β(Leu$_{121}$-Lys$_{122}$) Segment of Fibrinogen Is in a Region Essential for Plasminogen Binding by Fibrin Fragment E†

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ABSTRACT: It was shown previously that two sequentially nonidentical regions of human fibrin(ogen), present in fragments D and E, carry specific plasminogen-binding sites [Váradi, A., & Patthy, L. (1983) *Biochemistry 22*, 2440–2446]. Comparison of the affinity of a variety of fragment E species for immobilizd Lys-plasminogen revealed that fragment E_{3e} [($\alpha_{20/24-78}$, β_{54-122}, γ_{1-53})$_2$] possesses a strong plasminogen-binding site, whereas fragment E_{3t} [($\alpha_{20/24-78}$, β_{54-120}, γ_{1-53})$_2$] has 30-fold lower affinity for the affinant. Since the two fragments differ only in the β(Leu$_{121}$-Lys$_{122}$) segment, this suggests that residues β(Leu$_{121}$-Lys$_{122}$), present in the triple-helical connector region of fibrin(ogen), are essential for plasminogen binding by fragment E. Reduction and alkylation of fragment E_{3e} lead to the destruction of the plasminogen-binding site, indicating that none of the separated, alkylated polypeptide chains of the fragment are able to bind to plasminogen and probably the coiled-coil superstructure of the connector region is necessary for the maintenance of the plasminogen-binding site of fragment E.

Fibrin deposits formed intravascularly are continuously removed by the fibrinolytic enzyme system. The enzyme directly responsible for the proteolytic dissolution of insoluble fibrin polymer is plasmin, a serine protease with broad, trypsin-like sequence specificity. Despite this broad specificity, intravascularly the proteolytic action of plasmin is practically limited to fibrin polymer, since the formation of plasmin from its inactive proenzyme, plasminogen, occurs predominantly on fibrin. The molecular basis of this spatial restriction of plasminogen activation is that both plasminogen and plasminogen activator are bound to fibrin polymer, and fibrin greatly accelerates plasminogen activation by properly aligning the activator with plasminogen (Wiman & Collen, 1978; Hoylaerts et al., 1982). The specific binding of plasminogen and plasminogen activator to fibrin is thus of overwhelming importance for the regulation of the fibrinolytic process.

In our previous studies we have shown that plasminogen-binding sites of human fibrin(ogen) are located in two sequentially nonidentical regions of the molecule, present in the large proteolytic fragments E and D (Váradi & Patthy, 1983). The aim of the present investigation was the localization of the plasminogen-binding site within fragment E. A variety of fragment E species were prepared from cross-linked fibrin. Plasminolysis of human cross-linked fibrin yields fragments DD and E and α-chain polymer remnants as main high molecular weight terminal degradation products (Pizzo et al., 1973a,b). Three subspecies of fragment E are found in plasmic digests of cross-linked fibrin, E_1, E_2, and E_3. In early stages of digestion fragment E_1 and E_2 are formed, and these variants show affinity for fragment DD; thus, they are in DD·E complex (Olexa & Budzynski, 1979a,b). In terminal digests only fragment E_3 is present, which lost peptide regions essential for interaction with fragment DD (Olexa & Budzynski, 1979a,b). The covalent structures of fragments E_1, E_2, and E_3 have been determined (Olexa et al., 1981). The affinity of these chemically defined E species for Lys-plasminogen–Sepharose was compared in order to establish the structural requirement of plasminogen binding.

Experimental Procedures

Materials. Cyanogen bromide (Pierce), acetonitrile, HPLC[1] grade S (Rathburn), bovine pancreatic trypsin inhibitor, Trasylol (Bayer), bovine thrombin, Topostasin (Roche), streptokinase, Kabikinase (Kabi), carboxypeptidase A (Miles), and carboxypeptidase B (Worthington) were commercial preparations. Human Lys-plasminogen and Lys-plasminogen–Sepharose 4B were prepared as described previously (Váradi & Patthy, 1983). Human fibrinogen was prepared from plasminogen-depleted plasma by the plasma-fractionation procedure of Chen & Mosesson (1977).

Preparation of Fragment E Species of Cross-Linked Fibrin. Fibrinogen (98% clottable) was dissolved in 0.05 M Tris-HCl–0.15 M NaCl–0.01 M $CaCl_2$, pH 7.4, buffer at a final concentration of 10 mg/mL and was preincubated at 37 °C. Human plasminogen (0.025 mg/mL) and streptokinase (7.5 IU/mL) were added to this solution immediately before the addition of thrombin (8 NIH units/mL). Our fibrinogen preparation contained sufficient amount of factor XIII to form cross-linked fibrin when treated with thrombin. The reaction mixture was incubated at 37 °C, and the progress of digestion was analyzed by $NaDodSO_4$ gel electrophoresis as shown in Figure 1. Plasminolysis was arrested by the addition of Trasylol, 100 KIU/mL, ϵ-ACA,[1] 0.02 M, and EDTA, 0.02 M (all final concentrations). Fragments E_2 and E_{3e} were isolated from the 60-min digest. The digest was applied onto a Sephacryl S-200 column (3 × 140 cm) equilibrated with 0.3 M NH_4HCO_3 and eluted with the same buffer (Figure 2). Fragment E_{3e} was obtained in pure form from pool, B, whereas fragment E_2 was isolated from pool A (Figure 2) by the method of Olexa & Budzynski (1979a,b). Fragment E_{3t} was prepared from exhaustive plasmic digest of cross-linked fibrin by using a plasminogen concentration of 0.25 mg/mL and a streptokinase concentration of 75 IU/mL, and the digestion was arrested after 300 min (Olexa & Budzynski, 1979a).

Reduction and Alkylation of Fragment E_{3e}. Fragment E_{3e} (5 mg/mL) was dissolved in 0.2 M Tris-HCl–6 M guanidine hydrochloride, pH 7.4, buffer previously flushed with N_2. Dithioerythritol was added at a final concentration of 10 mM,

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[1] Abbreviations: Fragment E_{3e} [($\alpha_{20/24-78}$, β_{54-122}, γ_{1-53})$_2$] and fragment E_{3t} [($\alpha_{20/24-78}$, β_{54-120}, γ_{1-53})$_2$] are fragments isolated from early (E_{3e}) and terminal (E_{3t}) plasmic digests of cross-linked fibrin; the two E_3 fragments differ only in the β(Leu$_{121}$-Lys$_{122}$) segment. $NaDodSO_4$, sodium dodecyl sulfate; ϵ-ACA, ϵ-aminocaproic acid; EDTA, ethylenediaminetetraacetic acid; KIU, Kallikrein inhibitor unit; Lys-Pg, proteolytically modified plasminogen, Lys$_{77}$–Asn$_{790}$; CNBr, cyanogen bromide; HPLC, high-pressure liquid chromatography; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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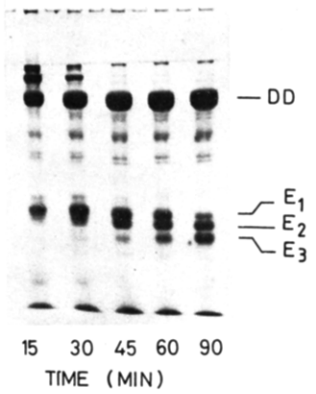

FIGURE 1: Digestion of human cross-linked fibrin with plasmin. Cross-linked fibrin was digested with plasmin as described under Experimental Procedures, and at intervals samples were withdrawn for gel electrophoresis on 6–16% linear polyacrylamide gradient–$NaDodSO_4$ slab gels. DD, E_1, E_2, and E_3 refer to fragments DD, E_1, E_2 and E_3, respectively. For preparative purposes digests of 60 min were used.

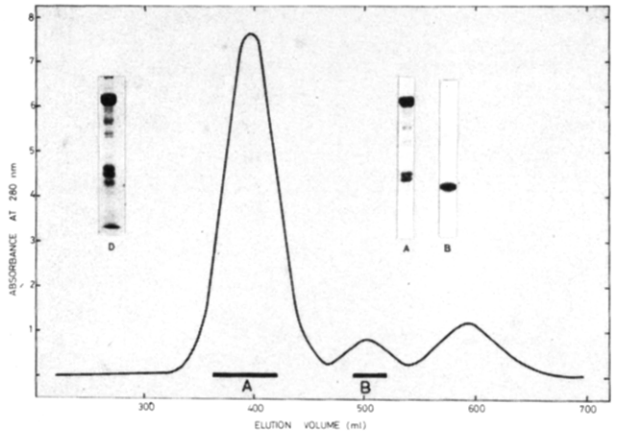

FIGURE 2: Isolation of fragment E_{3e} from the early plasmic digest of human cross-linked fibrin. Gel filtration of an early plasmic digest of cross-linked fibrin (60-min digest of Figure 1) was performed on a Sephacryl S-200 column (3 × 140 cm) equilibrated with 0.3 M NH_4HCO_3 buffer, and the fractions were pooled as shown by the solid bars. The $NaDodSO_4$ gel electrophoretic patterns of the digest (D), pool A (A) (DD·E complex), and pool B (B) (fragment E_{3e}) are also shown.

and the reaction mixture was incubated for 30 min at 25 °C. Alkylation was carried out with iodoacetic acid (30 mM) for 15 min at 25 °C in the dark, and then the reagents and salts were removed by gel filtration on a Sephadex G-25 column (3 × 45 cm) equilibrated with 0.1 M NH_4HCO_3.

Cyanogen Bromide Cleavage of Fragments E_{3e}. Cyanogen bromide cleavage was carried out by the method of Gross & Witkop (1961).

Digestion with Carboxypeptidase A and with Carboxypeptidase A plus B. Fragment E species were dissolved in 0.2 M NH_4HCO_3, 4 mg/mL, and were digested with an equal volume of carboxypeptidase A (0.1 mg/mL) for 90 min at 25 °C or with an equal volume of the mixture of carboxypeptidase A and carboxypeptidase B (0.1 and 0.5 mg/mL, respectively) under the same conditions. The digestion was arrested by the addition of an equal volume of 30% acetic acid, and the amino acids liberated were determined by amino acid analysis. Control samples not containing fragment E were also analyzed to determine the amino acids present in the digests due to the self-digestion of the proteases.

Amino-Terminal Amino Acid Analysis. Amino-terminal amino acid residues were determined by the dansylation method of Gray & Hartley (1963). The thin-layer chromatographic technique of Woods & Wang (1967) was used to identify the dansylated amino acids.

Affinity Chromatography and Competitive Affinity Chromatography. Affinity chromatographic experiments were carried out as described earlier (Váradi & Patthy, 1983). The relative plasminogen affinity of fragment E species was determined by competitive affinity chromatography on Lys-Pg–Sepharose 4B. Competitive affinity chromatography is based on the principle that if different proteins compete for the same sites of the affinant and the availability of these binding sites limits binding (i.e., the column is overloaded), then the ratio of the proteins in the bound fraction will be shifted in favor of the component with higher affinity according to the equation

$$[P_iL]/[P_1L] = K_i[P_i]/(K_1[P_1]) \quad (1)$$

where $[P_1]...[P_i]$ are the free concentrations of different proteins in equilibrium with the affinant, $K_1...K_i$ are the association constants of protein–affinant interactions, and $[P_1L]...[P_iL]$ are the concentrations of the protein–affinant complexes (Váradi & Patthy, 1983).

The sample containing an equimolar mixture of fragment E species to be compared (4-mL sample; protein concentration was 9 μM for both proteins) was continuously loaded onto the Lys-Pg–Sepharose column (0.4 mL; binding capacity of 3.6 nmol for fragment E species); i.e., the amount of each protein applied corresponded to 10-fold excess over the capacity of the column. After passage of the whole sample through the column, the column was washed with 5 bed volumes of buffer, and the bound proteins were eluted with buffer containing 10 mM ε-ACA. The composition of the sample, the fractions of the effluent, and the bound fraction were analyzed by high-pressure liquid chromatographic quantitation of C-terminal peptids of E β-chains removed by CNBr cleavage.

High-Pressure Liquid Chromatographic Determination of Small CNBr Peptides of E Fragments. Short peptides split off from the C-terminal part of the β-chains of fragment E species were separated, quantitated, and isolated for amino acid analysis by HPLC. After CNBr treatment, samples were diluted with 10 volumes of distilled water, lyophilized, and dissolved in 80–250 μL of 0.1% trifluoroacetic acid–acetonitrile, 65:35 (v/v). The chromatograph used was built up of an Altex pump (Model 110), a Rheodyne sample injector valve with a 20- or 50-μL sample loop (Model 70-10), and a variable wavelength detector, Spectrometer II (Laboratory Data Control). A reverse-phase LiChrosorb RP 18, 10 μm, 250 × 4.6 mm column (Merck) and a solvent of 0.1% trifluoroacetic acid–acetonitrile, 65:35 (v/v), were used. The effluent was monitored at 210 nm.

Gel Electrophoresis. Composition of the plasmic digests and the fractions of gel filtration were analyzed by 6–16% linear polyacrylamide gradient–$NaDodSO_4$ slab gel electrophoresis. The gels were stained with Coomassie brilliant blue G-250.

Amino Acid Analysis. Peptides and proteins were hydrolyzed in 6 N HCl in the presence of mercaptoacetic acid (Matsubara & Sasaki, 1969) at 110 °C for 24 h, and the composition of the acid hydrolysates was determined by amino acid analysis on a Biotronik LC 2000 analyzer.

Results

Comparison of Plasminogen Affinity of Fragments E_2 and E_{3f}. The plasminogen affinity of fragment E species isolated from plasmic digests of human cross-linked fibrin was compared to identify peptide regions essential for plasminogen

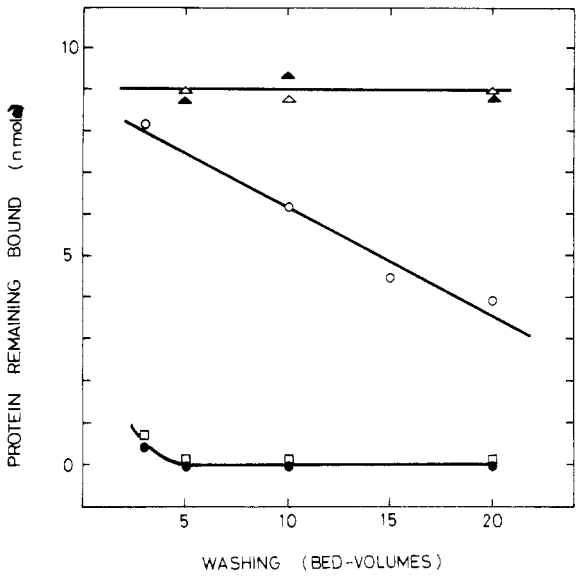

FIGURE 3: Influence of washing on the amount of fragment E variants and derivatives remaining bound to Lys-plasminogen–Sepharose. The Lys-plasminogen–Sepharose 4B column (1 mL) was overloaded with the protein in question, and after the column was washed with different bed volumes of buffer, the protein that remained bound was eluted with buffer containing 10 mM ϵ-ACA and quantiated. Fragments studied were fragment E_2 (△), fragment E_{3e} (▲), fragment E_{3t} (○), reduced–alkylated fragment E_{3e} (□), and cyanogen bromide treated fragment E_{3e} (●).

binding. Fragments E_2 and E_{3t} were found to bind to Lys-Pg–Sepharose and could be eluted with a low concentration (10 mM) of ϵ-ACA. As shown in Figure 3 the amount of fragment E_2 bound to the column is practically independent of the extent of washing whereas the amount of E_{3t} bound decreases upon prolonged washing, suggesting a weaker interaction of E_{3t} with the affinant. The binding capacity of the affinity column for these two species is practically the same when extrapolated to zero washing.

Fragments E_2 and E_{3t} are known to differ in that α_{17-20}, β_{121}, and γ_{54-63} are present in E_2 but absent from E_{3t} (Olexa et al., 1981); therefore, any of the above peptide segments can be responsible for the higher plasminogen affinity of fragment E_2. To decide which of these peptide segments participates in the construction of the plasminogen-binding site, a new E variant that has a structure intermediate between fragments E_2 and E_{3t} was prepared.

Preparation and Characterization of Fragment E_{3e}. We prepared an E_3 fragment from the early plasmic digest of human cross-linked fibrin. In the early stages of digestion fragments E_1 and E_2 predominate, but a small portion of the fragment already appears in E_3 form (Figure 1, 15–60 min). Whereas E_1 and E_2 are present in complex with fragment DD (Figure 2, pool A), the E_3 variant is eluted as a separate peak when the digest is gel filtered on a Sephacryl S-200 column (Figure 2, pool B), indicating that the regions responsible for the affinity of E fragments for fragment DD have already been lost from this E variant. In this respect the E_3 of the early digest is similar to fragment E_3 prepared from an exhaustive plasmic digest of cross-linked fibrin by the method of Olexa & Budzynski (1979a,b).

The amino acid analysis of the E_3 fragments isolated from early and terminal digests also failed to reveal any significant differences between the amino acid compositions of the two E_3 species (Table I). Similarly, N-terminal analysis of both E_3 variants identified lysine, tyrosine, valine, and histidine as amino termini, in harmony with the data obtained by Olexa et al. (1981) for E_3 isolated from the terminal digest. These studies thus revealed no difference between E_3 species prepared from early and terminal digests.

Table I: Amino Acid Composition of Fragment E_3 Species Isolated from Early (E_{3e}) and Terminal (E_{3t}) Plasmic Digests of Human Cross-Linked Fibrin[a]

amino acid	expected[b]	fragment E_{3e}	fragment E_{3t}
Asp	27	28.0	27.7
Thr	8	8.0	9.6
Ser	14	14.8	14.4
Glu	24	23.2	22.9
Pro	8	9.4	8.8
Gly	9	11.1	10.9
Ala	8	8.4	7.8
Cys	9	ND[c]	ND
Val	11	7.4	8.5
Met	2	1.9	1.5
Ile	6	4.4	4.6
Leu	16	14.6	14.4
Tyr	7	7.0	7.3
Phe	6	4.9	6.2
His	3	2.7	3.4
Lys	11	9.8	11.2
Arg	9	6.5	7.7
Trp	2	ND	ND

[a] The numbers represent moles of amino acid per mole of half-fragment. [b] Expected for α_{20-78}, β_{54-120}, and γ_{1-53} (Olexa et al., 1981). [c] ND, not determined.

Table II: Retention Times and Amino Acid Compositions of Peptides Released by Cyanogen Bromide Cleavage of Fragments E_2, E_{3e}, and E_{3t}[a]

amino acid[b]	fragment E_2	fragment E_{3e}	fragment E_{3t}
tyrosine	1.1	0.9	0.9
leucine	2.4	2.1	0.9
lysine	1.1	1.0	<0.05
retention time (min)	6.4	6.4	4.9

[a] The peptides were released by CNBr cleavage and were isolated by high-pressure liquid chromatography as described under Experimental Procedures. [b] The numbers represent moles of amino acid per mole of peptide.

The carboxy-terminal boundaries of fragment E_2 and fragment E_3 species were determined by carboxypeptidase digestions and by cyanogen bromide cleavage. After CNBr treatment, the small peptides released from the β-chains were isolated by high-pressure liquid chromatography, and the amino acid compositions of the isolated peptides were determined as shown in Table II. One can conclude from the data of Table II and from the known sequence of fibrinogen (Gårdlund, 1977a,b; Henschen & Lottspeich, 1977; Watt et al., 1978; Henschen et al., 1979; Doolittle et al., 1979) that fragment E_3 of the terminal digest has β-chains with a carboxy-terminal sequence of Met-Tyr-Leu_{120} in agreement with the results of Olexa et al. (1981), while the β-chains of fragment E_3 of the early digest and E_2 possess a carboxy-terminal sequence of Met-Tyr-Leu-Leu-Lys_{122}. In view of this structural difference between the E_3 species of early and terminal digests, the two species are referred to as E_{3e} and E_{3t}, respectively.

Whereas the simultaneous application of carboxypeptidase A and B released lysine, methionine, phenylalanine, tyrosine, and leucine from fragment E_{3e}, carboxypeptidase A alone was unable to remove any amino acids (Table III). These data indicate that removal of carboxy-terminal lysines was a prerequisite of the liberation of the neutral amino acids.

Table III: Amino Acids Released by Carboxypeptidase A and by Carboxypeptidase A plus B Digestion of Fragments E_{3e}, E_{3t}, and E_2

	carboxypeptidase A			carboxypeptidase A plus B		
	E_{3e}	E_{3t}	E_2	E_{3e}	E_{3t}	E_2
Met	<0.05[a]	2.2	<0.05	2.4	1.7	1.9
Ile	<0.05	<0.05	<0.05	<0.05	<0.05	0.8
Leu	<0.05	2.1	<0.05	4.5	2.0	5.1
Tyr	<0.05	3.8	<0.05	5.2	4.6	4.6
Phe	<0.05	1.9	<0.05	2.2	2.0	2.0
Lys	<0.05	0.05	<0.05	5.8	3.6	6.3

[a] The values indicate moles of amino acid released per mole of fragment.

In the case of fragment E_{3t} carboxypeptidase A released methionine, phenylalanine, tyrosine, and leucine (Table III). These amino acids are released from the carboxy-terminal sequence (Phe-Tyr-Met-Tyr-Leu_{120}) of the β-chains of E_{3t}; its α- and γ-chains are terminated by lysines (Olexa et al., 1981). Digestion of E_{3t} with a mixture of carboxypeptidase A and B yielded the same amino acids as in the case of E_{3e}, except for the difference in the quantity of leucines and lysines (Table III). This difference is explained by the presence of the $Leu\text{-}Lys_{120}$ segment in the β-chains of E_{3e} and its absence from E_{3t}. Since the structural difference between fragments E_{3e} and E_{3t} is restricted to the carboxy-terminal end of their β-chains, the covalent structure of fragment E_{3e} is $(\alpha_{20/24\text{-}78}, \beta_{54\text{-}122}, \gamma_{1\text{-}53})_2$, whereas that of fragment E_{3t} is $(\alpha_{20/24\text{-}78}, \beta_{54\text{-}120}, \gamma_{1\text{-}53})_2$.

Comparison of Plasminogen Affinity of Fragments E_{3e} and E_{3t}. Both fragments E_{3e} and E_{3t} are bound to Lys-Pg–Sepharose and can be specifically eluted with 10 mM ϵ-ACA. As shown in Figure 3 the quantity of E_{3e} bound to the affinant does not depend on the extent of washing whereas the amount of fragment E_{3t} bound decreases upon prolonged washing. This observation suggests that the interaction between fragment E_{3t} and the affinant is weaker than that between E_{3e} and the affinant. The binding capacity of the column for these E_3 species is practically the same when extrapolated to zero washing.

The quantitative difference in the plasminogen affinity of the two E_3 variants was determined by competitive affinity chromatography of an equimolar mixture of fragments E_{3e} and E_{3t} as described under Experimental Procedures. The ratio of the proteins in the sample, in the fractions of the effluent, and in the bound fraction was determined by HPLC quantitation of peptides Tyr-Leu and Tyr-Leu-Leu-Lys derived from E_{3t} and E_{3e}, respectively, after CNBr treatment of the different fractions. The HPLC chromatograms of the peptides released by CNBr cleavage of the sample and of the bound fraction are shown in Figure 4. The molar ratio of E_{3e}:E_{3t} in the bound fraction is 28 ± 4 whereas the free concentrations of the two fragments was equal. This shift indicates that the association constant of the E_{3e}–Lys-Pg–Sepharose interaction is about 30-fold higher than in the case of fragment E_{3t}.

Influence of Reduction and Alkylation of Fragment E_{3e} on Its Plasminogen Affinity. Fragments E, derived from the central nodule of fibrin(ogen), are composed of two sets of three nonidentical chains connected by a network of disulfide bridges (Kowalska-Loth et al., 1973). Reduction and alkylation of fragment E_{3e} achieved under denaturing conditions are accompanied by the loss of plasminogen binding affinity (Figure 3). The N-terminal analysis of the reduced alkylated fragment E_{3e} applied onto the Lys-Pg–Sepharose column and that of the protein material recovered in the nonbinding fraction revealed the same N-terminal amino acids, proving

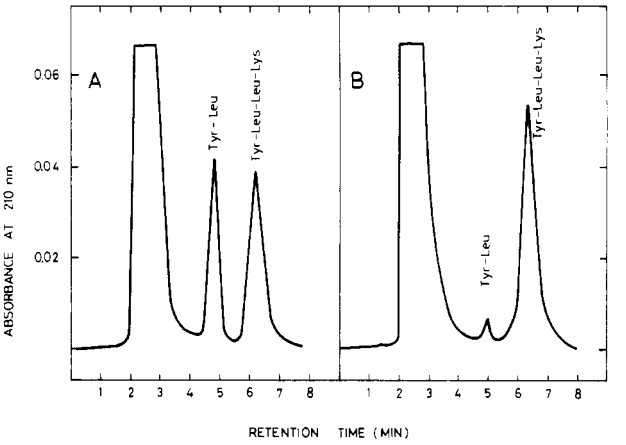

FIGURE 4: Competitive affinity chromatography of fragments E_{3e} and E_{3t} on Lys-plasminogen–Sepharose. An equimolar mixture (9:9 μM) of fragments E_{3e} and E_{3t} (4 mL) was continuously loaded onto the affinity column (0.4 mL). After passage of the sample through the column, the affinant was washed with 5 bed volumes of buffer, and the bound proteins were eluted with buffer containing 10 mM ϵ-ACA. The composition of the sample (A) and the bound proteins (B) was analyzed by high-pressure liquid chromatographic quantitation of the carboxy-terminal peptides of the E β-chains removed by cyanogen bromide cleavage. The dipeptide Tyr-Leu is derived from fragment E_{3t}, and the tetrapeptide Tyr-Leu-Leu-Lys is derived from fragment E_{3e}.

that none of the chains remained bound to the affinant.

Influence of CNBr Treatment of Fragment E_{3e} on Its Plasminogen Affinity. Fragment E species contain two methionine residues per half-molecule, residues αMet_{51} and βMet_{118}. CNBr treatment of fragment E species leads to the removal of peptide segments carboxy terminal to these residues (Olexa et al., 1981). CNBr cleavage of fragment E_{3e} was found to eliminate its plasminogen affinity (Figure 3). Control experiment revealed that cleavage of the peptide bonds was responsible for the loss of affinity, since exposing fragment E_{3e} to the harsh conditions of CNBr cleavage, but omitting CNBr, left the plasminogen affinity of the fragment unharmed.

Discussion

Fragment E_{3t} was found to show much weaker affinity for Lys-plasminogen–Sepharose than E_{3e} (Figure 3); thus, competitive affinity chromatography revealed that the association constant of the interaction between E_{3e} and the affinant is ~30-fold higher than that of E_{3t} (Figure 4). Since the structural difference between these two E species is that E_{3t} lacks one small segment, $\beta(Leu\text{-}Lys_{122})$, it is obvious that the above residues are important for forming a strong plasminogen-binding site of fragment E_{3e}.[2]

The $\beta(Leu\text{-}Lys_{122})$ segment essential for plasminogen binding of fragment E_{3e} is located in the interdomain connector region of fibrin(ogen) where the three nonidentical chains intertwine to form a triple-helical superstructure (Doolittle et al., 1978). The possible importance of the three-dimensional architecture of this coiled-coil region for plasminogen binding is suggested by our result that the disruption of this structure by reduction and alkylation under denaturing conditions was found to destroy the plasminogen-binding site of fragment E_{3e}: none of the constituent chains were detectably bound to the affinant (Figure 3). This could mean either that (i) none of

[2] In our previous work (Váradi & Patthy, 1983) we found that fragment E_3 has strong affinity for immobilized Lys-Pg. The fragment E_3 used in our previous work, however, turned out to be a mixture of E_{3e} and E_{3t} variants. In the light of the present investigations it is clear that only the E_{3e} component of the preparation has high affinity for Lys-Pg–Sepharose.

the chains are able to form such a binding site alone (i.e., structural elements belonging to different polypeptide chains are directly involved in the construction of a plasminogen-binding site of fragment E_{3e}) or that (ii) residues directly participating in plasminogen binding are located in a single polypeptide chain but their proper arrangement is maintained by peptide regions of other chain(s).

Our result that CNBr cleavage at αMet_{51} and βMet_{118} of fragment E_{3e} abolished its plasminogen affinity also points to the involvement of the triple-helical structure in the plasminogen-binding site since the peptide segments removed are part of this region.

D fragments, which also bind to Lys-Pg–Sepharose (Váradi & Patthy, 1983), also carry large regions of the triple-helical connector rod; therefore, one may speculate on the possible structural homology of plasminogen-binding sites of the E and D fragments.

Registry No. Plasminogen, 9001-91-6.

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