Inactivation of Plasminogen Activator Inhibitor-1 by Specific Proteolysis with Stromelysin-1 (MMP-3)*

Revised manuscript received in the laboratory of Dr. H. Nagase on July 29, 2000. This work was supported by a grant from the Flemish Fund for Scientific Research (KUL) and by the Belgian Science Policy (IUAP Contract P4/34). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

From the *Center for Molecular and Vascular Biology and ‡Laboratory for Pharmaceutical Biology and Phytopharmacology, University of Leuven, B-3000 Leuven, Belgium

H. Roger Lijnen‡, Begona Arza‡, Berthe Van Hoeff‡, Désiré Collen‡, and Paul J. Declerck‡

Matrix metalloproteinase-3 (MMP-3 or stromelysin-1) specifically hydrolyzes the Ser237–Ser338 (P10–P9) and Val341–Ile342 (P6–P5) peptide bonds in human plasminogen activator inhibitor-1 (PAI-1). Cleavage is completely abolished in the presence of the metal chelators EDTA or 1,10-phenanthroline. A stabilized active PAI-1 variant was also cleaved by MMP-3. At an enzyme/substrate ratio of 1/10 at 37 °C, PAI-1 protein cleavage occurred with half-lives of 27 or 14 min for active or stable PAI-1 and was associated with rapid loss of inhibitory activity toward tissue-type plasminogen activator with half-lives of 15 or 13 min, respectively. A substrate-like variant of PAI-1, lacking inhibitory activity but with exposed reactive site loop, was cleaved with a half-life of 23 min, whereas latent PAI-1 in which a major part of the reactive site loop is inserted into the molecule, was resistant to cleavage. Biospecific interaction analysis indicated comparable binding of active, stable, and substrate PAI-1 to both proMMP-3 and MMP-3 (Kₐ of 12–22 × 10⁶ M⁻¹), whereas binding of latent PAI-1 occurred with lower affinity (1.7–2.3 × 10⁶ M⁻¹). Stable PAI-1 bound to vitronectin was cleaved and inactivated by MMP-3 in a manner comparable with that of free PAI-1; however, the cleaved protein did not bind to vitronectin. Cleavage and inactivation of PAI-1 by MMP-3 may thus constitute a mechanism decreasing the antiproteolytic activity of PAI-1 and impairing the potential inhibitory effect of vitronectin-bound PAI-1 on cell adhesion and/or migration.

Plasminogen, the zymogen of the fibrinolytic system, is converted into the active enzyme plasmin by tissue-type (t-PA) or urokinase-type (u-PA) plasminogen activator. Both physiological plasminogen activators are inhibited mainly by plasminogen activator inhibitor-1 (PAI-1) (1). PAI-1 is a 50-kDa single chain glycoprotein consisting of 379 amino acids without disulfide bonds (2–4). It is a member of the serpin (serine proteinase inhibitors) superfamily, with reactive site (P1–P1) peptide bond Arg346–Met347 located in the strained reactive site loop (2–5). In contrast to other serpins, three different interconvertible conformations of PAI-1 have been described: an active conformation with inhibitory properties (forming a stable coherent complex with the target proteinase), a substrate conformation that is cleaved at P1–P1’ by its target proteinases (6), and a nonreactive latent conformation in which a major part of the reactive site loop is inserted into the molecule (7). PAI-1 is synthesized in the active conformation but converts spontaneously into the latent conformation with a half-life of 1–2 h under physiological conditions (8). PAI-1 is stabilized by binding to vitronectin (S protein), a protein present in the extracellular matrix and also detected in dimeric form in plasma (9).

ProMMP-3 (stromelysin-1) is secreted from different cell types as a 57-kDa single chain protein; conversion to MMP-3 involves removal of the NH₂-terminal prosequence of approximately 80 amino acids to yield the 45-kDa active enzyme (10). MMP-3 has a broad substrate specificity; it cleaves proteoglycans, fibronectin, procollagen type I, collagens types III, IV and IX, and laminin, and it may activate procollagenase and progelatinase B (10–13). It has been shown previously that MMP-3 specifically hydrolyzes some components of the fibrinolytic system, including fibrinogen (14), plasminogen (15), and urokinase-type plasminogen activator (16). In the present study, we report specific cleavage of PAI-1 by MMP-3, resulting in inactivation of the inhibitor.

MATERIALS AND METHODS

Proteins, Reagents, and Assays—Recombinant human proMMP-3 was produced in Escherichia coli, using the plasmid pET-psf STR (the expression vector pET-8c containing the cDNA of a COOH-terminally truncated proMMP-3; a kind gift of Dr. A. L. Marcy, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.) (10). It was purified and activated with p-aminophenyl-mercuric acetate, essentially as described (10). Full-length natural human MMP-3 was a kind gift of Dr. H. Nagase (Department of Biochemistry and Molecular Biology, University of Kansas Medical School, Kansas City, K5). Nonglycosylated recombinant active PAI-1 (45 kDa) was obtained and characterized as described (17); the preparation contained 79% active, 12% latent, and 9% substrate PAI-1, as determined by incubation with t-PA followed by SDS-PAGE and densitometric gel scanning (18). A stable PAI-1 mutant (with Asn230→His, Lys244→Thr, Gin245→Pro, Gin246→Leu, and Met251→Ile mutation) with about 80% inhibitory activity and half-life at 37 °C of more than 150 h (19, 20), and a substrate-like PAI-1 mutant (with the P12 residue Ala235 mutagenized to Pro) were obtained as described (17). Latent PAI-1 was obtained by incubation of active PAI-1 at 37 °C for 24–48 h (18). Substrate and latent PAI-1 did not have detectable inhibitory activity, as monitored by titration with two-chain t-PA. Two-chain t-PA was obtained by plasmin treatment of Actilyse® (Roche Molecular Biochemicals, Boehringer, Ingelheim, Germany) and characterized as described (21). PAI-1-resistant single chain t-PA (PAI-1) was obtained by reaction with Lys285 through Gly282), plasminogen, and plasmin were prepared as described (21).

Polymeric rat vitronectin, which binds human PAI-1 to a similar...
extent as monomeric human vitronectin,2 was obtained from Molecular Innovations Inc., (Royal Oak, MI). The chromogenic substrates S-2403 for plasmin and S-2288 for t-PA were purchased from Chromogenix (Antwerp, Belgium). β-Phe-Pro-Arg-CH₂-Cl (FAPACK) was from Calbiochem-Novabiochem (Nottingham, UK), and 1,10-phenanthroline was from Sigma.

SDS-PAGE without reduction or after reduction with 1% dithioerythritol was performed on 10–15% gradient gels using the Phast system (Amersham Pharmacia Biotech) and staining with Coomassie Brilliant Blue R or silver staining. Protein bands were quantitated by densitometric gel scanning using the Gel-scan accessory of the Beckman DU60 spectrophotometer. A protein calibration mixture was used consisting of phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). Association rate constants (kₐss in M⁻¹ s⁻¹) and dissociation rate constants (kₙs in s⁻¹) for the interactions between (pro)MMP-3 and different PAI-1 moieties were determined by biospecific interaction analysis using the BIACore instrument (Amersham Pharmacia Biotech). ProMMP-3 or MMP-3 were immobilized on the surface of sensor chip CM5 using the Amine Coupling kit (Amersham Pharmacia Biotech) at pH 4.0, and binding of the PAI-1 ligands was evaluated as described in detail elsewhere (22).

For NH₄-terminal amino acid sequence analysis, samples of PAI-1 treated with MMP-3 (E/S of 1/10 for 2 h at 37 °C) were run on a 10–20% Tris-Tricine gel (bis-tricine) and transferred to a 0.45-μm Immobilon™ P membrane (Millipore, Bedford, MA). Protein bands were visualized by brief staining with Amido Black and excised from the gel. Sequence analysis was performed on a Procise™, 492 Protein Sequencer (Applied Biosystems, Foster City, CA), with identification of amino acids by high performance liquid chromatography.

Human PAI-1 levels were measured by ELISA, using MA-7D4B7 for capture, and horseradish peroxidase-conjugated MA-7F5 for tagging (23). This ELISA recognizes active, latent, and complexed PAI-1, indicating that the monoclonal antibodies do not react with the COOH-terminal peptide removed after interaction with proteinases. PAI-1 activity was also monitored with an immunofunctional assay; therefore, excess human two-chain t-PA was added to the samples and after incubation for 10 min at 37 °C, t-PA:PAI-1 complex was determined by ELISA using MA-15H12 for capture, and horseradish peroxidase-conjugated MA-62E8 for tagging (24). A calibration curve was constructed using purified t-PA:PAI-1 complex. Figures were prepared using the curve fitting program of DeltaGraph.

**Proteolytic Cleavage of PAI-1 by MMP-3—Active, latent, substrate, or stable PAI-1 (final concentration, 3 μM) were incubated with MMP-3 (final concentration, 60–300 nm) at 37 °C in 50 mM Tris-HCl buffer, pH 7.5, containing 0.1 M NaCl, 10 mM CaCl₂, and 0.01% Tween 80. At different time intervals (0–120 min), samples were removed from the incubation mixtures, EDTA (final concentration, 25 mM) was added to stop the reaction, and SDS-PAGE was performed under nonreducing conditions. The amounts of the different molecular forms of PAI-1 in the samples were determined by densitometric scanning of the gels and expressed as a percentage of the total value.

In the experiments with active and stable PAI-1 moieties, PAI-1 activity was also monitored as a function of time. Therefore, samples were removed from the incubation mixtures, diluted 200–500-fold in 50 mM Tris-HCl buffer, pH 7.5, containing 35 mM NaCl and 0.01% Tween 80 and incubated with two-chain t-PA (final concentration, 20 nM) for 3 min at 37 °C. Residual t-PA activity was determined with S-2288 (final concentration, 0.5 mM) using a calibration curve constructed with two-chain t-PA (final concentration, 0–20 nM).

In addition, mixtures of vitronectin (final concentration, 6 μM) and stable PAI-1 (final concentration, 3 μM) were incubated in solution (50 mM Tris-HCl buffer, pH 7.5, containing 0.15 mM NaCl, 10 mM CaCl₂, and 0.01% Tween 80) for 0–120 min, before addition of MMP-3 (E/S of 1/10). The molecular forms of PAI-1 and vitronectin were monitored by SDS-PAGE under reducing conditions with silver staining, and PAI-1 activity was monitored by addition of two-chain t-PA, as described above.

In separate experiments, polymeric rat vitronectin was coated to blocking with albumin (3% in PBS for 30 min at room temperature), human vitronectin (0–100 ng/mL) were added in 50 mM Tris-HCl buffer, pH 7.5, containing 0.004% Tween 80. After incubation for 2 h at 37 °C and extensive washing of the plates, bound PAI-1 antigen was detected with horseradish peroxidase-conjugated MA-7F5.

**Effect of proMMP-3 on the Interaction of PAI-1 with t-PA**—The second-order rate constant for the inhibition of two-chain t-PA (final concentration, 10 nM) by active PAI-1 (final concentration, 17 nM) was determined at 25 °C in 50 mM Tris-HCl buffer, pH 7.5, containing 0.15 M NaCl and 0.01% Tween 80, by continuous monitoring of A₄₀₅ nm at pH 4.0, and binding of the PAI-1 ligands was evaluated as described in detail elsewhere (22). Addition of PAI-1 bound to proMMP-3 can still interact with t-PA, consecutive binding of both proteins to immobilized proMMP-3 was studied by biospecific interaction analysis. Therefore, a saturating concentration (500 nM) of t-PA or PAI-1-resistant t-PA (S-2288) was bound first to the proMMP-3, followed by active PAI-1 (500 nM). Additional binding of PAI-1 was determined by subtracting the binding curve for t-PA alone from that of t-PA plus PAI-1 and was compared with direct binding of PAI-1 (500 nM) to the insolubilized proMMP-3.

**RESULTS**

**Proteolytic Cleavage of PAI-1 by MMP-3—Inactivation of active PAI-1** (45 kDa) with MMP-3 (E/S ratio of 1/50 to 1/10) resulted in a time- and concentration-dependent conversion to a lower molecular mass fragment, compatible with removal of a small peptide (Fig. 1, inset). Addition of EDTA (final concentration, 25 mM) or 1,10-phenanthroline (final concentration, 1 mM) to the incubation mixture abolished the proteolytic cleavage, confirming that it is MMP-dependent. Densitometric scanning of SDS-PAGE with samples taken at different time points showed >80% cleavage within 60 min at E/S ratio of 1/10 (Fig. 1). To identify the cleavage site in PAI-1, it was digested with MMP-3 (E/S ratio of 1/10) for 2 h at 37 °C, as described above, and the reaction mixture was subjected to gel electrophoresis and NH₂-terminal amino acid sequence analysis, as described above. This yielded one major sequence (>90%) for the residual PAI-1 moiety, corresponding to the intact NH₂-terminal amino acid sequence (3), (Val-His-His-Pro-Pro-Ser), whereas for the
cleaved peptide fraction, one major sequence (>80%) was detected (Ile-Val-Ser-Ala-Arg-Met), compatible with cleavage of the Val^{[1]}-Ile^{[2]} (P6–P5) peptide bond, and a minor sequence (Xaa-Thr-Ala-Val-Ile) compatible with cleavage of the Ser^{[3]}-Ser^{[2]} (P10–P9) peptide bond.

Similar experiments with latent, stable, and substrate PAI-1 (E/S ratio of 1/10) revealed nearly quantitative hydrolysis of stable PAI-1 (>90% within 2 h), and somewhat more limited hydrolysis of substrate PAI-1 (75% within 2 h), whereas latent PAI-1 appeared fully resistant to cleavage (Fig. 2). Incomplete cleavage observed with active PAI-1 (Fig. 1) may thus be due to some conversion of active to latent PAI-1 during the experiment. The half-lives for protease cleavage (at E/S ratio of 1/10) determined from semi-logarithmic plots of residual PAI-1 versus time (not shown) were 27, 23, or 14 min for active, substrate, or stable PAI-1, respectively. Full-length natural glycosylated MMP-3 at E/S ratio of 1/10 also induced hydrolysis of active and stable PAI-1 (60 and 82% protein cleavage within 2 h, respectively; not shown).

Monitoring of PAI-1 activity in the experiments with active or stable PAI-1 indicated rapid loss of inhibitory activity upon addition of MMP-3; PAI-1 activity declined in parallel with the rate of proteolytic cleavage, with half-lives of 15 ± 2.6 or 13 ± 1.0 min for active or stable PAI-1, respectively (mean ± SD; n = 4). The faster disappearance of activity for the active PAI-1 as compared with protein cleavage is probably explained by partial conversion to latent PAI-1 during this experiment ($t_{1/2}$ of about 75 min under the conditions used). After 90 min of incubation, the residual PAI-1 activity was <10%; in agreement with these data, SDS-PAGE under nonreducing conditions did not reveal significant amounts of stable t-PA-PAI-1 complex (±7% of the sample without MMP-3) after addition of equimolar amounts of two-chain t-PA and incubation for 3 min at 37 °C (not shown).

In solution, MMP-3 cleaved vitronectin-bound stable PAI-1 as well as free stable PAI-1 in a time-dependent manner; protein cleavage at E/S ratio of 1/10 appeared somewhat slower in the presence of vitronectin (Fig. 3). PAI-1 activity, as monitored by addition of two-chain t-PA, decreased in a parallel manner, confirming proteolytic cleavage ($t_{1/2}$ of 20 or 27 min in the absence or the presence of vitronectin). Vitronectin itself was not cleaved by MMP-3 under these experimental conditions (Fig. 3, inset). Stable active PAI-1 as well as substrate PAI-1 bound in a concentration-dependent manner to vitronectin-coated microtiter plates as detected with horseradish peroxidase-conjugated MA-7F5 (Fig. 4). Substrate PAI-1 cleaved with t-PA (equimolar, 10 min at 37 °C) did not bind to vitronectin, whereas MMP-3 cleaved stable PAI-1 showed a low residual binding (<25% of untreated stable PAI-1), probably because of residual uncleaved protein. Binding of stable active PAI-1 and of MMP-3 cleaved PAI-1 to insolubilized MA-7F5 was comparable, as determined by biospecific interaction analysis ($K_D$ of 0.39 ± 0.01 × 10^6 or 0.37 ± 0.02 × 10^6 M; means ± S.D., n = 3).

**Binding of PAI-1 to (pro)MMP-3—Biospecific interaction analysis revealed comparable binding affinity of active, substrate, and stable PAI-1 to proMMP-3 ($K_D$ of 12 × 10^6 to 16 × 10^6 M^-1) and to MMP-3 ($K_D$ of 17 × 10^6 to 22 × 10^6 M^-1).**

**Effect of proMMP-3 on the Interaction of PAI-1 with t-PA—**

The second-order rate constant ($k = 1$ to $2 × 10^7$ M^{-1} s^{-1}) of the inhibition of two-chain t-PA (final concentration, 10 nM) by PAI-1 (final concentration, 17 nM) was not affected by the presence of proMMP-3 (final concentration, ≤20 μM) (data not shown).

The molecular interactions between proMMP-3, PAI-1, and t-PA were further investigated by biospecific interaction analysis. This revealed that single chain t-PA also binds to proMMP-3 ($k = 68 ± 4.8 × 10^6$ M^{-1} s^{-1}; $K_D = 10 ± 0 × 10^{-3}$ s^{-1} and $K_A = 6.6 ± 0.46 × 10^6$ M^{-1}); a PAI-1-resistant t-PA...
mutant (sct-PA-Δ(K296-G302)) bound with comparable affinity ($k_{\text{ass}} = 49 \pm 2.1 \times 10^{8} \text{M}^{-1} \text{s}^{-1}$ and $k_{\text{diss}} = 4.1 \pm 0.4 \times 10^{3} \text{s}^{-1}$ and $K_{d} = 12 \pm 0.7 \times 10^{6} \text{M}^{-1}$). When a saturating concentration of single chain t-PA was first bound to proMMP-3, additional subsequent binding of active PAI-1 could be observed (corresponding to 1050 resonance units, as compared with 460 resonance units for direct binding of the same PAI-1 concentration to the proMMP-3 on the sensor chip; data are the mean values of two independent experiments). In contrast, when the proMMP-3 was first saturated with sct-PA-Δ (K296-G302), subsequent binding of PAI-1 was equal to the direct binding of PAI-1 to proMMP-3 (410 resonance units versus 420 resonance units). These findings confirm that active PAI-1 bound to proMMP-3 can still interact with t-PA and suggest that the binding sites on proMMP-3 for t-PA and PAI-1 are different. In separate experiments it was confirmed that active-site blocked t-PA (treated with excess PPACK) binds to insolubilized PAI-1 ($k_{\text{ass}} = 67 \pm 7.0 \times 10^{8} \text{M}^{-1} \text{s}^{-1}$; $k_{\text{diss}} = 3.8 \pm 0.3 \times 10^{3} \text{s}^{-1}$, and $K_{d} = 18 \pm 2.6 \times 10^{6} \text{M}^{-1}$), whereas active-site blocked sct-PA-Δ (K2936-G302) showed no detectable binding to PAI-1 at a final concentration of 1 μM.

**DISCUSSION**

PAI-1 belongs to the serpin superfamily, which comprises about 40 members, mainly inhibitory but also some noninhibitory serpins (26). Serpins can be inactivated by proteinases that they do not inhibit (27, 28). Thus, it has been reported previously that MMP-3 (stromelysin-1) inactivates several serpins by cleavage of a specific peptide bond at or around the P1–P1′ reactive site, located in a loop structure situated 30–40 amino acids from the COOH-terminal end. MMP-3 inactivates α1-proteinase inhibitor (cleavage of P2–P1 Pro–Met) (29, 30), α1-antichymotrypsin (cleavage of P2′–P3′ Ala–Leu) (29), and antithrombin III (cleavage of P1–P1′ Arg–Ser) (29). α1-Proteinase inhibitor is also cleaved and inactivated by interstitial collagenase (MMP-1) (31) and by neutrophil collagenase (MMP-8) (32, 33), which both hydrolyze the Phe–Leu (P9–P8) peptide bond in α1-proteinase inhibitor but does not proteolyze PAI-1 (34). α1-Antichymotrypsin is also inactivated by MMP-1 and MMP-8, which both cleave the Ala–Leu (P2′–P3′) peptide bond (31, 33). Such interactions may constitute a mechanism whereby the local balance of proteinase inhibitory activity may be shifted in favor of proteolysis. Thus, inactivation of antithrombin III by neutrophil elastase was suggested to be a potential pathophysiological mechanism of thrombosis associated with inflammation (35), and inactivation of α1-proteinase inhibitor by MMPs may decrease the inhibitor concentration at the site of inflammation (29, 30, 33).

PAI-1, the main inhibitor of the physiological plasminogen activators t-PA and u-PA, plays an important (patho)physiological role in the hemostatic system. High PAI-1 levels are associated with several thrombotic disease states, including deep venous thrombosis and coronary artery disease (36, 37), an increased risk for reinfarction (38), atherosclerosis (39), and cardiovascular complications resulting from obesity or non-insulin-dependent diabetes mellitus (40, 41). Decreased levels or deficiency of PAI-1, resulting in bleeding after trauma or injury, have been reported in isolated cases (36, 37). Besides its role in hemostasis, PAI-1 is involved in a variety of other (patho)physiological processes, including sepsis, ovulation, embryogenesis, angiogenesis, and wound healing (42–46). High PAI-1 levels are also a strong prognostic indicator of relapse in human cancers (47).

 Whereas noninhibitory serpins react with their target proteinases resulting in cleavage of the P1–P1′ peptide bond without formation of a stable complex, inhibitory serpins form a stable, covalent complex after the initial 1/1 stoichiometric reversible complex (48, 49). PAI-1 is secreted in an active conformation that spontaneously converts into a nonreactive “latent” conformation under physiological conditions (8). Upon cleavage of the P1–P1′ reactive site peptide bond in active PAI-1, the new COOH-terminal end (P14–P1) inserts into β-sheet A to form strand s4A, whereas the new NH2-terminal (P1′–P10′) forms the β-strand s1C (50). Similarly, the noninhibitory substrate conformation in which the P1–P1′ bond is accessible for cleavage, has an insertion of residues P16–P3 into β-sheet A (51). In the latent conformation, the P14–P4 residues are inserted in the protein and form strand s4A of β-sheet A. The remainder of the reactive site loop (P3′–P10′) exists in an extended conformation on the surface of the protein with the P1–P1′ bond inaccessible for target proteinases (7).

Our findings on cleavage of different conformational forms of PAI-1 by MMP-3 are compatible with these structural data. The cleavage sites in PAI-1 in relation to the active site loop are indicated in Fig. 5. Active PAI-1 as well as a stable active PAI-1

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**TABLE I**

Apparent affinity constants of proMMP-3 and MMP-3 for binding to different molecular forms of PAI-1

| Ligand          | ProMMP-3 | MMP-3 |
|-----------------|----------|--------|
|                 | $k_{\text{ass}} \times 10^{7}$ | $k_{\text{diss}} \times 10^{3}$ | $K_{d} \times 10^{6}$ | $k_{\text{ass}} \times 10^{7}$ | $k_{\text{diss}} \times 10^{3}$ | $K_{d} \times 10^{6}$ |
| Active PAI-1    | 29 ± 1.7 | 1.8 ± 0.20 | 16 ± 1.9 | 47 ± 6.9 | 2.8 ± 0.54 | 17 ± 6.1 |
| Latent PAI-1    | 6.9 ± 0.64 | 3.0 ± 0.40 | 2.3 ± 0.45 | 9.9 ± 2.2 | 6.0 ± 0.66 | 1.7 ± 0.19 |
| Substrate PAI-1 | 20 ± 0.70 | 1.4 ± 0.11 | 15 ± 1.2 | 65 ± 4.7 | 3.0 ± 0.17 | 22 ± 0.76 |
| Stable PAI-1    | 15 ± 1.1 | 1.2 ± 0.13 | 12 ± 1.8 | 45 ± 6.5 | 2.6 ± 0.35 | 17 ± 4.7 |

Data are the means ± S.D. of three determinations.
moiety are efficiently cleaved by MMP-3; substrate PAI-1 is also cleaved, but latent PAI-1 is resistant to cleavage. Cleavage of the P10–P9 and P6–P5 bonds in PAI-1 is associated with inactivation of the serpin, as a result of removal of the P1–P1 reactive site peptide bond. These MMP-3 scissile peptide bonds in PAI-1 are located more NH2-terminally of the P1–P1 bond than in antithrombin III, α1-antichymotrypsin, or α1-proteinase inhibitor (29–34).

PAI-1 is stabilized by binding to vitronectin, which is present in plasma but also abundantly in the extracellular matrix (9). Native vitronectin accelerates the inactivation of human α-thrombin by PAI-1, which may be relevant for the control of extravascular proteolysis (52, 53). In the presence of vitronectin thereby competing with u-PA receptor-dependent or integrin-dependent binding of cells to the extracellular matrix (57–61). Thus, the blocking effect of PAI-1 on both u-PA receptor- and integrin-mediated cell adhesion is fully reversible with u-PA (57, 59, 62). Interpretation of the relevance of these findings is, however, complicated by the inhibitory effects of PAI-1, which modulate proteolytic activity in the cell environment (45). The exact role(s) of PAI-1 in cell migration, and the role of its inhibitory activity versus adhesion properties, remains to be further delineated. Cleavage by MMP-3 may, however, represent a mechanism not only decreasing the antiproteolytic activity of PAI-1 but also impairing the competitive inhibitory properties on cell adhesion and/or migration by reducing the binding of cleaved PAI-1 to vitronectin. It is unclear whether such a mechanism could play a role in processes such as wound healing and tumor progression.

Interestingly, PAI-1 associated with proMMP-3 can still functionally interact with t-PA, whereas interaction with active MMP-3 results in its neutralization. Taken together, cleavage and inactivation of PAI-1 by MMP-3 may constitute a mechanism whereby its inhibitory activity may be decreased in (patho)physiological conditions, allowing generation of enhanced proteolytic activity.

Acknowledgments—We are grateful to Dr. A. Rabijns (University of Leuven, Belgium) for advice in the preparation of Fig. 5. Skillful technical assistance by F. De Cock, E. Demarsin, L. Frederix, and H. Moreau is gratefully acknowledged.

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H. Roger Lijnen, Begona Arza, Berthe Van Hoef, Désiré Collen and Paul J. Declerck

J. Biol. Chem. 2000, 275:37645-37650.
doi: 10.1074/jbc.M006475200 originally published online August 30, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M006475200

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