The Course and Prerequisites of Lys-plasminogen Formation during Fibrinolysis†

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Received May 27, 1987; Revised Manuscript Received October 30, 1987

ABSTRACT: Plasmin-catalyzed modification of the native plasma zymogen Glu_1-plasminogen to its more reactive Lys_{78} form has been shown to be enhanced in the presence of fibrin. The aim of the present work has been to characterize the influence of fibrinopeptide release, fibrin polymerization, and plasmin cleavage of fibrin on the rate of Lys_{78}-plasminogen formation. ^{125}I-Labeled Glu_1- to Lys_{78}-plasminogen conversion was catalyzed by preformed Lys_{78}-plasmin, or by plasmin generated during plasminogen activation with tissue plasminogen activator or urokinase. The two forms of plasminogen were quantitated following separation by polyacrylamide gel electrophoresis in acetic acid/urea. Plasmin generated by plasminogen activator was monitored by a fixed-time amidolytic assay. The rate of Lys_{78}-plasminogen formation was correlated, in separate experiments, to the simultaneous, plasmin-catalyzed cleavage of ^{125}I-labeled fibrinogen or fibrin to fragments X, Y, and D. The radiolabeled components were quantitated after separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The results show that the formation of both bathroxobin-catalyzed des-A-fibrin and thrombin-catalyzed des-AB-fibrin leads to marked stimulation of Lys_{78}-plasminogen formation, whereas inhibition of fibrin polymerization, with Gly-Pro-Arg-Pro, abolishes the stimulatory effect. The rate of Lys_{78}-plasminogen formation varies markedly in the course of fibrinolysis. The apparent second-order rate constant of the reaction undergoes a transient increase upon transformation of fibrin to des-A(B) fragment X polymer and decreases about 10-fold to the level observed during fibrinogenolysis upon further degradation to soluble fragments Y and D. It is concluded that efficient conversion of Glu_1- to Lys_{78}-plasminogen requires formation of des-A or des-AB fragment X polymer, in the absence of significant quantities of fragments Y and D. Under these conditions, Lys_{78}-plasminogen formation is a potent stimulatory mechanism in fibrinolysis.

In mammalian organisms, the plasma zymogen Glu_1-plasminogen[1] may be activated to the serine protease plasmin by one of two distinct types of plasminogen activator: t-PA or u-PA. Plasmin is not itself a highly selective protease; however, it does demonstrate selectivity in its degradation of fibrin. This is related to the fact that plasminogen is activated in complex with the solid-phase fibrin substrate and to the fact that α_2-plasmin inhibitor in the surrounding plasma, and other extracellular fluids, rapidly inactivates plasmin dissociated from proteolytically solubilized fibrin (Lijnen & Collen, 1982; Aoki & Harpel, 1984; Müllertz, 1987). In addition to being a plasmin substrate, fibrin accelerates plasmin formation. This is particularly pronounced with t-PA, which binds strongly to fibrin.

During plasminogen activation, plasmin may catalyze the release of an N-terminal peptide (residues 1–77, and to a lesser extent, residues 1–68 and 1–78) from Glu_1-plasminogen, giving rise to the modified zymogen, Lys_{78}- (Met_{69}-, Val_{79}-) plasminogen (Claeys et al., 1973; Rickli & Otavsky, 1973; Robbins et al., 1972; Wiman & Wallén, 1973; Walter et al., 1974, Sodetz & Castellino, 1975; Summaria et al., 1975, Violand & Castellino, 1976). Lys_{78}-plasminogen is far more easily activated to plasmin (Claeys & Vermylen, 1974; Thorsen & Müllertz, 1974, Wallén & Wiman, 1975; Hoylearts et al., 1982; Rijken et al., 1982; Rånby, 1982) and has higher affinity to fibrin polymer than the native proenzyme (Thorsen, 1975; Moroi & Aoki, 1977; Rákóczi et al., 1978; Aoki et al., 1980; Lijnen et al., 1980; Suenson & Thorsen, 1981; Lucas et al., 1983a; Bok & Mangel, 1985). The rate of Lys_{78}-plasminogen formation has been shown to be accelerated by fibrin (when formed in the presence of plasminogen activators), both in the absence and in the presence of α_2-plasmin inhibitor (Müllertz et al., 1984; Thorsen et al., 1984; Takada et al., 1986).

It has recently been shown that des-A-fibrin protofibril formation (and to some extent lateral association of protofibrils) is of decisive importance to the effector function exerted by fibrin on t-PA-catalyzed plasminogen activation (Suenson & Petersen, 1986a). It has also been demonstrated that initial plasmin degradation of fibrin polymer enhances Glu_1-plasminogen and t-PA affinity to fibrin polymer and increases the rate of t-PA-catalyzed plasminogen activation (Suenson et al., 1982, 1984; Tran-Thang et al., 1984; Bok & Mangel, 1985; Harpel et al., 1985; Norrman et al., 1985; Suenson & Petersen, 1986a,b; van Zonnenveld et al., 1986; Higgins & Vehar, 1987).

† This work was supported by the Arvid Nilsson Foundation, the Danish Medical Research Council, and the Lilly Bentine Lund Foundation.

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[1] Abbreviations: Glu_1-plasminogen, native human plasminogen with N-terminal glutamic acid (residues 1–791); Lys_{78}-plasminogen, plasmin-modified plasminogen, mainly with N-terminal lysine (residues 78–790); Val_{443}-plasminogen, elastase-cleaved human plasminogen fragment with N-terminal valine (residues 443–790) [amino acid position numbering according to Forsgren et al. (1987)]; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; factor XIIIa, activated fibrin-stabilizing factor; tranexamic acid, *trans*-4-(aminomethyl)cyclohexane-1-carboxylic acid; Val-Leu-Lys-pNA, *N*-D-valyl-L-leucyl-L-lysine-4-nitroanilide; pNA, 4-nitroanilide; SDS, sodium dodecyl sulfate; ELISA, enzyme-linked immunosorbent assay; NIH unit, National Institutes of Health unit; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; IU, international units.

0006-2960/88/0427-2435$01.50/0

The aim of the present work has been to study the influence of fibrinopeptide release, fibrin polymerization, and plasmin-catalyzed degradation of the fibrin effector on Lys_{78}-plasminogen formation. More precise knowledge of the fibrin (-derived) effector, thus obtained, is a prerequisite to the study and evaluation of the possible physiological role of Lys_{78}-plasminogen formation in fibrinolysis. Preliminary reports of parts of this work have been presented (Suenson et al., 1982; Thorsen & Suenson, 1985).

Materials and Methods

Buffers and Chemicals. Tris-A was 0.05 M Tris-HCl and 0.10 M NaCl, adjusted to pH 7.7 (20 °C) in NaOH. Tris-A–gelatin was Tris-A containing 2.5 g/L gelatin from Difco Laboratories (Detroit, MI). Val-Leu-Lys-pDNA (S-2251) and tranexamic acid were from Kabi (Stockholm, Sweden).

Proteins and Peptides. Human Glu_1-plasminogen, Lys_{78}-plasminogen, Val_{443}-plasminogen, and thrombin were those described (Suenson & Thorsen, 1981; Thorsen et al., 1981; Suenson et al., 1984). Human u-PA with a specific activity of about 20 000 Plough units (=22 000 IU)/mg of protein was purchased from Leo Pharmaceuticals (Ballerup, Denmark). The enzyme was mainly in the $M_r \sim 54\,000$ form, as judged by SDS–polyacrylamide gel electrophoresis followed by fibrin overlay enzymography (Granelli-Piperno & Reich, 1978). A more highly purified preparation of u-PA with a specific activity of about 64 000 Plough units (=70 400 λU)/mg of protein was prepared and analyzed as described (Holmberg et al., 1978) and was a gift from Professor B. Åstedt, (University of Lund, Lund, Sweden). Human single-chain t-PA was purified from the serum-free medium of cultured human melanoma cells (Rijken & Collen, 1981). The preparation solely exhibited a double band with $M_r \sim 70\,000$ in SDS–polyacrylamide gel electrophoresis under reducing conditions. Its catalytic concentration was assayed according to Petersen et al. (1987) and calibrated against the reference preparation of t-PA (83/517) from the National Institute of Biological Standards and Control, London, England. Bathroxobin, aprotinin–Sepharose, and the synthetic polymerization site analogue Gly-Pro-Arg-Pro were those previously described (Suenson & Petersen, 1986a). Fibrinogen (deficient in both factor XIII and plasminogen) was purified from freshly drawn blood or further purified from commercial human fibrinogen, grade L, from Kabi (Stockholm, Sweden) (Suenson et al., 1984). The latter preparation was only used where specifically stated.

Lys_{78}-plasmin was prepared from 34 μM Glu_1-plasminogen in Tris-A with 50% glycerol and 26 mM lysine. The mixture was incubated for 2.5 h at 37 °C with 0.25 volume of packed u-PA–Sepharose, prepared as described (Suenson & Thorsen, 1981). Val_{443}-plasmin was prepared from 12.4 μM Val_{443}-plasminogen in Tris-A with 50% glycerol and incubated with 0.25 volume of packed u-PA–Sepharose for 120 min at 37 °C. Conversion of the two types of plasmin was checked by SDS–polyacrylamide gel electrophoresis under reducing conditions (Suenson & Thorsen, 1981). The Lys_{78}- and Val_{443}-plasmin preparations had intact light chains and intact heavy chain or Kringle 5, respectively.

Fibrinogen-derived fragment X was prepared from 1000 mL of 0.25 μM fibrinogen in Tris-A by incubation for 20 min at 20 °C with 4 nM Val_{442}-plasmin in the presence of 2.5 mM calcium chloride. Thereafter, plasmin activity was quenched by addition of 10 mL of aprotinin–Sepharose (Suenson & Petersen, 1986a). The suspension was stirred for 20 min prior to removal of the gel by filtration with a Seraclear filter (Technicon Instruments Corporation, Tarrytown, NY). The protein solution was concentratd at 25 °C to an absorbancy of $A_{280nm} = 10$, in an Amicon ultrafiltration cell with a YM-30 filter (both from Danvers, MA) prior to final purification by gel filtration using Ultrogel AcA 22 (L'Industrie Biologique Française, distributed by LKB, Stockholm, Sweden). SDS–polyacrylamide gel electrophoresis showed that the purified preparation contained X fragments with only trace amounts of fragments Y and D.

Fibrinogen-derived fragment E was prepared as described (Rupp et al., 1982).

Soluble fibrin degradation products (FDP_{CL}) were prepared from 26.5 μM fibrinogen (further purified from Kabi, grade L) incubated at 37 °C in Tris-A with 17 nM Lys_{78}-plasmin and 3×10^3 NIH units/L thrombin for 10 h. The nearly totally lysed reaction mixture was centrifuged and the supernatant denoted FDP_{CL}. SDS–polyacrylamide gel electrophoresis showed the preparation to contain a mixture of X, Y, and D fragments.

Determination of Protein Concentration. The concentrations of plasminogen, fibrinogen, and fibrinogen-derived fragment X were determined spectrophotometrically (Thorsen, 1975; Mihalyi, 1983). The catalytic concentration of both Lys_{78}- and Val_{443}-plasmin was measured at 37 °C by rate assay on Val-Leu-Lys-pNA using the kinetic constants determind by Wohl et al. (1980). The concentrations of u-PA and t-PA were expressed in molar concentrations by using 1 Plough unit of u-PA = 180 fmol (Christensen et al., 1982) and 1 IU of t-PA = 29 fmol (Petersen et al., 1987).

Polyacrylamide gel electrophoresis in SDS or acetic acid/urea at pH 3.2 was performed as previously described (Suenson & Thorsen, 1981).

^{125}I-Radioiodination of Glu_1-plasminogen and Fibrinogen. Glu_1-plasminogen was radioiodinated with $Na^{125}I$ [Hoechst, Frankfurt (Main), West Germany] by oxidation with *N*-chlorobenzenesulfonamide-derivatized polystyrene beads: Iodo-Beads (Pierce, Rockford, IL). Glu_1-plasminogen (800 μL), 94 μM in Tris A, was incubated with 10 μL, 37 MBq (=1 mCi), $Na^{125}I$ and three Iodo-Beads for 3 min at room temperature. Free iodide and protein in the iodination mixture were separated on Sephadex G-25 (Pharmacia, Uppsala, Sweden), equilibrated, and eluted with Tris-A. The specific activity of the preparation ranged from 245 to 321 MBq/μmol of protein, and the iodine substitution level ranged from 0.007 to 0.008 atom of iodine/protein molecule.

Fibrinogen was radioiodinated with solid-phase glucose oxidase/lactoperoxidase (Enzymo-Beads, Bio-Rad Laboratories, Richmond, CA). Three hundred microliters of the gel suspension was added to 800 μL of 32 μM fibrinogen, 300 μL of 11.5 mM β-D-glucose in distilled water, and 10 μL of 37 MBq $Na^{125}I$ and incubated for 15 min at 20 °C. Unbound radioiodide was removed as described above. The fibrinogen preparations, prepared in this manner, had a specific activity ranging from 212 to 506 MBq/μmol and an iodine substitution level of 0.006–0.007 atom of iodine/protein molecule. Radioiodination of fibrinogen using Iodo-Beads, or other chloramine T related methodology, resulted in total inhibition of thrombin-initiated polymerization.

Analysis of the Time Course of ^{125}I-Labeled Glu_1- to Lys_{78}-plasminogen Conversion during Fibrinogenolysis and Fibrinolysis. Correlation to the Catalytic Concentration of Plasmin. A mixture of ^{125}I-labeled Glu_1-plasminogen and fibrinogen (derivative) was incubated with Lys_{78}-plasmin or plasminogen activator in Tris-A. Where indicated, thrombin was added simultaneously with Lys_{78}-plasmin or plasminogen activator. At specified times of incubation at 37 °C, plasmin

amidolytic activity and the amount of Glu_1-plasminogen converted to Lys_{78}-plasminogen were measured.

Plasmin activity was determind by a fixed-time assay elaborated from that described by Sakata and Aoki (1982). Val-Leu-Lys-pNA (450 μL), 0.67 mM in Tris-A, was added to 100 μL of reaction mixtures containing insoluble fibrin (derivative) polymer or to 100-μL aliquots removed from soluble reaction mixtures. The mixtures were vigorously stirred with a plastic spatula and further incubated for 3 min at 37 °C. Plasmin cleavage of the synthetic tripeptide substrate was thereafter quenched by addition of 130 mg of urea and 100 μL of 9.6 M acetic acid, and pNA release was measured at A_{405nm}. The values were corrected for those found in corresponding reaction mixture with thrombin alone as well as those found in blanks where urea and acetic acid were added prior to tripeptide substrate. Apparent plasmin concentrations were calculated from the increase in pNA concentrations over the 3-min incubation periods, using the kinetic constants of Wohl et al. (1980). The coefficients of variation of single determinations ranged between 3.6% and 8% when calculated from 46 double determinations at three different levels between 3.5 and 138 nM. In reaction mixtures containing no insoluble fibrin derivative, Val-Leu-Lys-pNA amidolysis was found to occur at a constant rate throughout the 3-min incubation period. This was also the case with insoluble fibrin derivatives, at low concentrations where their turbidity could be canceled by dual-wavelength spectophotometry (Suenson & Petersen, 1986a). Maximally, 10% of the Val-Leu-Lys-pNA substrate was cleaved during incubation. No correction was made for the influence of fibrinogen or fibrin (derivatives) on plasmin amidolytic activity (Figures 3, 5B, and 7B). When intact fibrin (derived from 9 μM fibrinogen) was degraded to fragments X, Y, and D, the plasmin amidolytic activity varied from about 0.8 to 1.2 times that found in the absence of fibrin derivatives (see Figure 3 and Results). The fractional variations, probably due to competitive inhibition (Wiman et al., 1979) and allosteric effects (Brockway & Castellino, 1971; Christensen, 1978), were independent of plasmin concentration when tested in a 3–42 nM range.

Glu_1- to Lys_{78}-plasminogen conversion was measured in the same quenched reaction mixtures in which plasmin amidolytic activities were analyzed. Samples were subjected to acetic acid/urea–polyacrylamide gel electrophoresis, after which ^{125}I-labeled Glu_1- and Lys_{78}-plasminogen radioactivity was quantitated in excised gel sections as previously reported (Suenson & Thorsen, 1981). The coefficients of variation of single determinations of Glu_1- and Lys_{78}-plasminogen ranged between 3% and 33% (Müllertz et al., 1984). The highest coefficient of variation was observed with Lys_{78}-plasminogen when it constituted only a minimal fraction (≤0.03) of the total amount of plasminogen in the electrophoretic gel. The decrease in Glu_1-plasminogen radioactivity was in each case equal to the increase in Lys_{78}-plasminogen activity. No Lys_{78}-plasminogen formation could be detected during the 3-min incubation period elapsing from the addition of Val-Leu-Lys-pNA until plasmin amidolytic activity was quenched by addition of acetic acid/urea.

Assay of the Influence of Fibrin Polymerization on the Time Course of Glu_1- to Lys_{78}-plasminogen Conversion. Preformed Lys_{78}-plasmin and thrombin (or bathroxobin) were added simultaneously to a mixture of ^{125}I-labeled Glu_1-plasminogen and fibrinogen in Tris-A and incubated at 37 °C. Analogous reaction mixtures contained 4.6 mM Gly-Pro-Arg-Pro, which prevented polymerization for at least 90 min, as judged by turbidity measurements in a Beckman Acta IV spectophotometer at λ = 360 nm. At specified times of incubation the reactions were quenched by adding 2 volumes of 1.75 M acetic acid/10 M urea to reaction mixtures containing fibrin (derivative) polymer or to aliquots removed from soluble reaction mixtures. Each sample was subjected to acetic acid/urea–polyacrylamide gel electrophoresis after which ^{125}I-labeled Glu_1- and Lys_{78}-plasminogen radioactivity was quantitated as above.

Calculation of Apparent Rate Constants (k_{app}) of Lys_{78}-plasminogen Formation. Apparent rate constants (k_{app}) of Lys_{78}-plasminogen formation were calculated in order to obtain quantitative estimates with which to compare the efficacy of the fibrinogen effector, upon its conversion to fibrin and (subsequent) degradation by plasmin. Concurrent concentrations of Glu_1-plasminogen and plasmin were used in the calculations both in experiments with preformed plasmin (Figures 1, 2A, and 4) and in experiments where plasmin was continuously generated by activation of plasminogen (Figures 6A and 8A). The calculations were based on the assumption that the rate of Glu_1- to Lys_{78}-plasminogen conversion can be expressed by the equation

$$d[P]/dt = -d[S]/dt = k_{app}[E][S] \quad (1)$$

where P = Lys_{78}-plasminogen, S = Glu_1-plasminogen, E = plasmin, t = time, and k_{app} = apparent rate constant. Furthermore, it was assumed that the rate of Lys_{78}-plasminogen formation was unaffected by product inhibition. These assumptions were substantiated by experiments reported under Results.

By integrating eq 1, we obtain

$$\ln [S]_t/[S]_0 = -k_{app}[E]t \quad (2)$$

which can be transformed to

$$\log [S]_t = -k_{app}[E]t \ln 10 + \log [S]_0 \quad (3)$$

In experiments in which Glu_1- to Lys_{78}-plasminogen conversion was catalyzed by a constant concentration of Lys_{78}-plasmin, ([E], determined by rate assay prior to initiation of the experiments), log $[S]_t$ was plotted versus t (Figures 1, 2A, and 4). The k_{app} values (Table I) were then calculated from the slopes of linear curves according to eq 3 by using linear regression analysis.

In experiments where Glu_1- to Lys_{78}-plasminogen transformation was catalyzed by plasmin continuously generated by activation of plasminogen, the concentration of Lys_{78}-plasminogen ($[P]_t$) was plotted versus t (Figure 5A and 7A). The k_{app} values, at corresponding points in time, were then calculated from a modification of eq 1:

$$\Delta[P]/\Delta t = k_{app}[E]_{t,app}[S]_t \quad (4)$$

where Δ[P] = the increase in [P] over the time interval $\Delta t = t \pm 60$ s, as determind from the $[P]_t$ versus t plots, and where $[E]_{t,app}$ = apparent plasmin concentration, measured at t; $[S]_t$ = Glu_1-plasminogen concentration, measured at t (Figures 6A and 8A). The coefficients of variation of single determinations of k_{app} were 34% ($k_{app} \leq 10^4$ $M^{-1}\cdot s^{-1}$) and 23% ($k_{app} > 10^4$ $M^{-1}\cdot s^{-1}$), when calculated from 11 duplicate determinations at each level.

Assay of Fibrinogen or Fibrin Degradation to Fragments X, Y, and D during the Course of Glu_1- to Lys_{78}-plasminogen Conversion. Plasmin-catalyzed degradation of fibrinogen or thrombin-treated fibrinogen was analyzed in analogous reaction mixtures to those described above except that they contained ^{125}I-labeled fibrinogen and nonlabeled Glu_1-plasminogen. At specified times of incubation, 100-μL reaction mixtures containing fibrin polymer or 100-μL aliquots of

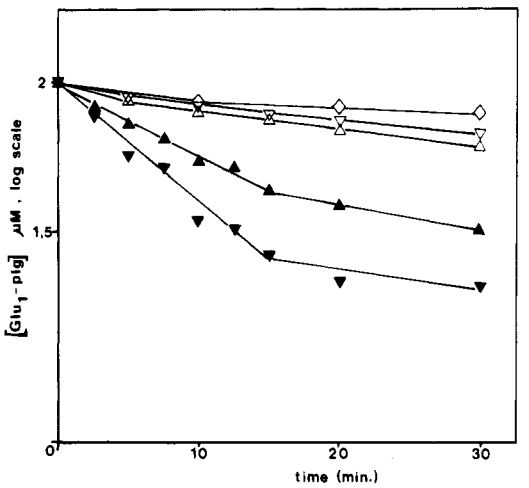

FIGURE 1: Influence of fibrinogen, fibrin, and their plasmin-cleaved derivatives on the time course of Glu_1- to Lys_{78}-plasminogen transformation, catalyzed by preformed Lys_{78}-plasmin. ^{125}I-Glu_1-plasminogen, 2 μM, was incubated at 37 °C with 11 nM preformed Lys_{78}-plasmin in the presence of 9 μM fibrinogen (Δ), 9 μM fibrinogen and 0.5×10^3 NIH unit/L thrombin (▲), 9 μM fragment X (▽), 9 μM fragment X and 0.5×10^3 NIH unit/L thrombin (▼), or 9 μM fragment E (◇). At specified times of incubation the remaining concentration of Glu_1-plasminogen was measured. Each point is the mean of duplicate determinations.

soluble reaction mixtures were mixed with 200 μL of 52.5 mM SDS and 9 M urea. ^{125}I-Fibrin(ogen) and its individual degradation products were separated electrophoretically in homogeneous 5% polyacrylamide gels, the individual bands were excised, and their radioactivity was quantified. Molar concentrations of fibrin(ogen) fragments X, Y, and D were calculated from their radioactivity by means of separate experiments, where radiolabeled fibrin(ogen) of known specific activity was partially converted to fragment X with preformed plasmin (after short periods of incubation) or completely converted to fragments D and E (after prolonged incubation). The specific activity of these fragments could thus be determined. That of fragment Y was estimated by subtracting the specific activity of fragment D from that of fragment X.

RESULTS

Time Course of Lys_{78}-plasminogen Formation during Fibrinogenolysis and Fibrinolysis, As Mediated by Preformed Lys_{78}-plasmin. Figure 1 demonstrates that the rate of plasmin-catalyzed Glu_1- to Lys_{78}-plasminogen transformation was slow when the reaction was initiated in the presence of fibrinogen or fibrinogen-derived fragments X or E. The rate of disappearance of Glu_1-plasminogen (reflecting the rate of Lys_{78}-plasminogen formation) could in all three instances be approximated to linear semilogarithmic plots reflecting apparent first-order kinetics. From the slope of these plots and the Lys_{78}-plasmin concentration of the reaction mixtures, k_{app} values were calculated and found to range between 0.29×10^4 and 0.56×10^4 $M^{-1} \cdot s^{-1}$ (Table I). Plasmin modification of Glu_1-plasminogen was associated with progressive degradation of fibrinogen or fragment X. At 30 min, Y and D fragments were dominant (not shown).

Thrombin-catalyzed cleavage and resultant polymerization of fibrinogen or fragment X resulted in a striking change in the kinetics of Lys_{78}-plasminogen formation (Figure 1). A biphasic pattern appeared, with a primary, rapid reaction rate followed by a slower one. The rate of the first phase was especially high in the presence of purified fragment X polymer. From the linear part of each phase of the semilogarithmic plots of Figure 1, and the Lys_{78}-plasmin concentration of the reaction mixtures, k_{app} values were calculated (Table I). Thrombin treatment of fibrinogen and fragment X increased the k_{app} (first phase) about 4- and 6-fold, respectively ($P < 0.001$). The difference in k_{app} (phase 1), in the fibrin and fragment X polymer systems, was likewise significant ($P < 0.001$). Transition from the first phase to the second resulted in a decrease in rate constants to that observed during fibrinogenolysis. The change in kinetics of Lys_{78}-plasminogen formation occurred at a stage of the associated fibrinolytic process where considerable amounts of fragments Y and D had been formed (Figure 2).

Table I: Apparent Second-Order Rate Constants (k_{app}) for Lys_{78}-plasminogen Formation during Fibrinogenolysis and Fibrinolysis, Catalyzed by Preformed Lys_{78}-plasmin[a]

effector moiety	$k_{app} \times 10^{-4}$ ($M^{-1} \cdot s^{-1}$) [mean ± SEM (n)]
fibrinogen	0.56 ± 0.085 (2)
fibrinogen-derived fragment X	0.52 ± 0.025 (2)
fibrinogen-derived fragment E	0.29 ± 0.035 (2)
fibrin	
phase 1	2.26 ± 0.11 (8)
phase 2	1.00 ± 0.13 (4)
fragment X polymer	
phase 1	3.37 ± 0.13 (6)
phase 2	0.62 ± 0.16 (2)

[a] The data are from the experiment of Figure 1 and other similar experiments where reaction mixtures initially contained 11 nM Lys_{78}-plasmin, 1 or 2 μM Glu_1-plasminogen, and 9 μM fibrin(ogen) (derivative). The k_{app} values were calculated according to eq 3 (see Materials and Methods) by using the slope of the linear first-order plots of the time course of Lys_{78}-plasminogen formation, in experiments initiated in the presence of fibrinogen or fibrinogen-derived fragments X or E. Alternatively, the slope of the linear first or second phases of the plots describing Lys_{78}-plasminogen formation in experiments initiated in the presence of thrombin-treated fibrinogen (fibrin), or thrombin-treated fragment X (fragment X polymer), was used. In each experiment the slope was estimated by linear regression analysis of 4–7 data points with fibrinogen derivatives ($r \leq -0.972$) or fibrin derivatives, phase 1 ($r \leq -0.990$), or of 3–4 data points with fibrin derivatives, phase 2 ($r \leq -0.894$).

The first-order kinetics of (phases of) Lys_{78}-plasminogen formation indicated by Figure 1 were compatible with the results of separate experiments, showing that the rate of Lys_{78}-plasminogen formation during fibrinogenolysis and in the two phases of fibrinolysis were proportional to the Glu_1-plasminogen concentration (0.2–2 μM) and to preformed Lys_{78}-plasmin concentrations (5–25 nM) (not shown).

The slow rates of Lys_{78}-plasminogen formation found during fibrinogenolysis and late in the course of fibrinolysis (Figure 1) were not related to Lys_{78}-plasmin instability. Thus, the rate of amidolysis of Val-Leu-Lys-pNA remained unchanged or increased during the course of the experiments (Figure 3). Furthermore, at least 90% of the heavy and light chains of ^{125}I-labeled Lys_{78}-plasmin were found to retain their apparent molecular weight (determined by SDS–polyacrylamide gel electrophoresis) throughout the duration of analogous experiments with equal reactant concentrations (not shown). Product inhibition could not explain the slow reaction rates either, as addition of 1 μM Lys_{78}-plasminogen to analogous reaction mixtures did not influence the rate of Lys_{78}-plasminogen formation (not shown).

Influence of Fibrinopeptide Release and Fibrin Polymerization on the Time Course of Lys_{78}-plasminogen Formation. Figure 4 shows that the rate of Lys_{78}-plasminogen formation followed a biphasic pattern, with an enhanced primary rate, regardless of whether fibrinogen was cleaved by thrombin, forming des-AB-fibrin, or by bathroxobin, forming des-A-fibrin. Under the conditions of these experiments, the k_{app} of the first phase was 1.4×10^4 $M^{-1} \cdot s^{-1}$ with des-A-fibrin and

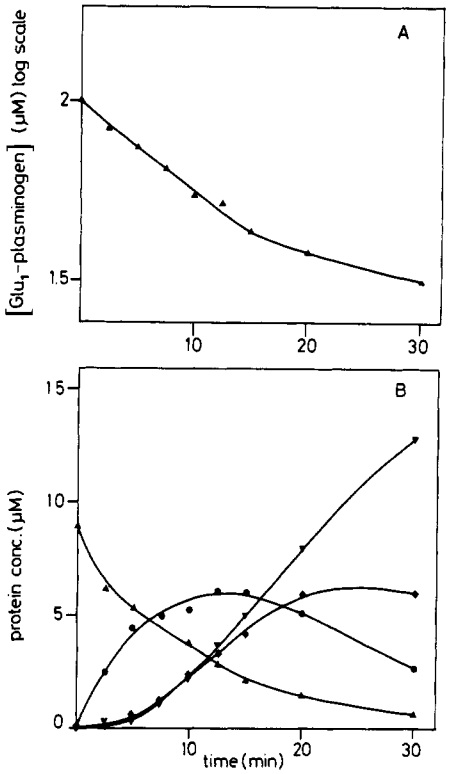

FIGURE 2: Correlation of the time course of Glu_1- to Lys_{78}-plasminogen transformation and simultaneous fibrin degradation, catalyzed by preformed Lys_{78}-plasmin. The data of (A) are from the experiment with fibrin (thrombin-treated fibrinogen) of Figure 1. The data of (B) are derived from an identical experiment, except that fibrin was ^{125}I-labeled, instead of Glu_1-plasminogen. At specificied times of incubation, plasmin-catalyzed transformation of ^{125}I-labeled fibrin (▲) to fragments X (●), Y (♦), and D (▼) was quantified. Each point is the mean of duplicate determinations.

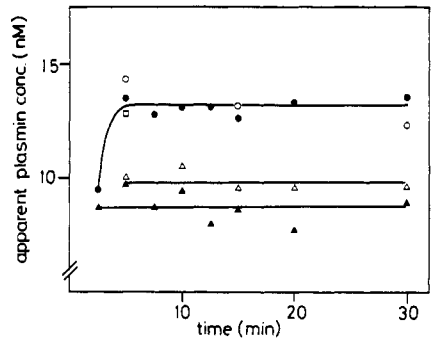

FIGURE 3: Influence of fibrinogen, fibrin, and their plasmin-cleaved derivatives on the amidolysis of Val-Leu-Lys-pNA, by preformed Lys_{78}-plasmin. The data are from the experiments of Figure 1. At specified times of incubation the apparent plasmin concentrations in the fibrinogen (△), fibrin (▲), fragment X (○), and fragment X polymer (●) systems were calculated from the results of duplicate fixed-time assays of plasmin amidolysis of Val-Leu-Lys-pNA (see Materials and Methods).

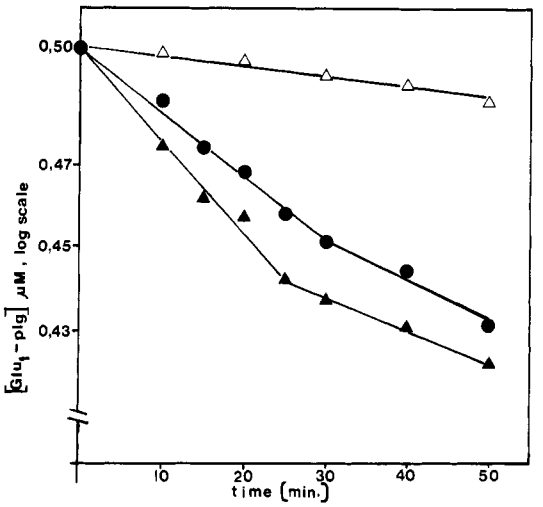

FIGURE 4: Influence of fibrinopeptide release and fibrin polymerization on the time course of Glu_1- to Lys_{78}-plasminogen conversion by preformed Lys_{78}-plasmin. ^{125}I-Labeled Glu_1-plasminogen, 0.5 μM, was incubated at 25 °C with 5 nM preformed Lys_{78}-plasmin in the presence of 1.5 μM fibrinogen with 0.5×10^3 NIH unit/L thrombin (▲), a corresponding concentration of bathroxobin (●), or 0.5×10^3 NIH unit/L thrombin and 4.6 mM Gly-Pro-Arg-Pro (△). (The latter prevented polymerization for at least 90 min as judged by turbidity measurements at $\lambda = 360$ nm.) At specified times of incubation the remaining Glu_1-plasminogen concentration was determined. Each point is the mean of duplicate determinations.

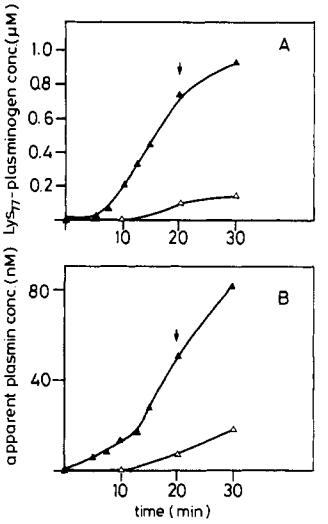

FIGURE 5: Time course of Lys_{78}-plasminogen and plasmin formation during fibrinogenolysis and fibrinolysis mediated by t-PA. ^{125}I-Glu_1-plasminogen, 2 μM, was incubated at 37 °C with 0.43 nM t-PA in the presence of 9 μM fibrinogen (△) or 9 μM fibrinogen and 0.5×10^3 NIH unit/L thrombin (▲). At specified times of incubation the concentration of Lys_{78}-plasminogen (A) and the apparent concentration of plasmin (B) generated were measured. All points represent the mean of duplicate determinations. The arrows mark the time of clot lysis.

2.0×10^4 $M^{-1} \cdot s^{-1}$ with des-AB-fibrin, when calculated as described in footnote *a* of Table I. Inhibition of fibrin polymerization by addition of Gly-Pro-Arg-Pro (Laudano et al., 1983) resulted in a marked decrease in rate of Lys_{78}-plasminogen formation (Figure 4). The k_{app} ($=0.24 \times 10^4$ $M^{-1} \cdot s^{-1}$) for Lys_{78}-plasminogen formation in the polymerization-inhibited system was at least about 6 times less than that of the first phase in the system with polymerized des-A- or des-AB-fibrin. The polymerization-inhibited system mainly contained des-A-fibrin monomer as it is well documented that inhibition of fibrin polymerization profoundly decreases the rate of thrombin-catalyzed fibrinopeptide B release (Blombäck et al., 1978; Hurlet-Jensen et al., 1982; Higgins et al., 1983). In order to ensure that Gly-Pro-Arg-Pro does not interfere with plasmin-catalyzed Glu_1- to Lys_{78}-plasminogen conversion, the experiment of Figure 4 was repeated without fibrin, using 0.3 mM tranexamic acid as an alternative effector and 10-fold higher plasmin concentrations, to obtain comparable rates of Lys_{78}-plasminogen formation. In these experiments (not shown), the presence of Gly-Pro-Arg-Pro did not alter the rate of plasmin modification of Glu_1-plasminogen.

Time Course of Lys_{78}-plasminogen Formation during Fibrinogenolysis and Fibrinolysis Mediated by t-PA. Figure 5 shows the time course of Lys_{78}-plasminogen (Figure 5A) and

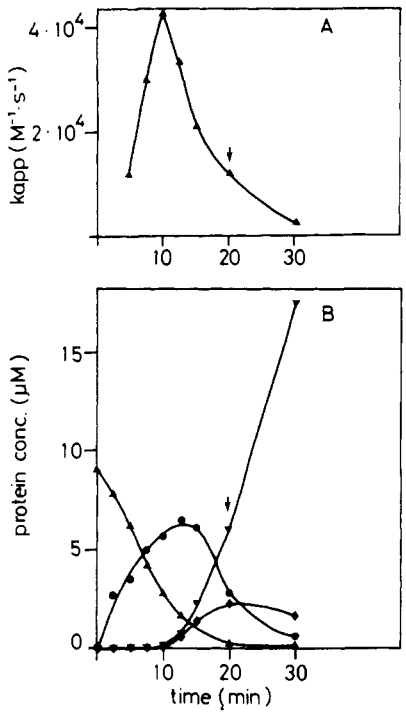

FIGURE 6: Variation in the apparent second-order rate constant (k_{app}) of Lys_{78}-plasminogen formation during fibrinolysis mediated by t-PA. Correlation to simultaneous plasmin cleavage of the fibrin effector. (A) k_{app} values of Lys_{78}-plasminogen formation versus time, derived from the progress curves of Lys_{78}-plasminogen and plasmin formation of Figure 5, as described under Materials and Methods, by using eq 4. (B) Simultaneous plasmin cleavage of ^{125}I-fibrin polymer (▲) to fragments X (●), Y (♦), and D (▼) versus time. The experiments were identical with those of Figure 5 with fibrin, except that fibrinogen was ^{125}I-labeled instead of Glu_1-plasminogen. Each point in (A) and (B) represents the mean of duplicate determinations. The arrows mark the time of clot lysis.

plasmin formation (Figure 5B) when activation of Glu_1-plasminogen with t-PA was initiated in the presence of fibrinogen or fibrin. The rate of formation of both Lys_{78}-plasminogen and plasmin increased markedly upon conversion of fibrinogen to fibrin.

In order to allow for the variation of plasmin concentration in the course of these experiments (and those of the following series of experiments with u-PA, Figures 7 and 8), the changes in the kinetics of Lys_{78}-plasminogen formation during fibrinolysis are visualized in Figure 6A (and Figure 8A) by plotting k_{app} versus time. (For further explanation see Materials and Methods.) The k_{app} increased at least 4-fold (to 4.2×10^4 M^{-1}·s^{-1}) over a 10-min period from the initiation of the experiment involving t-PA-mediated fibrinolysis (Figure 6A). This corresponds to a period of time in which fibrin was progressively degraded to fragment X polymer, and in which no degradation to lower molecular weight degradation products could be detected (Figure 6B). Thereafter, k_{app} gradually fell to the low levels seen during progressive degradation of fibrinogen.

With the fibrinogen effector, the k_{app} values decreased from 0.9×10^4 to 0.2×10^4 M^{-1}·s^{-1} in the course of the experiment. This was associated with the plasmin-catalyzed degradation of 9 μM fibrinogen to 5 μM fragment X, 1 μM fragment Y, and 1.5 μM fragment D (not shown).

Time Course of Lys_{78}-plasminogen Formation during Fibrinogenolysis and Fibrinolysis Mediated by u-PA. Figure 7 shows the time course of Lys_{78}-plasminogen (Figure 7A) and plasmin formation (Figure 7B) when activation of Glu_1-plasminogen with u-PA was initiated in the presence of fibrinogen, fibrin, and soluble fibrin degradation products

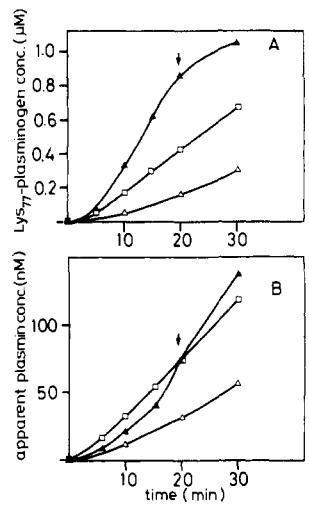

FIGURE 7: Time course of Lys_{78}-plasminogen and plasmin formation during fibrinogenolysis and fibrinolysis mediated by u-PA. ^{125}I-Glu_1-plasminogen, 2 μM, was incubated at 37 °C with 0.36 nM u-PA in the presence of 9 μM fibrinogen (△), 9 μM fibrinogen with 0.5×10^3 NIH unit/L thrombin (▲), or soluble fibrin degradation products, FDP_{CL}, consisting of a mixture of fragments X, Y, and D, prepared from 9 μM fibrinogen and 1 NIH unit/L thrombin (□). At specified times of incubation the concentration of Lys_{78}-plasminogen (A) and the apparent concentration of plasmin (B) generated were quantified. Each point represents the mean of duplicate determinations. The arrows mark the time of clot lysis.

consisting of a mixture of fragments X, Y, and D (FDP_{CL}). Similar results were obtained with the two u-PA preparations, containing respectively 22 000 and 70 400 IU/mg of protein. The results are comparable to those obtained with t-PA. Similar rates of plasmin formation were obtained in systems initiated in the presence of fibrin polymer and FDP_{CL}; however, the rate of Lys_{78}-plasminogen formation was lower with FDP_{CL}.

Figure 8A shows that the changes in the k_{app} values, reflecting changes in the kinetics of Lys_{78}-plasminogen formation in the course of plasmin-catalyzed degradation of fibrin, were essentially the same as those found when Glu_1-plasminogen was activated with t-PA (compare with Figure 6A). The k_{app} values of Lys_{78}-plasminogen formation increased from the initiation of the experiment to a peak level of 2.9×10^4 M^{-1}·s^{-1}, at which point $^1/_3$ of the fibrin effector was converted solely to fragment X polymer. Thereafter, k_{app} values gradually fell to the levels seen during the degradation of fibrinogen.

With the fibrinogen effector the k_{app} of Lys_{78}-plasminogen formation decreased from 1.0×10^4 to 0.3×10^4 M^{-1}·s^{-1} in the course of fibrinogenolysis, in a manner similar to that seen in t-PA-activated systems (Figure 6A).

DISCUSSION

The rate of t-PA-catalyzed activation of Glu_1-plasminogen increases in the presence of fibrin as compared to fibrinogen, the second-order rate constant increasing approximately 60-fold (Hoylearts et al., 1982). A further 7–22-fold increase in the second-order rate constant of plasmin formation is found upon transformation of Glu_1- to Lys_{78}-plasminogen in the course of early fibrinolysis (Rånby, 1982; Hoylearts et al., 1982; Suenson & Petersen, 1987). Glu_1- to Lys_{78}-plasminogen transformation is therefore potentially a very potent amplification mechanism in fibrinolysis.

In both plasma systems and purified systems, transformation of fibrinogen to fibrin has been shown to be a prerequisite for rapid Lys_{78}-plasminogen formation (Suenson et al. 1982; Müllertz et al., 1984; Thorsen et al., 1984; Takada et al., 1986). The present study indicates that the stimulatory effect

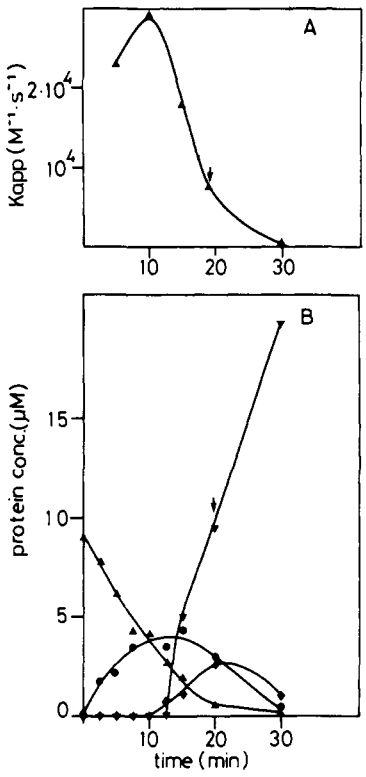

FIGURE 8: Variation in the apparent second-order rate constant (k_{app}) of Lys_{78}-plasminogen formation during fibrinolysis mediated by u-PA. Correlation to simultaneous plasmin cleavage of the fibrin effector. (A) k_{app} values of Lys_{78}-plasminogen formation versus time, derived from the progress curves of Lys_{78}-plasminogen and plasmin formation of Figure 7, as described under Materials and Methods, by using eq 4. (B) Simultaneous plasmin cleavage of the ^{125}I-labeled fibrin effector (▲) to fragments X (●), Y (♦), and D (▼) versus time. The experiment was identical with that of Figure 7 with fibrin, except that fibrinogen was ^{125}I-labeled instead of Glu_1-plasminogen. Each point in (A) and (B) represents the mean of duplicate determinations. The arrows mark the time of clot lysis.

of fibrin is dependent on a sequence of at least three distinguishable steps: thrombin-catalyzed fibrinopeptide release, fibrin polymerization, and early plasmin-catalyzed degradation of fibrin polymer to yield fragment X polymer. These effector transformations result in an at least 6-fold increase in rate of Lys_{78}-plasminogen formation (Table I).

Conversion of fibrinogen to des-A-fibrin (catalyzed by bathroxobin), as well as to des-AB-fibrin (catalyzed by thrombin), leads to a marked increase in the rate of Lys_{78}-plasminogen formation (Figures 1 and 4), indicating that the release of fibrinopeptide B is not essential to the stimulatory effect of fibrin on Lys_{78}-plasminogen formation, just as it is not essential to the enhancing effect of fibrin on the direct activation of Glu_1-plasminogen to Glu_1-plasmin by t-PA or u-PA (Norrman et al., 1985; Suenson & Petersen, 1986a).

The polymerization site analogue Gly-Pro-Arg-Pro inhibits the stimulation of Lys_{78}-plasminogen formation by fibrin, suggesting that protofibril formation, and possibly their subsequent lateral association and gelation (Hantgan et al., 1983), is essential to the stimulatory effect of fibrin (derivatives) on Lys_{78}-plasminogen formation. Fibrin polymerization has previously been shown to be essential to its stimulatory effect on t-PA-catalyzed plasminogen activation (Suenson & Petersen, 1986a). The inhibitory effect of the tetrapeptide is not due to interference with plasmin-catalyzed Glu_1- to Lys_{78}-plasminogen conversion (see Results). However, the possibility remains that Gly-Pro-Arg-Pro might interfere with plasminogen binding to the fibrin effector and thus quench its effector function. This is however unlikely, as the plasminogen binding sites of fibrin most probably involve lysine residues (Radcliff, 1983; Brownlee, 1983), with which the tetrapeptide amino acids presumably hardly interact (Root-Bernstein, 1982). In relation to the importance of polymerization, it is of interest to note the congenital dysfibrinogenemia of a family with recurrent venous thromboembolic disease, designated the Dusard syndrome (Soria et al., 1983). This abnormal fibrinogen has been shown to exhibit defective polymerization (decreased polymer light scattering), despite normal rates of fibrinopeptide release, as well as defective Lys_{78}-plasminogen binding and defective stimulation of t-PA-catalyzed plasminogen activation (Soria et al., 1983; Lijnen et al., 1984). The molecular defect has not been localized.

The rate of Lys_{78}-plasminogen formation varies in the course of plasmin-catalyzed degradation of the fibrin polymer effector. This is the case regardless of whether Lys_{78}-plasminogen is formed as the result of catalysis by a constant concentration of preformed plasmin (Figures 1, 2, and 4, Table I), or by plasmin gradually formed by t-PA- or u-PA-catalyzed activation of plasminogen (Figures 6A and 8A). The effect is therefore not dependent on the plasminogen activator moiety but is probably exerted on the Glu_1-plasminogen and possibly also the plasmin reactant. The rate of Lys_{78}-plasminogen formation, catalyzed by preformed plasmin, is higher in experiments initiated in the presence of fragment X polymer than when initiated in the presence of intact fibrin (Figure 1, Table 1). Furthermore, conversion of fibrin to X polymer during activation of Glu_1-plasminogen by t-PA leads to a higher rate of Lys_{78}-plasminogen formation as reflected by an increase in k_{app} values (Figure 6A). Although not statistically significant, a similar tendency is noted in experiments with u-PA (Figure 8A). The increase in k_{app}, seen upon conversion of fibrin to fragment X polymer, is most likely underestimated in the experiments presented in Figures 1, 6, and 8, as the methodology employed does not allow measurements of initial rates of Lys_{78}-plasminogen formation at a stage where fibrin is essentially intact. Upon further fibrinolysis k_{app} values decrease markedly. This decrease is related to the formation of fragments Y and D and is found despite constant concentrations of fragment X in the reaction mixtures (see Figures 6 and 8, t = 10–20 min). In contrast to the results obtained with t-PA (and possibly with u-PA), no early increase in rate of Lys_{78}-plasminogen formation was associated with the conversion of fibrin to fragment X polymer by preformed Lys_{78}-plasmin (compare Figure 6A and 8A with Figure 2A). This may be explained by differences in the kinetics of fibrin degradation as reflected by the earlier appearance of fragments Y and D during fibrinolysis mediated by preformed plasmin than by activator (compare Figure 2B with Figures 6B and 8B). The combined results indicate that X polymer produces maximal stimulation of Lys_{78}-plasminogen formation and that further degradation to Y and D fragments compromise its effector function. Glu_1-plasminogen has been shown to have affinity to fragment D (Nieuwenhuizen et al., 1983; Vâradi & Patthy, 1983; Christensen, 1984). Thus, domains in fragment D may compete with similar domains in fibrin and fragment X polymer without furnishing the additional effector interactions of importance to Glu_1- to Lys_{78}-plasminogen transformation. Additional explanations may be fragment Y and D interference with polymer structure and fragment X polymer heterogeneity, specific early fragment X polymer moieties perhaps stimulating plasmin cleavage of Glu_1-plasminogen to a greater extent than those that appear later.

Lucas et al. (1983b) demonstrated a stimulatory effect of fibrinogen and fibrinogen-derived fragment E on Glu_1- to

Lys_{78}-plasminogen conversion, as compared to a simple buffer system, or fibrinogen-derived fragment D. We find the stimulatory effect of equal molar concentrations of fibrinogen and fibrinogen-derived fragment E to be between 6- and 11-fold lower than that found with fragment X polymer (Table I).

Among the best studied effectors of Glu_1-plasminogen transformation to Glu_1-plasmin or Lys_{78}-plasminogen are lysine and its analogues (Castellino, 1981). The binding of these ligands to a class of weak affinity sites in Glu_1-plasminogen results in conformational changes in the molecule and in stimulation of both Glu_1-plasminogen activation and Glu_1- to Lys_{78}-plasminogen transformation (Marcus et al., 1978, 1979). Although both conversion reactions are stimulated by L-lysine, they are not stimulated in parallel with regard to ligand concentrations (Violand et al., 1978).

In analogy, fibrin and particularly fragment X polymer form complexes with Glu_1-plasminogen. Although Glu_1-plasminogen binding to intact fibrin seems to involve only high-affinity lysine binding sites (as well as non-lysine-dependent sites) (Suenson et al., 1984; Christensen, 1984; Bok & Mangel, 1985; Suenson & Petersen, 1987), Glu_1-plasminogen binding to initially plasmin degraded fibrin (fragment X polymer) also involves weak lysine binding sites (Suenson et al., 1984). Complex formation involving these sites might, as is the case with ω-amino carboxylic acids, result in a conformational change in the Glu_1-plasminogen moiety, thus increasing the rates of both Glu_1-plasmin and Lys_{78}-plasminogen formation. The increase and subsequent decrease in rate of Lys_{78}-plasminogen formation in the course of fibrinolysis, as reflected by k_{app} values, are associated with similar, but not parallel, changes in the rate of Glu_1-plasmin formation. Previous work has indicated that both t-PA- (Suenson & Petersen, 1986a) and u-PA- (Thorsen et al., 1984; Suenson et al., 1984), catalyzed activation of Glu_1-plasminogen is stimulated over a wider range of fibrin degradation than plasmin-catalyzed Lys_{78}-plasminogen formation. In the present study, plasminogen activation catalyzed by u-PA is seen to occur at approximately equal rates in the presence of fibrin polymer and plasmin-solubilized fibrin degradation products (FDP_{CL}) (Figure 7B), whereas the rate of Lys_{78}-plasminogen formation is markedly lower in the soluble system (Figure 7A). The difference in tolerated range of effector degradation exhibited by the two reactions may reflect differences in their dependency on fragment X polymer concentration, as is the case with the lysine effector (Violand et al., 1978).

In the presence of optimal ω-amino carboxylic acid concentrations (e.g., 1 mM tranexamic acid) the second-order rate constant of plasmin conversion of Glu_1-plasminogen to Lys_{78}-plasminogen at 37 °C is 4.4×10^3 $M^{-1} \cdot s^{-1}$ (Petersen & Suenson, 1986). This value is similar to the k_{app} of Lys_{78}-plasminogen formation during fibrinogenolysis and approximately 8-fold lower than the k_{app} observed in the presence of fragment X polymer (Table I, Figures 6A and 8A).

In blood or plasma, fragment X polymer formation can be inhibited by factor XIIIa catalyzed intermolecular fibrin cross-linking and cross-linking of the α_2-plasmin inhibitor to fibrin (Gaffney & Whitacker, 1979; Sakata et al., 1984). These mechanisms, as well as hampered diffusion into clots or thrombi, may account for the following observations: (1) plasminogen activator added to preformed plasma clots leads to far less efficient plasminogen activation and plasmin cleavage of fibrin than when activator is present during fibrin formation (Brommer, 1984), and (2) hardly any Lys_{78}-plasminogen is formed in the presence of preformed plasma clots in vitro, or preformed thrombi in vivo, upon addition of activators (Holvoets et al., 1985). The above mechanisms may therefore also account for the fact that t-PA concentrations 3 to 4 orders of magnitude higher than those found under physiological conditions are required to achieve effective thrombolytic therapy.

Plasmin-catalyzed cleavage of fibrin-forming fragment X polymer leads to removal of the C-terminal part of the α chains (approximately 400 residues), which most probably contain stimulatory domains for the activation of factor XIII by thrombin (Credo et al., 1981; Carmassi & Chung, 1982), and to which cross-linking of α_2-plasmin inhibitor and interchain α–α chain cross-linking occur (Kimura & Aoki, 1986; Doolittle, 1984). Activation of factor XIII and the inhibitory effects of factor XIIIa on fibrin resolution would thus be hampered upon X polymer formation.

The combined results of our work and a number of the above-sited studies indicate that the role of the plasmin system under physiological conditions may be that of dissolving small early fibrin polymers, in competition with ongoing thrombin action (Nossel, 1982), rather than that of a follow-up process, initiated subsequent to full-scale fibrin formation and aimed at lysing preformed cross-linked blood clots and thrombi.

From the present work it is concluded that the potent stimulatory mechanism of fibrinolysis which efficient conversion of Glu_1- to Lys_{78}-plasminogen represents requires the presence of des-A- or des-AB fragment X polymer. The stimulatory effect of X polymer on Lys_{78}-plasminogen formation is comparable to but not identical with that previously described for direct activation of Glu_1-plasminogen to Glu_1-plasmin, the stimulatory effect on Lys_{78}-plasminogen formation apparently requiring higher concentrations of the X polymer effector and demonstating greater sensitivity to further plasmin-catalyzed degradation of the effector to fragments Y and D.

ACKNOWLEDGMENTS

Dr. Lars Christian Petersen in thanked for fruitful discussions and comments regarding the manuscript. The excellent technical assistance of Birgitte Korsholm and Onita Lützen is gratefully acknowledged.

Registry No. PA, 105913-11-9; plasminogen, 9001-91-6.

REFERENCES

Aoki, N., & Harpel, P. C. (1984) *Semin. Thromb. Hemostasis 10*, 24–41.

Aoki, N., Sakata, Y., Matsuda, M., & Tateno, K. (1980) *Blood 55*, 483–488.

Blombäck, B., Hessel, B., Hogg, D., & Therkildsen, L. (1978) *Nature (London) 275*, 501–505.

Bok, R. A., & Mangel, W. F. (1985) *Biochemistry 24*, 3279–3286.

Brockway, W. J., & Castellino, F. J. (1971) *J. Biol. Chem. 246*, 4641–4647.

Brommer, E. J. P. (1984) *Thromb. Res. 34*, 109–115.

Brownlee, M., Vlassara, H., & Cerami, A. (1983) *Diabetes 32*, 680–684.

Carmassi, F., & Chung, S. I. (1982) *Haemostasis 11 (Suppl. 1)*, 2 (Abstract 4).

Castellino, F. J. (1981) *Chem. Rev. 81*, 431–446.

Christensen, U. (1978) *Biochim. Biophys. Acta 526*, 194–201.

Christensen, U. (1982) *Thromb. Haemostasis 48*, 24–26.

Christensen, U. (1984) *Biochem. J. 223*, 413–421.

Claeys, H., & Vermylen, J. (1974) *Biochim. Biophys. Acta 342*, 351–359.

Claeys, H., Molla, A., & Verstraete, M. (1973) *Thromb. Res. 3*, 515–523.

Credo, R. B., Curtis, C. G., & Lorand, L. (1981) *Biochemistry 20*, 3770–3778.
Doolittle, R. F. (1984) *Annu. Rev. Biochem. 53*, 195–229.
Forsgren, M., Råden, B., Israelson, M., Larsson, K., & Hedén, L.-O. (1987) *FEBS Lett. 213*, 254–260.
Gaffney, P., & Whitacker, A. N. (1979) *Thromb. Res. 14*, 85–94.
Granelli-Piperno, A., & Reich, E. (1978) *J. Exp. Med. 148*, 223–234.
Hantgan, R., McDonagh, J., & Hermans, J. (1983) *Ann. N.Y. Acad. Sci. 408*, 344–366.
Harpel, P. C., Chang, T.-S., & Verderber, E. (1985) *J. Biol. Chem. 260*, 4432–4440.
Higgins, D. L., & Vehar, G. A. (1987) *Biochemistry 26*, 7786–7791.
Higgins, D. L., Lewis, S. D., & Shafer, J. A. (1983) *J. Biol. Chem. 258*, 9276–9282.
Holmberg, L., Lecander, I., Persson, B., & Astedt, B. (1978) *Biochim. Biophys. Acta 544*, 128–137.
Holvoets, P., Lijnen, H. R., & Collen, D. (1985) *J. Biol. Chem. 260*, 12106–12111.
Hoylaerts, M., Rijken, D. C., Lijnen, H. R., & Collen, D. (1982) *J. Biol. Chem. 257*, 2912–2919.
Hurlet-Jensen, A., Cummins, H. Z., Nossel, H. L., & Liu, C. Y. (1982) *Thromb. Res. 27*, 419–427.
Kimura, S., & Aoki, N. (1986) *J. Biol. Chem. 261*, 15591–15598.
Laudano, A. P., Cottrell, B. A., & Doolittle, R. F. (1983) *Ann. N.Y. Acad. Sci. 408*, 315–329.
Lijnen, H. R., & Collen, D. (1982) *Semin. Thromb. Hemostasis 8*, 2–10.
Lijnen, H. R., Hoylaerts, M., & Collen, D. (1980) *J. Biol. Chem. 255*, 10214–10222.
Lijnen, H. R., Soria, J., Soria, C., Collen, D., & Caen, J. P. (1984) *Thromb. Haemostasis 51*, 108–109.
Lucas, M. A., Fretto, L. J., & McKee, P. A. (1983a) *J. Biol. Chem. 258*, 4249–4256.
Lucas, M. A., Straight, D. L., Fretto, L. J., & McKee P. A. (1983b) *J. Biol. Chem. 258*, 12171–12177.
Marcus, G., De Pasquale, J. L., & Wissler, F. C. (1978) *J. Biol. Chem. 253*, 727–732.
Marcus, G., Priore, R. L., & Wissler, F. C. (1979) *J. Biol. Chem. 254*, 1211–1216.
Mihalyi, E. (1983) *Ann. N.Y. Acad. Sci. 408*, 60–70.
Moroi, M., & Aoki, N. (1977) *Thromb. Res. 10*, 851–856.
Müllertz, S. (1987) *Fibrinolysis 1*, 3–12.
Müllertz, S., Thorsen, S., & Sottrup-Jensen, L. (1984) *Biochem. J. 223*, 169–177.
Nieuwenhuizen, W., Verheijen, J. H., Vermond, A., & Chang, G. T. G. (1983) *Biochim. Biophys. Acta 755*, 531–533.
Norrman, B., Wallén, P., & Rånby, M. (1985) *Eur. J. Biochem. 149*, 193–200.
Nossel, H. L. (1981) *Nature (London) 291*, 165–167.
Petersen, L. C., & Suenson, E. (1986) *Biochim. Biophys. Acta 883*, 313–325.
Petersen, L. C., Handest, P., Brender, J., Selmer, J., Jørgensen, M., & Thorsen, S. (1987) *Thromb. Haemostasis 57*, 205–211.
Radcliff, R. (1983) *Biochim. Biophys. Acta 743*, 422–430.
Rákóczi, I., Wiman, B., & Collen, D. (1978) *Biochim. Biophys. Acta 540*, 295–300.
Rånby, M. (1982) *Biochim. Biophys. Acta 704*, 461–469.
Rickli, E. E., & Otavsky, W. I. (1973) *Biochim. Biophys. Acta 295*, 381–384.
Rijken, D. C., & Collen, D. (1981) *J. Biol. Chem. 256*, 7035–7041.
Rijken, D. C., Hoylaerts, M., & Collen, D. (1982) *J. Biol. Chem. 257*, 2920–2925.
Robbins, K. C., Barnabe, P., Arzadon, L., & Summaria, L. (1972) *J. Biol. Chem. 247*, 6757–6762.
Root-Bernstein, R. S. (1982) *J. Theor. Biol. 94*, 885–894.
Rupp, C., Sievi, R., & Furlan, M. (1982) *Thromb. Res. 27*, 117–121.
Sakata, Y., & Aoki, N. (1982) *J. Clin. Invest. 69*, 536–542.
Sakata, Y. Mimuro, J., & Aoki, N. (1984) *Blood 63*, 1394–1401.
Sodetz, J. M., & Castellino, F. J. (1975) *J. Biol. Chem. 250*, 3041–3049.
Soria, J., Soria, C., & Caen, J. P. (1983) *Br. J. Haematol. 53*, 575–586.
Suenson, E., & Thorsen, S. (1981) *Biochem. J. 197*, 619–628.
Suenson, E., & Petersen, L. C. (1986a) *Biochim. Biophys. Acta 870*, 510–519.
Suenson, E., & Petersen, L. C. (1986b) *Fibrinolysis 1*, Abstract 11.
Suenson, E., Thorsen, S., & Kok, P. (1982) *Haemostasis 11 (Suppl. 1)*, 2 (Abstract 2).
Suenson, E., Lützen, O., & Thorsen, S. (1984) *Eur. J. Biochem. 140*, 513–522.
Suenson, E., Lind, B., & Petersen, L. C. (1987) in *Fibrinogen 2, Biochemistry, Physiology and Clinical Relevance: Proceedings of the Fibrinogen Workshop* (Lowe, G. D. O., Douglas, J. T., Forbes, C. D., & Henschen, A., Eds.) Glasgow, Scotland, April 6–8, 1987, pp 297–302, Excerpta Medica, Amsterdam.
Summaria, L., Arzadon, L., Barnabe, P., & Robbins, K. C. (1975) *J. Biol. Chem. 250*, 3988–3995.
Takada, A., Watahiki, Y., & Takada, Y. (1986) *Thromb. Res. 41*, 819–827.
Thorsen, S. (1975) *Biochim. Biophys. Acta 393*, 55–65.
Thorsen, S., & Müllertz, S. (1974) *Scand. J. Clin. Lab. Invest. 34*, 167–176.
Thorsen, S., & Suenson, E. (1985) *Thromb. Haemostasis 54*, 266 (Abstract 1575).
Thorsen, S., Clemmensen, I., Sottrup-Jensen, L., & Magnusson, S. (1981) *Biochim. Biophys. Acta 668*, 377–387.
Thorsen, S., Müllertz, S., Suenson, E., & Kok, P. (1984) *Biochem. J. 223*, 179–187.
Tran-Thang, C., Kruithof, E. K. O., & Bachmann, F. (1984) *J. Clin. Invest. 74*, 2009–2016.
van Zonneveld, A. J., Veerman, H., & Pannekoek, H. (1986) *J. Biol. Chem. 261*, 14214–14218.
Vâradi, A., & Patthy, L. (1983) *Biochemistry 22*, 2440–2446.
Violand, B. N., & Castellino, F. J. (1976) *J. Biol. Chem. 251*, 3906–3912.
Violand, B. N., Byrne, R., & Castellino, F. J. (1978) *J. Biol. Chem. 253*, 5395–5401.
Wallén, P., & Wiman, B. (1975) in *Proteases and Biological Control* (Reich, E., Rifkin, D. B., & Shaw, E., Eds.) pp 291–303, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Walther, P. J., Steinman, H. M., Hill, R. L., & McKee, P. A. (1974) *J. Biol. Chem. 249*, 1173–1181.
Wiman, B., & Wallén, P. (1973) *Eur. J. Biochem. 36*, 25–31.
Wiman, B., Lijnen, H. R., & Collen, D. (1979) *Biochim. Biophys. Acta 579*, 142–154.
Wohl, R. C., Summaria, L., & Robbins, K. C. (1980) *J. Biol. Chem. 255*, 2005–2013.