleavage is followed by the removal of the remaining propeptide amino acids by stromelysin 1 (Murphy et al., 1987; Suzuki et al., 1990; Knäuper et al., 1993). Stromelysin 1 also activates progelatinase B. This is a biphasic process that starts with an initial cleavage at the bait region followed by a second cleavage to remove the rest of the propeptide (Ogata et al., 1992).

Progelatinase A (EC 3.4.24.24, 72 kDa gelatinase, type IV collagenase) has proved resistant to activation by either serine proteinases or stromelysin 1 (Okada et al., 1990; Strongin et al., 1993; Lees et al., 1994), but its propeptide can be removed by matrilysin (Crabbe et al., 1994a) and membrane-type MMP (MT-MMP; Sato et al., 1994). The expression of these two MMPs is upregulated in fibroblasts stimulated by concanavalin A, which explains why the stimulation leads to an increase in the amount of activated gelatinase A present (Overall & Sodek, 1990). Fibroblasts treated with concanavalin A also produce more interstitial collagenase (Overall & Sodek, 1990), so here we examine the possibility that this MMP is another activator of progelatinase A.

MATERIALS AND METHODS

Materials. Recombinant forms of human progelatinase A, (Δ418−631)progelatinase A, human interstitial procollagenase, (Δ243−450)procollagenase, human prostromelysin 1, and human tissue inhibitor of metalloproteinases 1 (TIMP-1), and the active site mutant of human progelatinase A, proE^{375}→A, were all purified from medium conditioned by the relevant transfected mouse myeloma cell line as previously described (Crabbe et al., 1993, 1994b; Murphy et al., 1992a, 1991; Koklitis et al., 1991). Heparin (from porcine intestinal mucosa) was from Sigma.

Proenzyme Activation. Maximum gelatinase A activity was generated by the incubation of a 1 μM solution of the proenzyme at 23 °C for 4 h in the presence of 1 mM APMA. The APMA was then removed by gel filtration using Sephadex G-25. Interstitial procollagenase (2 μM) was activated by its incubation with 5 μg/mL *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated porcine trypsin for 45 min at 37 °C in 0.1 M Tris-HCl, 0.1 M NaCl, 0.01 M $CaCl_2$, and 0.05% (v/v) Brij 35, pH 7.5. The trypsin was then inactivated by the addition of either phenylmethanesulfonyl fluoride (PMSF) to 1 mM or a 4-fold excess of soybean trypsin inhibitor (SBTI). "Fully active" collagenase (F^{81} collagenase) was prepared by performing the above reaction in the presence of 0.05 μM trypsin-activated stromelysin 1 (Murphy et al., 1987). The activation of progelatinase A by collagenase was performed at 37 °C in 63 mM Tris-HCl, 65 mM NaCl, 10 mM $CaCl_2$, and 0.025% (v/v) Brij 35, pH 7.5, unless otherwise stated.

Enzyme Assay. Matrix metalloproteinase activity was assayed by following the increase in fluorescence that accompanied hydrolysis of the synthetic substrate (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-[3-(2,4-dinitrophenyl)-L-2,3 diaminopropionyl]-Ala-Arg-NH_2 (McaPLGLDpaAR), as previously described (Knight et al., 1992; Murphy et al., 1992b) and with a gelatinase A concentration in the assay of 100 pM. Interstitial collagenase is only weakly active against this substrate (Knight et al., 1992).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). Samples were run under reducing conditions on either 10−20% or 4−20% precast polyacrylamide gels (Daichii, Tokyo) and stained using Coomassie blue R-250. Samples to be analyzed on zymograms were run without prior boiling and under nonreducing conditions on 10% polyacrylamide gels containing 1 mg/mL gelatin. Gelatinase activity was then visualized according to standard procedures (Kleiner & Stetler-Stevenson, 1994).

Protein Concentration. Progelatinase A concentration was determined by absorbance at 280 nm using $\epsilon = 122\,800\ M^{-1}\ cm^{-1}$. The concentration of activated collagenase was determined by active site titration using known amounts of purified recombinant human TIMP-1 (Crabbe et al., 1992).

N-Terminal Amino Acid Sequencing. Proteins were separated by SDS−PAGE run under reducing conditions using the precast 4−20% polyacrylamide gels and then transferred to a poly(vinylidene difluoride) membrane (Immobilon PSQ, Millipore). After staining with Ponceau S the relevent bands were excised and sequenced on a 470A protein sequencer (Applied Biosystems) with an on-line 120A HPLC.

RESULTS

The Activation of Progelatinase A by Collagenase. The ability of collagenase to activate progelatinase A was monitored by following the increase in the rate of hydrolysis of a synthetic substrate and the loss of the progelatinase A propeptide by reduced SDS−PAGE (Figure 1). At the concentration of progelatinase A tested there was no detectable activation by autolysis, but the addition of trypsin-activated collagenase led to an increase in activity that was mirrored by the conversion of the M_r 72 000 proenzyme to an M_r 66 000 species that had the N-terminal amino acid sequence YNF. This identifies it as being identical to APMA-activated gelatinase A, which has lost the propeptide by hydrolysis of the N^{80}-Y^{81} peptide bond (Collier et al., 1988; Stetler-Stevenson et al., 1989a; Figure 7A). The rate and extent of activation were dependent upon the concentration of collagenase present and started with an initial lag phase that was followed by a period of more rapid production of active gelatinase A. The highest observed activity in the 1 μM collagenase incubation (38% of maximum) was not increased with incubations of longer than 6 h, even though all the progelatinase A was eventually processed. This is a result of the intrinsic instability of activated gelatinase A when it is incubated at 37 °C (Okada et al., 1990; Crabbe et al., 1994). It is also noticeable that the collagenase, which initially migrated on SDS−PAGE as a doublet of M_r ~41 000, was itself altered during the incubation such that it eventually ran as a single band of slightly lower M_r. Amino acid sequencing of the collagenase revealed that at the start of the incubation it had a major N-terminal sequence of VMKQPRXGVP and a minor sequence of XGVPDVAQF. This shows that the activation of procollagenase by trypsin ended with cleavage at either K^{66}-V^{67} or R^{72}-C^{73} within the propeptide domain (Whitham et al., 1986; Figure 7B). The single collagenase band present at the end of the incubation had the N-terminal sequence FVLTEGNPR, demonstrating

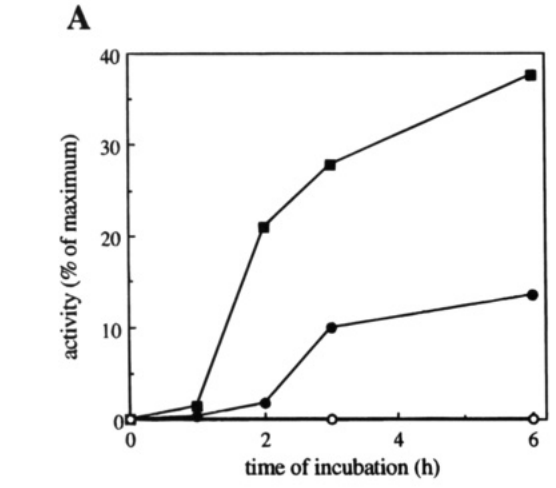

FIGURE 1: The activation of progelatinase A by collagenase. (A) Progelatinase A (1 μM) was incubated with either (○) no additions or (●) 0.2 μM or (■) 1 μM trypsin-activated interstitial collagenase. At various time points aliquots of the various incubations were removed and assayed for MMP activity as described in Materials and Methods. (B) Aliquots taken from the 0.2 μM collagenase (lanes 2−6) and 1 μM collagenase (lanes 7−11) incubations described above were analyzed on a 10−20% polyacrylamide gel. Lane 1, marker proteins; lanes 2 and 7, 0 h; lanes 3 and 8, 1 h; lanes 4 and 9, 2 h; lanes 5 and 10, 3 h; lanes 6 and 11, 6 h. The positions of migration of collagenase and SBTI are as shown.

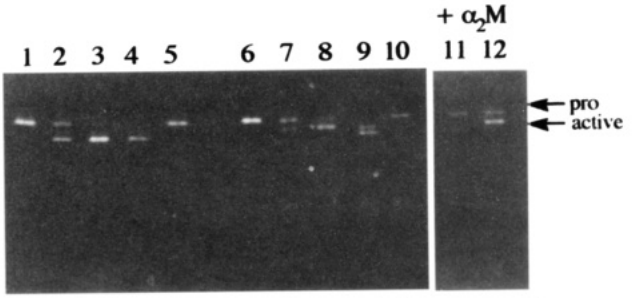

FIGURE 2: Zymogram of progelatinase A cleavage by collagenase. Progelatinase A at a concentration of either 4 μM (lanes 1−5) or 0.04 μM (lanes 6−10) was incubated with 1 μM trypsin-activated collagenase at 37 °C in 0.1 M Tris-HCl, 0.1 M NaCl, 10 mM $CaCl_2$, and 0.05% (v/v) Brij 35, pH 7.5. At various time points samples were removed, diluted using the same buffer to a gelatinase A concentration of 0.8 nM, and analyzed by zymography. Lanes 1 and 6, 0 h; lanes 2 and 7, 2 h; lanes 3 and 8, 8 h; lanes 4 and 9, 24 h; lanes 5 and 10, 24 h in the absence of collagenase. α_2-Macroglobulin was added to a concentration of 0.1 μM to the diluted samples taken after 8 h of the 4 μM (lane 11) and 0.04 μM (lane 12) gelatinase A/collagenase incubations, and the mixtures were incubated for 4 h at 23 °C before their analysis by zymography. The positions of migration of progelatinase A (pro) and propeptide-lacking gelatinase A (active) are as indicated.

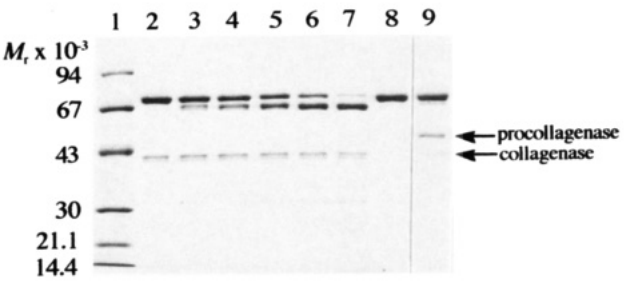

FIGURE 3: Cleavage of proE^{375}→A by collagenase. ProE^{375}→A (4 μM) was incubated with collagenase (1 μM) at 37 °C, and at various time points samples were analyzed by SDS−PAGE on a 4−20% polyacrylamide gel. Lane 1, marker proteins; lane 2, 0 h; lane 3, 1 h; lane 4, 2 h; lane 5, 4 h; lane 6, 8 h; lane 7, 24 h; lane 8, 24 h with no collagenase; lane 9, 4 h with 1 μM procollagenase.

that hydrolysis of its Q^{80}-F^{81} bond had somehow occurred during the 6 h incubation. F^{81} as the N-terminus has previously been shown to be the determining feature of collagenase that is fully active against native type I collagen (Suzuki et al., 1990). None of the molecular weight changes were observed if the reaction was performed in the presence of 4 μM TIMP-1, a specific inhibitor of matrix metalloproteinase activity (results not shown). The activation by collagenase was also followed at two starting concentrations of progelatinase A by using gelatin zymography (Figure 2). At a progelatinase A concentration of 4 μM the proenzyme was converted to the propeptide-lacking, active enzyme as before but at 0.04 μM the reaction proceeded via the formation of an intermediate. The propeptide-lacking species did eventually become visible at this low gelatinase A concentration but only after 24 h of incubation. In order to determine whether or not the intermediate was active, we took advantage of the fact that the inhibitor α_2-macroglobulin only binds to active proteinases and the complexes so formed are not destroyed by SDS and are too large to enter a 10% polyacrylamide gel (Ogata et al., 1992). Unlike the inactive progelatinase A, therefore, propeptide-lacking gelatinase A was removed from its normal position of migration by its incubation with α_2-macroglobulin (Figure 2; lane 11). This contrasts with the intermediate, which was not removed by the inhibitor (lane 12) and must, therefore, have retained the latency of the proenzyme. Insufficient quantities of the intermediate were generated for N-terminal amino acid sequencing.

The Action of Collagenase on ProE^{375}→A. In order to determine the relative contributions of collagenase and gelatinase A to the activation process, we studied the effect of collagenase on proE^{375}→A. This is a point mutant of progelatinase A that has had a catalytically essential glutamic acid residue replaced by an amino acid that allows less than 0.01% of the normal activity when the propeptide is removed but has little or no effect on conformation (Crabbe et al., 1994b). The results in Figure 3 show that proE^{375}→A was also cleaved by activated collagenase but that the final product was not the M_r 66 000 propeptide-lacking gelatinase A but an M_r 68 000 species likely to be the equivalent of the inactive intermediate seen on the zymogram. Amino acid sequencing of the M_r 68 000 species formed after 4 h of the incubation revealed it to possess the N-terminal sequence LFVLKDTLKKM. Thus, collagenase activity is only responsible for the hydrolysis of the N^{37}-L^{38} peptide bond situated within the progelatinase A propeptide (Collier et al., 1988; Figure 7A). This cleavage, however, appears to be a prerequisite for the other events to occur. The trypsin-

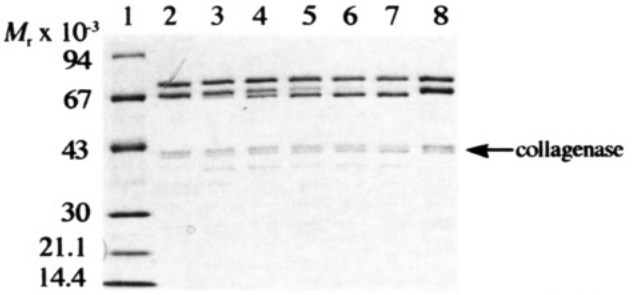

FIGURE 4: Processing of the M_r 68 000 intermediate by (Δ418−631)gelatinase A. ProE^{375}→A (4 μM) was treated with 1 μM collagenase for 4 h at 37 °C before the solution was incubated at the same temperature in the presence of 0.25 μM APMA-activated (Δ418−631)gelatinase A. At various time points during the incubation, aliquots were removed and analyzed by SDS−PAGE on a 4−20% polyacrylamide gel. Lane 1, marker proteins; lane 2, 0 min; lane 3, 2 min; lane 4, 5 min; lane 5, 10 min; lane 6, 20 min; lane 7, 40 min; lane 8, 40 min with no (Δ418−631)gelatinase A. Activated (Δ418−631)gelatinase A migrates at M_r = 36 000 but is not visible on this gel.

activated collagenase migrated as an M_r ~41 000 doublet on SDS−PAGE during the entire course of its incubation with proE^{375}→A, which suggests that the previously observed hydrolysis of its Q^{80}-F^{81} bond must have been catalyzed by gelatinase A activity. Collagenase activated by a combination of trypsin and stromelysin 1 (F^{81} collagenase) also cleaved proE^{375}→A to just the M_r 68 000 species and did so at a rate equal to that of collagenase activated by trypsin alone (results not shown).

Processing of the M_r 68 000 Intermediate. The wild-type progelatinase A preparation used in these studies had 0.1% of the activity of APMA-activated gelatinase A. This low starting activity probably arose from contaminating active gelatinase A that had copurified with the proenzyme (Crabbe et al., 1993) and might be responsible for initially cleaving the inactive, M_r 68 000 intermediate to the M_r 66 000 active form. To test this hypothesis, APMA-activated (Δ418−631)gelatinase A was added to the products of a 4 h incubation of proE^{375}→A with collagenase. (Δ418−631)-Gelatinase A is a deletion mutant that lacks the C-terminal domain but has full catalytic activity (Murphy et al., 1992). The results in Figure 4 show that it was able to completely convert the M_r 68 000 intermediate, but not proE^{375}→A, to an M_r 66 000 species after only approximately 20 min of incubation at 37 °C. The N-terminal sequence of the M_r 66 000 species was YNFFPRKPKW, which demonstrates that cleavage at N^{80}-Y^{81} had indeed occurred (Figure 7A).

Effect of Heparin on Activation. Heparin is able to enhance the normally slow autolytic activation of progelatinase A (Crabbe et al., 1993). The results in Figure 5 show that it was also able to accelerate the rate, and therefore extent, of activation of progelatinase A by collagenase. With the enzyme concentrations selected, the maximum enhancement was achieved in the range 100−500 μg/mL heparin, such that the highest observed activity (53% of maximum) occurred after 40 min of incubation at 37 °C. The presence of heparin, therefore, increases the rate of activation approximately 10-fold. The increase in activity was again mirrored by an increased conversion of wild-type progelatinase A to the M_r 66 000 active enzyme, and the same heparin concentrations were also able to enhance the conversion of proE^{375}→A to the M_r 68 000 intermediate (Figure 5B). This suggests that the rate of activation was accelerated by

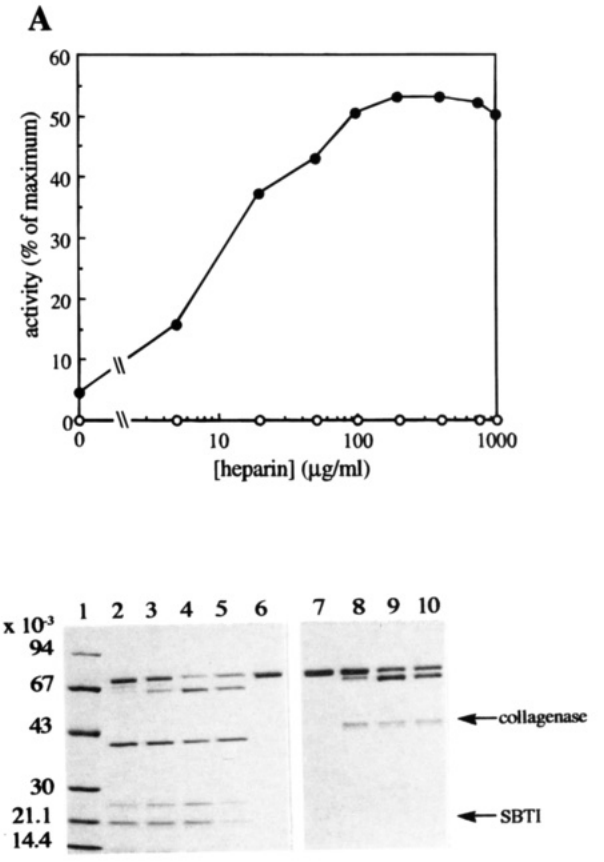

FIGURE 5: The effect of heparin on the activation of progelatinase A by collagenase. (A) Progelatinase A (1 μM) was incubated with varying heparin concentrations at 37 °C for 40 min in either (●) the presence or (○) the absence of 1 μM collagenase, after which the MMP activity was assayed. (B) Samples taken from the incubations described above (lanes 2−6) or from 1 h incubations at 37 °C of proE^{375}→A in either the presence or absence of 1 μM collagenase and varying concentrations of heparin (lanes 7−10) were analyzed by SDS−PAGE on a 4−20% polyacrylamide gel. Lane 1, marker proteins; lane 2, no heparin + collagenase; lane 3, 5 μg/mL heparin + collagenase; lane 4, 100 μg/mL heparin + collagenase; lane 5, 1 mg/mL heparin + collagenase; lane 6, 100 μg/mL heparin, no collagenase; lane 7, 100 μg/mL heparin, no collagenase; lane 8, 1 μg/mL heparin + collagenase; lane 9, 100 μg/mL heparin + collagenase; lane 10, 1 mg/mL heparin + collagenase.

increases in the rate of cleavage of both N^{37}-L^{38} by collagenase and N^{80}-Y^{81} by autolysis. In all cases, however, the stimulatory effect of heparin was reduced if it was present at concentrations of greater than 500 μg/mL. In the absence of collagenase, heparin had no effect on either progelatinase A or proE^{375}→A within the incubation time monitored. Heparin also had no noticeable effect on the rate of either (Δ418−631)progelatinase A activation by collagenase or proE^{375}→A cleavage to the M_r 68 000 intermediate when catalyzed by trypsin-activated (Δ243−450)collagenase (results not shown). Both of these truncated enzymes have a functional active site but differ from their full-length equivalents in that they are unable to bind to heparin−Sepharose CL 6B at pH 7.5 (Murphy et al., 1992a; Crabbe et al., 1993; results not shown).

Heparin at 100 μg/mL was able to accelerate the gelatinase A-catalyzed conversion of trypsin-activated collagenase to F^{81} collagenase (Figure 6). In the absence of heparin, 1 μM of APMA-activated gelatinase A took 20 min to cleave 1 μM of the M_r ~41 000 collagenase doublet (V^{67}/C^{73}) to the

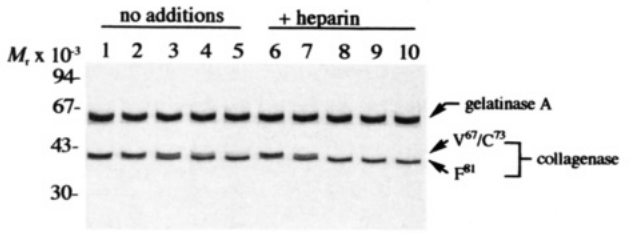

FIGURE 6: The effect of heparin on the processing of collagenase by gelatinase A. Trypsin-activated interstitial collagenase (1 μM) was incubated with APMA-activated gelatinase A (1 μM) at 37 °C in either the presence or absence of 100 μg/mL heparin as indicated. At various time points during the incubation, samples were removed and analyzed by SDS−PAGE using a 10−20% polyacrylamide gel. Lanes 1 and 6, 0 min; lanes 2 and 7, 1 min; lanes 3 and 8, 5 min; lanes 4 and 9, 10 min; lanes 5 and 10, 20 min.

single, lower M_r species that has F^{81} as its N-terminus, while in its presence, the reaction was completed after 1−5 min. This accelerated rate was still approximately 20 times slower than the same cleavage catalyzed by stromelysin 1 although, in this case, there was no heparin enhancement (results not shown). Like stromelysin 1, gelatinase A was unable to cause any significant activation of procollagenase (results not shown).

DISCUSSION

The results presented in this report demonstrate that interstitial collagenase can promote the activation of progelatinase A. This is accomplished by the cleavage of a single peptide bond (N^{37}−L^{38}) that aligns approximately with the propeptide bait regions described previously for procollagenase, prostromelysin 1, promatrilysin, and progelatinase B (Suzuki et al., 1990; Nagase et al., 1990; Crabbe et al., 1992; Ogata et al., 1992). Progelatinase A is unlike the other MMPs, however, in that it is not processed by a serine proteinase but by other members of its enzyme family. Collagenase does not directly activate gelatinase A because it is unable perform a second cleavage at the N^{80}−Y^{81} peptide bond marking the end of the propeptide; an unexpected result because, in work detailing its cleavage site specificity, collagenase was shown to refuse phenylalanine but not tyrosine residues in the P_1' position (Netzel-Arnett et al., 1991). Instead, the collagenase cleavage has the effect

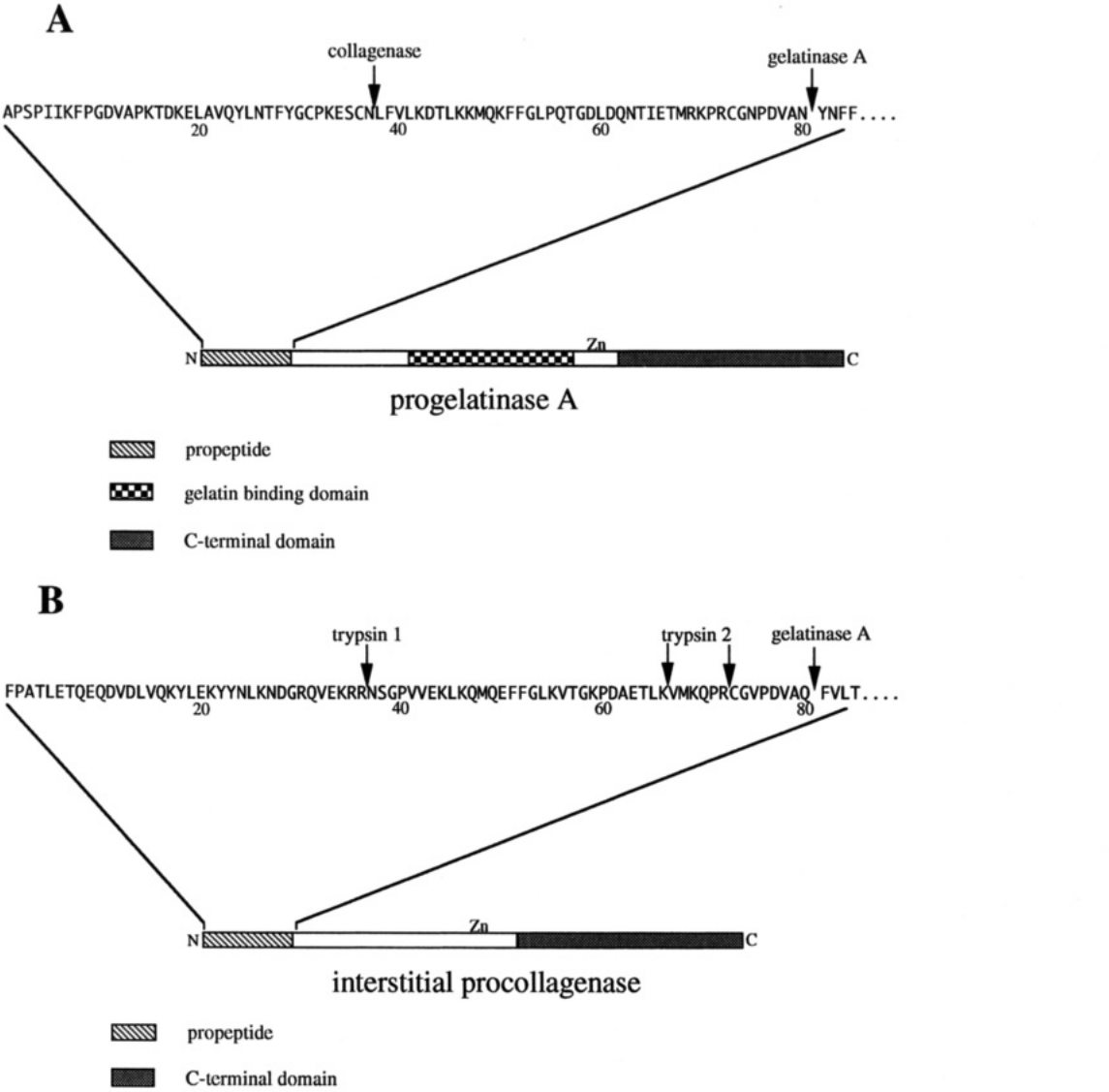

FIGURE 7: Points of cleavage during the activation of progelatinase A and interstitial procollagenase. (A) The amino acid sequence of progelatinase A that encompasses its N-terminal propeptide [from Collier et al. (1988)]. (B) The amino acid sequence of interstitial procollagenase that encompasses its N-terminal propeptide [from Whitham et al. (1986)]. The trypsin 1 cleavage site is that described by Grant et al. (1987) as being the initial event in the activation. The trypsin 2 cleavage sites are those described in this report as the final products of the trypsin activation.

of significantly increasing the susceptibility of the N^{80}-Y^{81} peptide bond to autolysis. This bond is subject to autolysis when the whole propeptide is present, for previous studies have shown that 50% cleavage of $proE^{375}$→A at N^{80}-Y^{81} could be brought about by one-fifth the amount of APMA-activated (Δ418−631)gelatinase after 16 h (Crabbe et al., 1994b). Here we have shown that, at equivalent enzyme concentrations, the M_r 68 000 intermediate is completely converted to the M_r 66 000 form in just 20 min. This represents an approximately 100-fold increase in rate and demonstrates that amino acids 1−37 are crucial in protecting the proenzyme against autolytic activation. The inability of the intermediate to form complexes with α_2-macroglobulin reveals that the loss of these amino acids is not sufficient to activate the enzyme. This is not without precedent, for in the activation of both progelatinase B by stromelysin 1 and neutrophil procollagenase by trypsin, the initial bait region cleavage also produces an inactive but unstable intermediate (Ogata et al., 1992; Knäuper et al., 1993). Cleavage at the bait region will not remove the conserved propeptide cysteine, and it is possible that enough of the propeptide is retained for the cysteine−catalytic zinc interaction to remain intact. We propose that the activating cleavage at N^{80}-Y^{81} is initially catalyzed by the small amount of active gelatinase A already present in the purified progelatinase A preparation. This would explain why there was a lag phase in the activation for there will be an initial buildup of the inactive intermediate until there is enough active gelatinase A present to make the second part of the reaction almost instantaneous. Lowering the concentration of progelatinase A will tend to extend this lag phase, resulting in a buildup of intermediate sufficient for its visualization by zymography. *In vivo*, the initiating gelatinase A activity might be generated by matrilysin, an MMP that is able to completely remove the gelatinase A propeptide (Crabbe et al., 1994a). Alternatively, the initiating activity might belong to stromelysin 1, which can hydrolyze N^{80}-Y^{81} if the N^{37}-L^{38} peptide bond has first been cleaved (results not shown).

The incubation of procollagenase with trypsin produced two lower M_r forms possessing either V^{67} or C^{73} as the N-terminus. Although they retain the conserved propeptide cysteine, both forms are likely to be active because plasmin-activated collagenase has L^{65} as its N-terminus (Suzuki et al., 1990). Collagenase treated with plasmin or trypsin alone displays only approximately 15% of the collagenolytic activity of F^{81} collagenase, the fully active form generated by an additional cleavage catalyzed by stromelysin 1 (Murphy et al., 1987; Suzuki et al., 1990). F^{81} as the N-terminal amino acid is a key requirement for full activity because APMA treatment produces collagenase with V^{82} and L^{83} as N-termini and this has only 40% of the collagenolytic activity of F^{81} collagenase (Suzuki et al., 1990). The ability of active gelatinase A to cleave the Q^{80}-F^{81} bond of trypsin-treated collagenase might, therefore, represent a significant additional contribution made by this MMP to the breakdown of the extracellular matrix. F^{81} collagenase is not necessarily able to transfer its increased collagenolytic activity to other substrates because it was no more active against $proE^{375}$→A than the collagenase activated by trypsin alone. This meant that unlike other reciprocated activations such as the one performed by factor XII and plasma kallikrein (Tankersley & Finlayson, 1984), the process described in this report was not self-amplifying.

Much of the recent work on progelatinase A activation has centered on its ability to be performed by the membranes of stimulated fibroblasts (Brown et al., 1990; Murphy et al., 1992b; Strongin et al., 1993). The activation is specific for gelatinase A and is prevented by the TIMPs (Ward et al., 1991), which suggests that it is the result of MMP activity. During the activation, an intermediate is generated and its N-terminal amino acid sequence revealed that it must also have arisen from cleavage of the N^{37}-L^{38} peptide bond (Strongin et al., 1993). Interstitial collagenase, however, is probably not responsible because we have been unable to demonstrate its presence on these membranes (results not shown). It is more likely that, in this instance, the cleavage is catalyzed by MT-MMP, an MMP whose expression correlates with the ability of cells to activate progelatinase A (Sato et al., 1994). Progelatinase A activation is the only known function of MT-MMP, and this property, in conjunction with its cell surface localization, has led to the proposal that it is a key mediator of cell invasion through basement membranes (Sato et al., 1994). The reciprocated mechanism of activation described in this report does nevertheless tally with the fact that interstitial collagenase and gelatinase A are sometimes coordinately expressed (Overall & Sodek, 1990; Welgus et al., 1990; Kataoka et al., 1993) and suggests that, in other physiological settings, these two MMPs might indeed activate each other. The combination of F^{81} collagenase and activated gelatinase A would certainly be a potent matrix-degrading force because the initial cleavage of the native collagen triple helix by the collagenase will unravel its structure and allow the gelatinase to rapidly degrade it to smaller fragments. This cooperative mechanism of type I collagen degradation has previously been shown to be performed by the complexes of gelatinase B and interstitial collagenase secreted by monocytes (Goldberg et al., 1992).

The activation of progelatinase A by collagenase is a relatively slow process when compared with the activation of other MMPs by the serine proteinases. Procollagenase activation by plasmin, for example, is completed within 30 min of incubation at 37 °C (Suzuki et al., 1990). Similarly, gelatinase A is a poor activator of collagenase when compared to stromelysin 1. The ability of heparin to significantly increase the rate of both of these reactions is, therefore, of importance if this reciprocated mechanism of activation is to be regarded as having any physiological significance. Heparin presumably works by acting as a template that positions active collagenase and progelatinase A together such that the reactions have an increased chance of occurring. High heparin concentrations reduce the effect because the reactants will tend to become separated again. This mechanism of action is supported by the fact that heparin failed to enhance the rate of propeptide cleavage when C-terminal domain deletion mutants of either collagenase or progelatinase A were examined. These mutants retain a functional active site but have lost the heparin-binding site possessed by their full-length equivalents. It is not known whether there exists a physiological equivalent of heparin, although it is worth noting that it is made and released by mast cells, which are often found at sites of matrix remodeling or breakdown where collagenase and gelatinase A activities would be required (Bromley & Woolley, 1984; Dabbous et al., 1986). The involvement of mast cells is made additionally attractive by the fact that

chymase, one of the serine proteinases they produce, can activate interstitial procollagenase in a process that is also stimulated by heparin (Lees et al., 1994; Saarinen et al., 1994). A complication not investigated in this report is that progelatinase A is commonly secreted as a mixture of free proenzyme and proenzyme complexed with the inhibitor TIMP-2 (Stetler-Stevenson et al., 1989b; Goldberg et al., 1989). The proenzyme/TIMP-2 complex can itself act as an inhibitor of MMP activity (Kolkenbrock et al., 1990), so for collagenase to be an effective activator of progelatinase A it will probably be necessary that it is present at concentrations higher than those of any TIMP-2.

In conclusion, therefore, we have shown that interstitial collagenase can promote the activation of gelatinase A and that active gelatinase A can then cleave collagenase to its fully active form. This reciprocated mechanism of activation is the first of its kind to be described for the MMPs.

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