Proteinase Specificity and Functional Diversity in Point Mutants of Plasminogen Activator Inhibitor 1*

(Received for publication, January 16, 1997, and in revised form, February 25, 1997)

Ann Gils and Paul J. Declerck§

From the Laboratory for Pharmaceutical Biology and Phytopharmacology, Faculty of Pharmaceutical Sciences, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Plasminogen activator inhibitor-1 (PAI-1) is a unique member of the serpin superfamily. The alternative behavior of PAI-1 as an inhibitor, a non-inhibitory substrate, or a non-reactive latent form has been shown to be dependent on the initial conformation. In this study, we have evaluated the effect of a substitution outside the reactive site loop (P18) or in the reactive site loop (P6 and P10) on proteinase specificity and conformational transitions in PAI-1. Wild-type PAI-1 (wtPAI-1) revealed the same conformational distribution pattern towards tissue-type plasminogen activator (t-PA) as towards urokinase-type plasminogen activator (u-PA) (i.e. 53 ± 6.9% active, 36 ± 6.8% latent, and 12 ± 1.9% substrate). Inactivation of wtPAI-1 resulted in the conversion of the labile active form into the latent form while the stable substrate form remained unchanged. PAI-1-P6 (Val → Pro at P6) revealed a target specificity for t-PA (39 ± 7% versus 3 ± 2% of the theoretical maximal value toward t-PA and u-PA, respectively), PAI-1-P10 (Ser → Pro at P10) was 4-fold more active toward u-PA than toward t-PA, and PAI-1-P18 (Asn → Pro at P18) exhibited inhibitory properties exclusively toward u-PA (41 ± 10%). Surprisingly, inactivation of these mutants revealed functional and conformational transitions distinct from those observed for wtPAI-1. Inactivation of PAI-1-P6 (Val → Pro) resulted in a total conversion of the active form into the latent form and in a partial conversion of the substrate form into the latent form. The active forms of both PAI-1-P10 (Ser → Pro) and PAI-1-P18 (Asn → Pro) are also labile but, in contrast to the active form of wtPAI-1, convert into substrate forms. Based on the existence of various conformations of PAI-1, we propose an alternative reaction scheme describing the putative interactions between serpins and their target proteinases. The unusual conformational and functional flexibility of PAI-1 that, according to the current study, appears not to be restricted to the reactive site loop further underlines the importance of potential structural rearrangements (e.g. upon binding to cofactors) in PAI-1 (or serpins in general) for its functional behavior at particular biological sites.

* This work was supported in part by a grant from the Research Fund K.U.Leuven (OT/94/27). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Laboratory for Pharmaceutical Biology and Phytopharmacology, Faculty of Pharmaceutical Sciences, Katholieke Universiteit Leuven, E. Van Evenstraat 4, B-3000 Leuven, Belgium. Tel.: 32-16-32 34 31; Fax: 32-16-32 34 60; E-mail: paul.declerck@farm.kuleuven.ac.be.

‡ Senior Research Associate of the National Fund for Scientific Research (Belgium).

§ To whom correspondence should be addressed: Laboratory for Pharmaceutical Biology and Phytopharmacology, Faculty of Pharmaceutical Sciences, Katholieke Universiteit Leuven, E. Van Evenstraat 4, B-3000 Leuven, Belgium. Tel.: 32-16-32 34 31; Fax: 32-16-32 34 60; E-mail: paul.declerck@farm.kuleuven.ac.be.

Plasminogen activator inhibitor 1 (PAI-1), a glycoprotein with an apparent molecular weight of 50,000 (1), is the most important physiological inhibitor of tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) and inhibits both proteinases very rapidly with second-order rate constants of more than $2 \times 10^7 \text{M}^{-1} \text{s}^{-1}$ (2, 3). Increased plasma levels of PAI-1 have been shown to correlate with an increased risk for cardiovascular disease (4; for review, see Ref. 5).

PAI-1 is synthesized as an active molecule that converts spontaneously to a latent form that can be partially reactivated by denaturants such as guanidinium chloride, sodium dodecyl sulfate or urea (6). In addition, a stable non-inhibitory form with substrate properties has been identified (7–9). PAI-1 is a member of the serine proteinase inhibitor (serpin) superfamily (10–13). The serpins comprise more than 40 single-chain proteins, each containing 370–390 residues with an overall amino acid homology of approximately 35% (14, 15). All serpins have the same highly ordered tertiary structure consisting of three beta sheets, A, B, and C, nine g-helices, and a reactive site loop containing residues P16 to P10', which are highly variable (16). The reactive site loop is situated 30–40 amino acids from the carboxyl-terminal end and provides a “bait” peptide bond (P1-P1') that mimics the normal substrate of the target proteinase (17). In PAI-1, the Arg346-Met347 bond has been identified as the P2-P4' bond (18). In latent PAI-1, the amino-terminal part of the reactive site loop (P16-P1) is inserted into beta sheet A forming s4A and rendering P1-P1' inaccessible to the target proteinase. The substrate form of PAI-1 reacts with its target proteinases, e.g. t-PA or u-PA, resulting in a cleavage of the P1-P1' bond but, in contrast to the active form, without formation of a covalent complex and without inhibition of the proteinase. The target specificity of serpins is mainly determined by the nature of the residues at the P1 and P1' position (19–27). However, residues at positions P3 and P2 (26, 28–30) or the presence of cofactors (31) have also been reported to influence the target specificity of serpins.

The alternative behavior of PAI-1 as an inhibitor, a non-inhibitory substrate or a non-reactive latent form, has been shown to be mainly dependent on the initial conformation of this serpin (7). However, in general, the functional behavior of serpins (i.e. inhibition versus substrate) is often explained by the occurrence of one reactive conformation resulting in the generation of common intermediates until the branching point between substrate and inhibitory pathways (32). According to this hypothesis, the product distribution (i.e. complex formation or cleaved serpin) depends on the reaction conditions in-
flaunting this branching (32–35).

In the current study, the characterization of PAI-1 variants carrying proline mutations at positions P6, P10, or P18 reveals (a) a t-PA target specificity for PAI-1-P6(Val → Pro) and a u-PA target specificity for PAI-1-P10(Ser → Pro) and PAI-1-P18(Asn → Pro). (b) an unexpected transition of the active form of PAI-1-P10(Ser → Pro) and PAI-1-P18(Asn → Pro) into a substrate form, and (c) the existence of two distinct substrate populations in PAI-1-P6(Val → Pro). These observations have allowed us to draw an alternative reaction scheme for the interactions between serpins and their target proteinases.

**EXPERIMENTAL PROCEDURES**

**Materials and Methods**—The chromogenic substrate S-2403 was obtained from Chromogenix (Möln达尔, Sweden). t-PA (predominantly single chain) was a kind gift from Boehringer Ingelheim (Brussels, Belgium); low molecular weight two-chain u-PA (tcu-PA) was prepared from single-chain u-PA (scu-PA) kindly provided by Dr. Lijnen (University of Leuven, Belgium). Alternatively, u-PA (consisting of high and low molecular weight tcu-PA in a 75:25 ratio) kindly provided by Bournonville Pharma (Braine l’Alleud, Belgium) was used.

**Construction, Expression, and Purification of PAI-1 and PAI-1 Variants—**PAI-1-P6(Val → Pro) at P6 (PAI-1-P6) and PAI-1-P10(Val → Pro) at P10 were constructed as described before (36). PAI-1-P18 (Asn → Pro) at P18 was constructed in a similar way using the synthetic oligonucleotide 5′-CACCGTGGCACTCTCGGGAAGTCTCAGTTT-3′ to obtain the desired mutation. Expression and purification of wtPAI-1 and variants was performed in Escherichia coli as described earlier (37).

**Measurement of PAI-1 Activity—**PAI-1 activity was determined using the method described by Verheijen (38) by adding a fixed amount of t-PA or u-PA to the PAI-1 containing samples. t-PA was calibrated versus the international reference preparation for t-PA (NISSC 86/670). All PAI-1 activity data are expressed as percentage of the theoretical maximal activity, i.e. ~745,000 units/mg (t-PA inhibitory units) and 120,000 units/mg (u-PA inhibitory units) calculated on the basis of a specific activity for t-PA of 500,000 units/mg and for u-PA of 100,000 units/mg and molecular masses of 67, 54, and 45 kDa for t-PA, u-PA, and PAI-1, respectively.

**Determination of the Conformational Distribution of PAI-1 and PAI-1 Variants—**Samples of wtPAI-1 and PAI-1 mutants were incubated with a 2-fold molar excess of t-PA or u-PA. PAI-1 samples were diluted with phosphate-buffered saline (PBS; 140 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4) to a concentration of 0.25 mg/ml. t-PA and u-PA were diluted with PBS to a concentration of 0.7 mg/ml and 0.5 mg/ml, respectively. Equal volumes of t-PA (or u-PA) and PAI-1 were mixed and incubated at 37 °C for 2 h. The reaction was terminated by adding SDS (final concentration of 1%) and heating during 30 s at 100 °C. Reaction products were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent densitometric scanning with the Imagemaster™ (Pharmacia, Uppsala, Sweden).

**Kinetic Analysis of the Inhibition of t-PA and u-PA by PAI-1 and PAI-1 Variants—**The rate of inhibition of t-PA (predominantly single chain) and u-PA (consisting of high and low molecular weight tcu-PA in a 75:25 ratio) by PAI-1 and PAI-1 variants was determined as follows. u-PA and t-PA were used at concentrations of 0.2 nm, and PAI-1 and PAI-1 variants were used at concentrations of 0.8, 1.2, or 1.6 nm (pseudo first-order conditions). Residual u-PA and t-PA activity was quantified (38) after blocking the reaction by addition of a 200-fold molar excess of the monoclonal antibodies MA-899D4 (for u-PA activity) and MA-867C10 (for t-PA activity). Both antibodies inhibit PAI-1 activity very rapidly and completely, without interference on the u-PA or the t-PA activity assay.

**Inactivation of PAI-1 Samples—**PAI-1 samples (except PAI-1-P18) were inactivated by diluting the samples to a final concentration of 150–190 µg/ml using the appropriate diluent (containing Tween 80 and Na2HPO4) to obtain a buffered solution with 45 mM phosphate, 70 mM NaCl, and 0.01% Tween 80, pH 7.4, followed by incubation at 37 °C for 24 h. Under these conditions, PAI-1-P18 exhibited extensive precipitation. Inactivation of PAI-1-P18 was, therefore, carried out at a concentration of 125 µg/ml in PBS containing 0.01% Tween 80 and 2 mM glutathione and incubation at 4 °C for 60 h.

The half-lives for inactivation were calculated with the program GraphPad Prism™ using ‘one-phase exponential decay’ according to the equation $Y = \text{Span} \times e^{-k \times t} + \text{Plateau}$. The half-life of the decay is then equal to $0.693/k$. 

![Figure 1](http://www.jbc.org/) Inhibitory activity of wtPAI-1 and PAI-1 variants.

Inhibitory activity is expressed as percentage of the theoretical maximum and was determined toward t-PA (black bar) and toward u-PA (hatched bar). wt, wtPAI-1; P6, PAI-1-P6(Val → Pro); P10, PAI-1-P10 (Ser → Pro); and P18, PAI-1-P18(Asn → Pro). Mean ± S.D., n = 3–6.

**Statistical Analysis—**The statistical significance of differences was evaluated using Student’s t-test; p values < 0.05 were considered non-significant.

**RESULTS**

**Determination of Specific Inhibitory Activity against t-PA and u-PA—**wtPAI-1 revealed similar activities toward t-PA (68 ± 10% (mean ± S.D., n = 6)) as toward u-PA (68 ± 6%) (Fig. 1). PAI-1-P6(Val → Pro) was significantly less active (p < 0.0001) toward t-PA (3.9 ± 2.2%) than toward t-PA (45 ± 10%) (n = 6). In contrast, PAI-1-P10(Ser → Pro) and PAI-1-P18(Asn → Pro) were significantly less active (p < 0.01) toward t-PA (5.9 ± 0.33 and 0.20 ± 0.04%, respectively) than toward u-PA (19 ± 4.9 and 41 ± 10%, respectively, n = 3).

**Reaction Products Formed after Incubation of wtPAI-1 and PAI-1 Mutants With t-PA and u-PA at 37 °C—**Incubation of wtPAI-1 at 37 °C with a 2-fold molar excess of t-PA revealed the formation of t-PA-PAI1 complexes (49 ± 6%, mean ± S.D., n = 4), small amounts of cleaved derivative (13 ± 1%), and residual non-reactive material (38 ± 5%) (Fig. 2A and Table I). Under these conditions, the amount of complexes formed with PAI-1-P6(Val → Pro), PAI-1-P10(Ser → Pro), and PAI-1-P18(Asn → Pro) were compatible with their respective activity data. In addition, for all these mutants, a decreased inhibitory activity was associated with an increased (3–10-fold, p < 0.0001 versus wtPAI-1) substrate behavior. Surprisingly, in contrast to wtPAI-1, PAI-1-P6(Val → Pro), and PAI-1-P10(Ser → Pro), virtually no latency was observed for PAI-1-P18(Asn → Pro), demonstrating that the absence of inhibitory activity of this mutant toward t-PA was totally attributed to substrate behavior. (Fig. 2A, Table I)

The amount of complexes formed after incubation of PAI-1-P10 with a 2-fold molar excess of u-PA (Fig. 2B, Table I) was compatible with the u-PA inhibitory activity data. Comparison of the amount of complexes formed in the presence of either t-PA or u-PA further confirmed the proteinase specificity (i.e. 4-fold higher activity toward u-PA than toward t-PA). In agreement with the activity data, no detectable complexes were formed between PAI-1-P18(Asn → Pro) and t-PA, whereas up to 20% complex formation could be observed in the presence of u-PA. Complex formation of PAI-1-P6(Val → Pro) with t-PA was 10–15-fold higher compared with complex formation with u-PA. Importantly, from the data in Table I and Fig. 2, it can be deduced that, for each mutant, the differences in proteinase specificity (with respect to inhibitory activity) were mainly attributed to a concomitant inverse difference in substrate behavior. In all cases, no proteinase-dependent differences in non-reactive material could be observed.

**Reaction Products Formed after Inactivation of wtPAI-1 and PAI-1 Mutants Followed by Incubation with t-PA and u-PA—**As expected, inactivation of wtPAI-1 occurred with a half-life of 60 ± 20 min (mean ± S.D., n = 6) and was associated with a concomitant increase in latent PAI-1. The amount
FIG. 3. SDS-PAGE of wtPAI-1 and PAI-1 variants after incubation at 37 °C (wtPAI-1, PAI-1-P6(Val → Pro), PAI-1-P10 (Ser → Pro) or at 4 °C (PAI-1-P18(Asn → Pro)) and addition of a 2-fold molar excess of t-PA (a) or u-PA (B). Lane 1, wtPAI-1; lane 2, PAI-1-P6(Val → Pro); lane 3, PAI-1-P10(Ser → Pro); and lane 4, PAI-1-P18(Asn → Pro). The solid arrowhead indicates the migration position of intact PAI-1. The arrow indicates the migration position of the serine proteinase/PAI-1 complex. The open arrowhead indicates the migration position of cleaved PAI-1.

TABLE II
Functional behavior of inactivated wtPAI-1 and PAI-1 mutants versus t-PA and u-PA

|          | Complexed | Non-reactive | Cleaved |
|----------|-----------|--------------|---------|
| versus t-PA |           |              |         |
| wtPAI-1   | <2        | 90 ± 1       | 10 ± 1  |
| PAI-1-P6(Val → Pro) | <2        | 83 ± 1       | 17 ± 1  |
| PAI-1-P10(Ser → Pro) | 11 ± 4   | 19 ± 9       | 70 ± 7  |
| PAI-1-P18(Asn → Pro) | 27 ± 1   | <2           | 73 ± 1  |

of substrate remained unchanged. (Fig. 3 and Table II)

Inactivation of PAI-1-P6(Val → Pro) occurred with a similar half-life (64 ± 18 min) and a conversion of the active form to the latent form. In contrast to what was observed for wtPAI-1, the decrease in inhibitory activity was partly associated with a decrease in substrate behavior. However, as observed for wt-PAI-1, a significant amount (~15%) remained as stable substrate. From the time-dependent changes in inhibitory and substrate forms of PAI-1-P6(Val → Pro), it could be calculated that the conversion of this unstable substrate form of PAI-1-P6(Val → Pro) to latent PAI-1-P6(Val → Pro) occurred with a different half-life than the conversion of inhibitory PAI-1-P6(Val → Pro) to latent PAI-1-P6(Val → Pro) (t_{1/2} = 118 ± 34 min versus 64 ± 18 min, p < 0.05). In contrast to wtPAI-1 and PAI-1-P6(Val → Pro), loss of inhibitory activity of PAI-1-P10(Ser → Pro) or PAI-1-P18(Asn → Pro) was almost exclusively associated with an increase in substrate behavior (Fig. 3 and Table II).

Kinetic Analysis of the Inhibition of t-PA and u-PA by PAI-1 and PAI-1 Variants—The second-order rate constants of inhibition of t-PA and u-PA by PAI-1 and PAI-1 variants are shown in Table III. The t-PA specific mutant PAI-1-P6(Val → Pro) as well as the u-PA specific mutant PAI-1-P10(Ser → Pro) inhibit t-PA and u-PA with similar rate constants of ~10^2 m^{-1} s^{-1}, showing that the kinetics of inhibition are not related with the target specificity of the PAI-1 variants.

DISCUSSION

Serpins form a large group of proteins with important regulatory properties toward a wide variety of serine proteinases. Naturally occurring deficiencies and/or mutants with aberrant functional properties often give rise to severe pathologies. Even though from a structural point of view serpins are very similar, functionally they can be divided into two groups: (a) inhibitory serpins forming stable covalent complexes with their target proteinases, and (b) non-inhibitory serpins that are susceptible to cleavage upon reaction with their putative target proteinase. Today, a wide variety of mutants has been characterized (39) with the aim to delineate domains that are important for the
structure-function relationship in serpins. The reactive site loop (P16 to P10) is considered one of the most important domains regarding the interaction between the serpin and its target proteinase. The importance of the P3 to P1 region for target specificity of serpins has been reported extensively before (19–30, 40). While a basic residue at the P1 position of PAI-1 is required for inhibition of u-PA (25), the presence of neutral or hydrophobic amino acids at this position does not affect t-PA inhibition properties (27). Inhibition of u-PA by PAI-1 still occurs when P1 is substituted with any amino acid except proline (25). Alteration of the residues at position P2 and P3 of PAI-1 revealed the possibility for target specific inhibition, with t-PA being more tolerant than u-PA for structural diversity at the P2 and P3 positions (30). The present study describes the functional effects of PAI-1 proline mutations that are remote of the P3-P1 region. The current study reveals that substitution of the residue at position P6 by a proline (PAI-1-P6(Val → Pro)) results in a t-PA target specificity, whereas substitution by a proline at position P10 or P18 yields an increased u-PA target specificity (PAI-1-P10(Ser → Pro)) or even a PAI-1 variant with exclusively u-PA inhibitory properties (PAI-1-P18(Asn → Pro)). The target-specific mutants inhibit t-PA and u-PA with similar rate constants of ~10^7 M^-1 s^-1, illustrating that the kinetics of inhibition do not contribute to this target specificity. To the best of our knowledge, this is the first report of modulation of the proteinase specificity of serpins by mutations remote of the P3-P1 region. Analysis of the reaction products formed between the mutants studied and t-PA or u-PA also revealed significant changes in the inhibitor versus substrate ratio compared with wtPAI-1. The behavior of a serpin as an inhibitor or as a substrate has been reported to be influenced (in part) by external reaction conditions such as temperature, ionic strength, presence of cofactors, etc. (32–34, 41). These effects are generally explained through the existence of a branched pathway as shown in Fig. 4. It should be realized that in those studies, even though large conformational changes could be excluded, minor conformational changes in the reactive site loop could have influenced this reaction behavior. In addition, recent studies have demonstrated that decreased kinetics of insertion (through mutations at position P14) (42, 43) are associated with an increased substrate behavior. This might also explain the substrate-type properties of various serpin mutants in which bulky or charged residues have been introduced in the reactive site loop. (36, 37, 43–47). However, for the serpin PAI-1, it had also been shown that, in the wild-type molecule, different initial conformations exist, which predetermine the reaction pathway (7). The mutants described in the current study further substantiate these findings and provide evidence for the existence of two different substrate conformations. Indeed, inactivation of PAI-1-P6(Val → Pro) results in a disappearance of part of the substrate-reacting population with a different half-life compared with the active form. Based on the branched pathway mechanism, conversion of the one (and only) reactive form would have resulted in a perfectly parallel decrease of substrate and inhibitor reaction. Importantly, even though the substrate behavior of PAI-1-P6(Val → Pro) decreases during incubation at 37 °C, a significant portion appears to be stable (as observed previously with wtPAI-1), clearly demonstrating the existence of both a labile and a stable substrate conformation of PAI-1-P6(Val → Pro). The existence of an initial conformation pathway responsible for the formation of cleaved serpin (PAI-1), independent of the possible phenomenon of “branching,” is further proven by the observation that inactivation of PAI-1-P10(Ser → Pro) and PAI-1-P18(Asn → Pro) results in an increased substrate behavior demonstrating that the active, inhibitory form is converted to a distinct, substrate form. The proposed conformational transitions are further substantiated by the observation of changes in tryptophan fluorescence characteristics upon inactivation of these mutants (data not shown).

Taken together, these observations demonstrate that, in contrast to what is generally accepted, more conformational changes do occur. We propose an alternative reaction scheme of the interaction of the different conformations (I_a, I_b, and I_c) of the PAI-1 variants with their target proteinases (E). Alterations in the ratio of complexed/cleaved are a direct consequence of relative changes in the ratio of the initial conformations I_a and I_c. wt, P6, P10, and P18 represent wtPAI-1, PAI-1-P6(Val → Pro), PAI-1-P10(Ser → Pro), and PAI-1-P18(Asn → Pro), respectively, and indicate the pathways particularly observed with the respective variants.

### Table III

| Inhibition of t-PA | Inhibition of u-PA |
|-------------------|-------------------|
| wtPAI-1           | 0.8 ± 0.2 × 10^7  |
| PAI-1-P6(Val → Pro) | 1.1 ± 0.1 × 10^7  |
| PAI-1-P10(Ser → Pro) | 1.4 ± 0.3 × 10^7  |
| PAI-1-P18(Asn → Pro) | NA 4.3 ± 0.8 × 10^7 |

**Mean ± S.D., n = 3–7. NA, not applicable.**

**k_1**

**E + I**

**E + F**

**P6 & wt**

**P6**

**P10 & P18**

**k_4**

**E + I***

**FIG. 4. Branched pathway of serpins.** E and I represent proteinase and inhibitor, respectively. The common intermediate (EI*) that is formed can react according to either one of two pathways, as an inhibitor which forms a complex with the target proteinase (EI*) or as a substrate that is cleaved by its proteinase (E). Relative changes in k_3 and k_4 then lead to an altered complexed/cleaved ratio (adapted from Gettins, et al. (48)).

**FIG. 5. Alternative reaction scheme of the interaction of the different conformations (I_a, I_b, and I_c) of the PAI-1 variants with their target proteinases (E).** Alterations in the ratio of complexed/cleaved are a direct consequence of relative changes in the ratio of the initial conformations I_a and I_c. wt, P6, P10, and P18 represent wtPAI-1, PAI-1-P6(Val → Pro), PAI-1-P10(Ser → Pro), and PAI-1-P18(Asn → Pro), respectively, and indicate the pathways particularly observed with the respective variants.
in the $k_r/k_a$ ratio in the branched reaction pathway. On the other hand, it should also be realized that our data obviously do not allow us to exclude the possible existence of a branched pathway under certain conditions with certain serpins. Therefore, this possibility is still included in our alternative reaction scheme. However, in this respect, it is important to note that the hypothesis of a branched pathway responsible for the formation of two possible reaction products (complexed or cleaved), first proposed in 1991 (32) and applied in later studies, was based on indirect evidence.

In conclusion, our study reveals (a) three target specific PAI-1 variants in which in each variant only one residue, located outside the P3 to P1 region, is replaced by a proline; (b) the existence of two different substrate populations in the PAI-1-P6(Val → Pro) mutant; and (c) a conformational transformation from the active form into a substrate form for PAI-1-P10(Ser → Pro) and PAI-1-P18(Asn → Pro). These observations lead to the conclusion that the reaction pathway between PAI-1 and its target protease is much more complex than originally anticipated based on the previously proposed branched pathway for serpins.

REFERENCES

1. Van Mourik, J. A., Lawrence, D. A. & Loskutoff, D. J. (1984) J. Biol. Chem. 259, 14914–14921
2. Alessi, M. C., Declerck, P. J., De Mol, M., Neltes, L. & Collen, D. (1988) Eur. J. Biochem. 175, 531–540
3. Lawrence, D. A., Strandberg, L., Grundstrom, T. and Ny, T. (1989) Eur. J. Biochem. 186, 523–533
4. Hamsten, A., Wiman, B., de Faire, U. & Blombäck, M. (1985) N. Engl. J. Med. 313, 1557–1563
5. Declerck, P. J., Juhan-Vague, I., Vaughan, D. E. & Collen, D. (1992) J. Biol. Chem. 267, 11935–11966
6. Urano, T., Strandberg, L., Johansson, L. B. & Ny, T. (1992) Eur. J. Biochem. 209, 985–992
7. Munch, M., Heegaard, C. W. & Andreasen, P. A. (1995) Biochim. Biophys. Acta 1202, 29–37
8. Pannekoek, H., Veerman, H., Lambers, H., Diergaard, P., Verweij, C. L., Van Zonneveld, A. J. & Van Mourik, J. A. (1986) EMBO J. 5, 2539–2544
9. Ny, T., Sawdey, M., Lawrence, D., Milan, J. L. & Loskutoff, D. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 6776–6780
10. Ginsburg, D., Zeheh, K., Young, A. Y., Rafferty, U. M., Andreasen, P. A., Nielsen, L., Dán, Le, R. V. & Gelehrter, T. D. (1986) J. Clin. Invest. 78, 1673–1680
11. Andreasen, P. A., Riccio, A., Weizieder, K. G., Douglas, R., Sartorio, R., Nielsen, L. S., Blasi, F. & Dano, K. (1986) FEBS Lett. 209, 213–218
12. Carrell, R. W. & Boswell, D. R. (1986) in Protease Inhibitors (Barret, A. J., and Salvesen, G., eds.) pp. 403–425, Elsevier Scientific Publishing Co., Amsterdam
13. Huber, R. & Carrell, R. W. (1989) Biochemistry 28, 8951–8966
14. Sprang, S. R. (1992) Trends Biochem. Sci. 17, 49–50
15. Laskowski, M. & Kato, I. (1985) Annu. Rev. Biochem. 49, 593–626
16. Lindahl, T. L., Ohlsson, P. I. & Wiman, B. (1990) Biochem. J. 265, 109–113
17. Owen, M. C., Brennan, S. O., Lewis, J. H. & Carrell, R. W. (1983) N. Engl. J. Med. 309, 694–698
18. Holmes, W. E., Liinen, H. R., Neltes, L., Klufa, C., Nieuwenhuis, H. K., Bijman, D. C. & Collen, D. (1987) Science 238, 209–211
19. Erjument, H., Lane, D. A., Panico, M., Di Marzo, V. & Morris, H. R. (1988) J. Biol. Chem. 263, 5589–5593
20. Aukas, K. S., Pemberton, P. A., Rosen, F. S., Carrell, R. W., Lachmann, P. J. & Harrison, R. A. (1988) Biochem. J. 253, 615–618
21. Rubin, H., Wang, Z., Nickbarg, E. B., McLarney, S., Naidoo, N., Schoenberger, O. I., Johnson, J. L. & Cooperman, B. S. (1995) J. Biol. Chem. 265, 1199–1207
22. Derchin, V. M., Blind, M. A. & Tollefsen, D. M. (1990) J. Biol. Chem. 265, 5623–5628
23. Sherrman, P. M., Lawrence, D. A., Yang, A. Y., Vandenberg, E. T., Paielli, D., Sot, S. T., Shore, D. J. & Ginsburg, D. (1992) J. Biol. Chem. 267, 5788–5795
24. Phillips, J. E., Cooper, S. T., Potter, E. E. & Church, F. C. (1994) J. Biol. Chem. 269, 16696–16700
25. Sherrman, P. M., Lawrence, D. A., Verhamme, I. M., Paielli, D., Shore, D. J. & Ginsburg, D. (1995) J. Biol. Chem. 270, 9301–9306
26. Jallat, S., Carvallo, D., Tessler, L. H., Roelkink, D., Roitsch, C., Ogushi, F., Crystal, R. G. & Courtney, M. (1986) Protein Eng. 1, 29–35
27. Patston, P. A., Rood, N., Schafferli, J. A., Biachoff, R., Courtney, M. & Schapira, M. (1990) J. Biol. Chem. 265, 10786–10791
28. York, J. D., Li, P. & Gardell, S. J. (1991) J. Biol. Chem. 266, 8495–8500
29. Ehrlich, H. J., Gebbink, R. K., Keijer, J., Linders, M., Preisser, K. T. & Pannekoek, H. (1990) J. Biol. Chem. 265, 13029–13035
30. Patston, P. A., Gettins, P. G. W., Beechem, J. & Shapiro, M. (1991) Biochemistry 30, 8876–8882
31. Cooperman, B. S., Stavriri, E., Nickbarg, E., Ramorla, E., Schechter, N. M. & Rubin, H. (1993) J. Biol. Chem. 268, 23616–23625
32. Schechter, N. M., Jordan, L. M., James, A. M., Cooperman, B. S., Wang, Z. & Rubin, H. (1993) J. Biol. Chem. 268, 23626–23633
33. van Meijer, M. & Pannekoek, H. (1985) Fibrinolysis 9, 263–276
34. Audenaert, A.-M., Knockaert, I., Collen, D. & Declerck P. J. (1994) J. Biol. Chem. 269, 19559–19564
35. Gila, A., Knackaert, I. & Declerck P. J. (1996) Biochemistry 35, 7474–7481
36. Verheijen, J. H., Chang, G. T. G. & Kluft, C. (1984) Biochemistry 23, 20293–20301
37. Tucker, H. M., Mottonen, J., Goldsmith, E. J. & Gerard, R. D. (1995) Trends Biochem. Sci. 20, 593–626
Proteinase Specificity and Functional Diversity in Point Mutants of Plasminogen
Activator Inhibitor 1
Ann Gils and Paul J. Declerck

J. Biol. Chem. 1997, 272:12662-12666.
doi: 10.1074/jbc.272.19.12662

Access the most updated version of this article at http://www.jbc.org/content/272/19/12662

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 24 of which can be accessed free at http://www.jbc.org/content/272/19/12662.full.html#ref-list-1