Immunochemical Characterization of a Low Affinity Lysine Binding Site within Plasminogen*

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A degradation product corresponding to the fourth kringle has been isolated from porcine elastase digests of human plasminogen, and an antiserum has been utilized to immunochemically characterize this derivative. This antiserum bound radiiodinated kringle 4 and plasminogen, but 0.2 M 6-aminohexanoic acid markedly reduced the binding of these ligands. Furthermore, introduction of three ω-aminocarboxylic acids, trans-4-aminomethylcyclohexanecarboxylic acid, 6-aminohexanoic acid, and lysine into the radioimmunoassay produced a concentration-dependent inhibition of the binding of either kringle 4 or plasminogen by the antiserum. The concentration of these derivatives producing 50% inhibition was reflective of the dissociation constant of the ω-aminocarboxylic acids for the low affinity lysine binding within kringle 4. Modification of kringle 4 by reduction and alkylation, cyanogen bromide cleavage, or chymotrypsin degradation markedly decreased or abolished its capacity to interact with lysine-Sepharose and caused a concomitant decrease in its capacity to be bound by the antiserum. Taken together, these observations suggest that an available and functionally intact lysine binding site is required for kringle 4 and kringle 4-containing derivatives to be bound by the antiserum. Only kringle 4-containing derivatives of plasminogen interacted with the antibody, indicating that the lysine binding sites are not identical despite the structural similarities between the plasminogen kringles. The conversion of plasminogen to plasmin did not alter the exposure of the kringle 4 region, but formation of the plasmin-α2-antiplasmin complex resulted in increased accessibility of this region to antibody and indicates that the conformation of plasmin is perturbed by its interaction with the inhibitor. Fibrinogen and α2-antiplasmin did not inhibit the binding of kringle 4 by its antiserum, suggesting that the affinity of these molecules for the low affinity lysine binding site within kringle 4 is less than 10^5 M^-1. In addition, the lysine binding site of kringle 4 appears to be uncomplexed when plasminogen is within plasma.

Plasminogen is the zymogen form of plasmin, the primary fibrinolytic enzyme of mammalian blood. Effective fibrinolysis requires efficient regulation of plasmin activity, and this is achieved by the level of plasminogen activators and plasmin inhibitors as well as by structural features inherent to the plasminogen molecule itself. The capacity of plasminogen to reversibly bind ω-aminocarboxylic acids such as lysine, 6-aminohexanoic acid, and trans-4-aminomethylcyclohexanecarboxylic acid is mediated by specific "lysine binding sites" (1-7), and these sites appear to play an important role in regulating interaction of plasmin with its primary substrate, fibrin (8, 9), and its primary inhibitor, α2-antiplasmin (10, 11). Markus et al. (5) demonstrated that plasmin contains two classes of binding sites with respect to affinity for 6-aminohexanoic acid: one high affinity site with a Kd of 9 μM and approximately five low affinity sites with a Kd of 5 mM. In addition, in comparing the binding of 6-aminohexanoic acid to native Glu-plasminogen and modified Lys-plasminogen, which arises from limited degradation in the NH2-terminal region of Glu-plasminogen, significant differences were noted (12).

The lysine binding sites of plasminogen reside in the region from which the heavy chain of plasmin is derived (13). The entire plasminogen molecule has now been sequenced (14, 15), and the lysine binding sites are associated with the first four of the five "kringle-like" structures of plasminogen (15). These kringles are envisioned as looped disulfide structures of 80-90 amino acids and are highly homologous in sequence to one another and to the nonthrombin region of prothrombin (15, 16). When plasminogen is subjected to limited digestion with porcine elastase, three major derivatives are obtained. Elastase degradation product I contains kringles 1, 2, and 3 and retains capacity to interact with lysine derivatives. EDP II, a fragment of M. = 10,000-12,000 corresponds to kringle 4 and contains a single low affinity lysine binding site. EDP III consists of kringle 5 and the remaining carboxyl-terminal region of plasminogen. EDP III does not bind to lysine-Sepharose and may be activated to form a low molecular weight plasmin (7, 15). In the present study, we describe an antiserum to EDP II which appears to be entirely specific for the lysine binding site within this fragment. The antiserum has been utilized to characterize the interaction of ω-aminocarboxylic acids with kringle 4 and to compare this lysine binding site as expressed by isolated EDP II or when contained in the plasminogen molecule.

MATERIALS AND METHODS

Plasminogen—Glu-plasminogen was isolated from fresh human plasma or from Cohn's Fraction III by affinity chromatography on lysine-Sepharose, followed by molecular exclusion chromatography on Bio-gel A1.5m or Ultrogel AcA44 (3, 17). Lys-plasminogen was

1 The abbreviations used are: EDP (I, II, III), elastase (porcine) degradation products of plasminogen; AMCA, trans-4-aminomethylcyclohexanecarboxylic acid; SDS, sodium dodecyl sulfate; ABC-33%, antigen binding capacity derived from the antiserum dilution binding 33% of the ligand.
isolated from Fraction III, but the extraction and isolation were performed at 22 °C (12). By NH2-terminal analysis (18), glutamic acid was the only NH2-terminal residue in Glu-plasminogen preparations, and Lys-plasminogen contained predominantly lysine as well as traces of methionine, valine, and serine as NH2 terminal. These NH2 terminals are consistent with the reported properties of Glu- and Lys-plasminogen (19-21).

**Elastase Degradation Products**—Limited digestion of Glu-plasminogen with porcine elastase was performed according to the method of Sotrup-Jensen et al. (15). Briefly, 150 mg of Glu-plasminogen in 10 ml of 0.3 M NH4HCO3, pH 8.3, containing 1 mg of pancreatic trypsin inhibitor was incubated for 3.25 h in 22 °C in 0.45 mg of porcine elastase. Diisopropylfluorophosphate was added to a final concentration of 0.15 mM, and after 15 min, solid NH4HCO3 was added to adjust the final bicarbonate concentration to 0.55 M. Following an overnight incubation, the digest was applied to a column (2.5 × 90 cm) in 0.1 M NH4HCO3, pH 8.3. The major high molecular weight derivative was EDP I (see Fig. 1B), and the second major fraction containing EDP II was lyophilized, redissolved in 0.1 M NaH2PO4, pH 6.0 and eluted with 0.15 M NaHCO3 containing 0.15% acetic acid, and further purified by high pressure liquid chromatography on a analytical C18 (reverse-phase) micro-Bondapak column (Waters, Milford, MA). Solvent A was 0.1 M NaH2PO4, 0.1 M NaHCO3, and Solvent B was 60% acetonitrile/40% Solvent A. A 25-min gradient was employed from 70%/30% to 50%/50% Solvent A/Solvent B under 1.0 ml/min. Elution was monitored at 212 nm.

**Plasmin(ogen) Derivatives**—Plasmin was converted to plasmin with either urokinase or streptokinase, and complete conversion was established by SDS-polyacrylamide gel electrophoresis under reducing conditions. The plasmin–α2-antiplasmin complex was formed in a 10% excess of α2-antiplasmin and was purified by affinity chromatography on lysine-Sepharose followed by molecular exclusion chromatography on Ultrogel AcA44 (22, 23). The plasmin light and heavy chains were isolated following mild reduction and alkylation of plasmin. The light chain was obtained as the unbound fraction from lysine-Sepharose, and the heavy chain was eluted with 0.2 M 6-aminohexanoic acid (13). Purity of all derivatives was assessed by SDS-polyacrylamide gel electrophoresis, and a homogeneity of >90% for each derivative was indicated by the staining patterns.

**Modification of EDP II—EDP II. 1 mg in 1.0 ml of 8.0 M urea, 0.5 M Tris, pH 8.2, was reduced with 0.15 M 2-mercaptoethanol for 1 h at 22 °C and/or alkylated at 4 °C for 1 h with 0.52 M iodoacetamide.** Cyanoacrylamide cleavage of EDP II was performed by incubation of EDP II (1.0 mg/ml) in 70% formic acid and 0.04 M cyanoacrylamide for 16 h at 22 °C. Excess reagents were removed by dialysis in 1,000-dalton cut-off tubing versus 0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2. Enzymatic digestion of EDP II with trypsin or chymotrypsin was performed in 0.15 M NaCl, 0.1 M sodium phosphate buffer, pH 7.2, at 37 °C for 1 or 16 h at enzyme/substrate ratios of 1.3:1 or 10:1 (w/w). Under each condition, a second EDP II sample containing approximately 50,000 cpm of [3H]-EDP II was treated in parallel and was utilized to assess binding of EDP II on columns of lysine-Sepharose (0.9 × 10 cm) on the basis of radioactivity (10–30 mg of lysine/ml of Sepharose 2B).

**Radioimmunoassay for EDP II—Rabbit antiserum to EDP II was prepared by injecting 100 μg of EDP II initially in complete Freund’s adjuvant, followed by biweekly injections in incomplete Freund’s adjuvant.** An early bleeding following the fourth immunization was utilized for this study. EDP II was radioiodinated by a modified chloramine-T procedure (24). To 50 μg of EDP II in 50 μl of 0.1 M sodium phosphate, pH 7.2, 1.1 ×10^6 cpm of carrier-free 125I (17 Ci/mg, Amersham) and 10 μg of chloramphenicol were added. After 5 min at 22 °C, 7.5 mg of sodium metabisulfite were added. Bovine serum albumin (1%) was added to bring the final volume to 600 μl, and the sample was injected repeatedly to remove free iodine. The capacity of EDP II to bind to lysine-Sepharose was 80–90%, and greater than 90% of the radioactivity was precipitated by 15% trichloroacetic acid.

The radioimmunoassay system utilized was of the double antibody type, employing goat anti-rabbit immunoglobulin to achieve precipitation in a 0.04 M borate buffer, pH 8.3, containing 0.05 M NaCl and 2% normal rabbit serum (25). 125I-EDP II or 3H-Glu-plasminogen at 0.7 nm, inhibitors, and antiserum were added in 0.5-ml additions, and after an 18-h incubation at 4 °C, 0.5 ml of optimally diluted second
chased from Sigma and AMCA was from Kabi, Stockholm, Sweden. Porcine elastase, chymotrypsin, and trypsin were from Worthington.

RESULTS

Plasminogen with glutamic acid as the NH₂ terminus was isolated from human plasma or Cohn's Fraction III and subjected to limited elastase digestion. When the digest was chromatographed on lysine-Sepharose (Fig. 1A), the unbound material contained EDP III and the bound protein was eluted with 0.2 M 6-aminohexanoic acid. This eluate was subjected to molecular exclusion chromatography on Sephadex G-75 (Fig. 1B), and two major as well as two minor components were resolved. The second major peak, eluting from 350 to 380 ml, contained EDP II and was further purified by reverse phase chromatography on high pressure liquid chromatography (Fig. 1C). The final product was homogeneous as judged by its high pressure liquid chromatography elution pattern and by polyacrylamide gel electrophoresis in the presence of urea and SDS (Fig. 1D). The molecular weight of the isolated derivative was estimated to be 11,000, consistent with the reported value of EDP II (7). The identity of the isolated derivative as EDP II was based upon its amino acid composition (Table I), which is in reasonable agreement with that predicted from the published sequence of EDP II, and by identification of valine as the only detectable NH₂-terminal amino acid (7, 15).

Antiserum to EDP II was raised in rabbits and utilized to develop a double antibody radioimmunoassay system. Both 125I-EDP II and 125I-Glu-plasminogen were effectively bound by the antiserum (Fig. 2). At a 1/50 dilution of the antiserum, greater than 85% of each ligand was bound, and the ABC-33% was 1.4 nmol of EDP II and 0.8 nmol of Glu-plasminogen bound/ml of antiserum. When 6-aminohexanoic acid at a final concentration of 0.2 M was included in the assays, binding of either ligand by the antiserum was markedly reduced. The reductions represented at least a 35-fold decrease in the antigen binding capacity of the antiserum for the ligands in the presence of 6-aminohexanoic acid.

With numerous other antisera to plasminogen derivatives and to unrelated proteins, 6-aminohexanoic acid had no effect on antibody binding. The interaction of 6-aminohexanoic acid with the lysine binding site of EDP II could result in the observed inhibition of 125I-EDP II and 125I-Glu-plasminogen binding by anti-EDP II, and this possibility was explored in detail. Serial dilutions of lysine, 6-aminohexanoic acid, and AMCA were introduced into the radioimmunoassay as potential inhibitors for the binding of 125I-EDP II by anti-EDP II.

As shown in Fig. 3, each derivative produced a concentration-dependent inhibition curve and caused complete inhibition at high concentrations. The concentrations required for 50% competitive inhibition were 5.0, 0.24, and 0.047 mM for lysine, 6-aminohexanoic acid, and AMCA, respectively; the series AMCA > 6-aminohexanoic acid > lysine is consistent with their relative potency as antifibrinolytic agents (4). Generically similar results were obtained utilizing 125I-Glu-plasminogen as the ligand with anti-EDP II, and the concentrations of the lysine analogues required for 50% inhibition are summarized in Table II. In each case, a higher concentration of the ω-aminocarboxylic acid was required for 50% inhibition of 125I-Glu-plasminogen binding, but the relative inhibitory ca-

### Table I

| Amino acid composition of EDP II | Determined residues/mol | Predicted residues/mol |
|--------------------------------|-------------------------|-----------------------|
| Lysine                         | 6.0                     | 6                     |
| Histidine                      | 2.9                     | 3                     |
| Arginine                       | 4.1                     | 4                     |
| Threonine                      | 10.7                    | 11                    |
| Serine                         | 7.8                     | 8                     |
| Glutamic acid                  | 7.7                     | 7                     |
| Proline                        | 6.2                     | 6                     |
| Glycine                        | 7.5                     | 7                     |
| Alanine                        | 3.4                     | 3                     |
| Valine                         | 2.1                     | 2                     |
| Methionine                     | 2.0                     | 2                     |
| Isoleucine                     | 0.25                    | 0                     |
| Leucine                        | 2.4                     | 2                     |
| Tyrosine                       | 5.1                     | 5                     |
| Phenylalanine                  | 1.0                     | 1                     |

* From the sequence of Sottrup-Jensen et al. (15).

### Table II

| Concentrations of ω-aminocarboxylic acids required to produce 50% inhibition of the binding of 125I-EDP II and 125I-Glu-plasminogen by anti-EDP II | Concentration required for 50% inhibition* |
|--------------------------------------------------------------------------------|------------------------------------------|
| 125I-EDP II                                                                | 125I-Glu-plasminogen                     |
| Lysine                                                                     | 6.6                                      |
| 6-Aminohexanoic acid                                                        | 0.28                                     |
| AMCA                                                                       | 0.022                                    |

* Average of four determinations.
iodoacetate, its binding to lysine-Sepharose and anti-EDP I1. When EDP I1 was subjected to either 2-mercaptoethanol and alkylation, both activities were completely abolished. Plasmin moiety during complexation with a2-antiplasmin inhibition of AMCA was assessed. In the heavy chain of plasmin (a 4.3-fold excess) and Glu-plasminogen, the heavy (A) chain of plasmin, and Glu-plasminogen competitively inhibited, whereas EDP I1, EDP I11, and the light (B) chain of plasmin did not inhibit. Higher concentrations of the heavy chain of plasmin (a 4.3-fold excess) and Glu-plasminogen (a 9.5-fold excess) were required to give similar inhibition as EDP I1, suggesting that regions within spatial proximity of EDP I1 may hinder the accessibility of antibody to the EDP I1 determinants in the larger derivatives.

Modulation of EDP I1 determinants during the conversion of plasminogen to plasmin and complexation of plasmin with α2-antiplasmin was assessed (Fig. 5). The inhibition curves of Glu-plasminogen, Lys-plasminogen, and plasmin were not significantly perturbed during the activation of the zymogen. A 3.3-fold lower concentration of the plasmin-α2-antiplasmin complex was required to produce inhibition similar to the free plasminogen molecules. This is consistent with the interpretation that conformational modulations of the plasmin moiety during complexation with α2-antiplasmin increase the exposure of the EDP II region.

The capacity of chemical and enzymatic modification of EDP II to modulate its ability to interact with anti-EDP II and to bind to lysine-Sepharose was evaluated (Table III). When EDP II was subjected to either 2-mercaptoethanol or iodoacetate, its binding to lysine-Sepharose and anti-EDP II was minimally altered. However, when subjected to reduction and alkylation, both activities were completely abolished.

Cyanogen bromide cleavage in 70% formic acid partially diminished the capacity of EDP II to bind to lysine-Sepharose and reduced its inhibitory capacity in radioimmunoassay 8-fold, whereas exposure of EDP II to formic acid alone was without significant effect. The loss of lysine-binding activity following reduction and alkylation is consistent with the recent report of Lerch and Rickli (29) although they found that CNBr also completely destroyed this function. As they reported, we also observed cleavage at only one of the two methionyl residues based upon the polyacrylamide gel patterns of reduced and nonreduced samples. Chymotryptic digestion for 1 h at 37 °C completely inhibited the capacity of EDP II to bind to anti-EDP II and lysine-Sepharose, but tryp tic digestion for 18 h at 37 °C did not alter either activity. Digestion of EDP II by chymotrypsin to derivatives of molecular weights of less than 4000 was verified by polyacrylamide gel electrophoresis in the presence of SDS and urea, whereas EDP II incubated with trypsin for 18 h was unchanged. (The resistance of EDP II to tryptic degradation was verified with four different trypsin preparations.) Thus, in all cases, modifications which reduced the capacity of EDP II to bind to lysine-Sepharose resulted in a parallel reduction in its capacity to be bound by anti-EDP II.

The lysine binding sites participate in the interaction of plasminogen with fibrinogen and α2-antiplasmin (8-11). The preceding data suggest that when the lysine binding site of EDP II is occupied, neither EDP II nor plasminogen will

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**Fig. 4.** Competitive inhibition by plasminogen derivatives of the binding of 125I-EDP II by anti-EDP II. The anti-EDP II dilution used bound 48% of the 125I-EDP II (0.18 nM) present in the radioimmunoassay.

**Fig. 5.** Competitive inhibition by plasminogen derivatives and the plasmin-α2-antiplasmin complex of the binding of 125I-EDP II by anti-EDP II. The anti-EDP II dilution used bound 48% of the 125I-EDP II (0.38 nM) present in the radioimmunoassay.

**Table III**

Effect of chemical and enzymatic modifications of EDP II on its capacity to inhibit 125I-EDP II binding by anti-EDP II and to bind to lysine-Sepharose

| Modifying agent | Concentration Binding to lysine-Sepharose | Binding to EDP II |
|-----------------|------------------------------------------|------------------|
|                 | nM | % of unmodified EDP II | % of unmodified EDP II |
| None            |    | 100.0                  | 100.0             |
| 2-Mercaptoethanol | 7.4 | 97.3                   | 97.3              |
| Iodoacetate     | 9.3 | 91.2                   | 91.2              |
| 2-Mercaptoethanol + iodoacetate | >1000 | 0.0                 | 0.0               |
| Fornic acid     | 3.7 | 90.4                   | 90.4              |
| Fornic acid + CNBr | 32.0 | 36.1                 | 36.1              |
| Chymotrypsin (1 h at 37 °C) | >1000 | 0.0                 | 0.0               |
| Trypsin (18 h at 37 °C) | 4.2 | 103.1                 | 103.1             |

*Conditions of modification are detailed under "Materials and Methods."

50% relative inhibition of the binding of 125I-EDP II by anti-EDP II.

The binding of unmodified 125I-EDP II was assigned a value of 100%.


**Immunochrometry of a Lysine Binding Site of Plasminogen**

**FIG. 6.** Competitive inhibition of the binding of $^{125}$I-EDP II anti-EDP II by plasminogen diluted either in borate buffer, pH 8.3, or in a 1/10 dilution of plasminogen-depleted plasma. The plasma was depleted of plasminogen on lysine-Sepharose and contained approximately 10% of the plasminogen concentration of normal plasma.

react with anti-EDP II. On this basis, the capacity of fibrinogen and $\alpha_2$-antiplasmin to inhibit the binding of $^{125}$I-EDP II by anti-EDP II was assessed; but, at concentrations as high as $10^{-5}$ M, these molecules were not inhibitory. Interaction of the lysine binding site of EDP II with other plasma components was also assessed (Fig. 6). Isolated Glu-plasminogen was diluted in buffer or in plasminogen-depleted plasma. If the lysine binding site in the EDP II region of plasminogen is occupied in the plasma milieu, serial dilutions of plasminogen in plasminogen-depleted plasma should be less inhibitory than those in buffer. In contrast, dilutions in plasminogen-depleted plasma were slightly more inhibitory. This may be due to small amounts of residual plasminogen in the plasminogen-depleted plasma, or to interactions at other sites of plasminogen which secondarily increase the exposure of the EDP II region.

**DISCUSSION**

The $\omega$-aminocarboxylic acids exert profound effects upon the conformation of plasminogen (30-32) and dissociate the noncovalent complexes of plasminogen with fibrinogen (8, 9) and $\alpha_2$-antiplasmin (10, 11). In addition, the kinetics of plasminogen activation (30) and of the interaction of plasmin with $\alpha_2$-antiplasmin (11) are markedly affected by the $\omega$-aminocarboxylic acids. Taken together, these data point to an important role of lysine binding sites in regulating the activation and subsequent interactions of plasmin with its primary substrate and inhibitor. The lysine binding sites reside in the heavy chain region of plasminogen (13) and have been further localized in a recent study to specific enzymatic degradation products (7, 15). EDP II corresponds to the fourth kringle of plasminogen and contains a single low affinity lysine binding site (7). In this study, we have characterized an antiserum to EDP II which appears to require an unoccupied and functionally intact lysine binding site within EDP II for recognition by anti-EDP II. This conclusion is based on several lines of evidence. First, binding of $^{125}$I-EDP II or $^{125}$I-Glu-plasminogen by anti-EDP II was markedly inhibited by 0.2 M 6-aminohexanoic acid, representing at least a 35-fold reduction in the antigen binding capacity of the antiserum. Second, three $\omega$-aminocarboxylic acids produced concentration-dependent inhibition binding of EDP II and Glu-plasminogen by anti-EDP II, and the relative inhibitory capacity of lysines < 6-aminohexanoic acid < AMCA is consistent with the apparent affinity of these $\omega$-aminocarboxylic acids for the lysine binding sites (5, 6) and their potency as antifibrinolytic agents (4). Third, modifications, including reduction and alkylation, cyanogen bromide cleavage, and chymotryptic digestion, which reduced or abolished the capacity of EDP II to interact with lysine-Sepharose, markedly reduced the capacity of EDP II to be bound by anti-EDP II. The sensitivity of antibody binding to unfolding of EDP II induced by reduction and alkylation suggests that the recognized antigenic determinant(s) is conformationally dependent. Interaction of $\omega$-aminocarboxylic acids with the lysine binding site of EDP II could directly block the capacity of antibody to bind to the key amino acids comprising this determinant, or could sufficiently perturb the conformation of the antigenic locus so that it could no longer be recognized by antibody.

An argument may be developed to suggest that the concentrations of the $\omega$-aminocarboxylic acids required for 50% relative inhibition of the binding of either EDP II or Glu-plasminogen by anti-EDP II are the values of the dissociation constants of the $\omega$-aminocarboxylic acids for the lysine binding site within EDP II. The reactions occurring in the radioimmunoassay may be described as two equilibria in which LBS is the lysine binding site of EDP II, I is the $\omega$-aminocarboxylic acid and Ab is anti-EDP II:

$$K_c = \frac{[LBS\cdot Ab]}{[LBS]}$$

At the concentration I producing 50% relative inhibition in the radioimmunoassay, $[I]_{50\%}$, the concentration of free LBS is equal to [LBS-I] independent of the effective concentration of LBS available in the reaction; i.e. uncomplexed with antibody. Thus,

$$K_c = \frac{[LBS][I]}{[LBS]\cdot I}$$

By ultrafiltration, Markus et al. (5) estimated the average dissociation constant of the low affinity lysine binding sites of Glu-plasminogen for 6-aminohexanoic acid to be 5 mM, and Lerch et al. (7) determined the dissociation constant of 6-aminohexanoic acid for isolated EDP II to be 0.036 mM by equilibrium dialysis. By the immunochemical analysis in this study, the concentrations of 6-aminohexanoic acid required for 50% inhibition of binding were 1.49 mM with $^{125}$I-Glu-plasminogen and 0.28 mM with $^{125}$I-EDP II. Thus, these values are in reasonable agreement (in one case, 3.3-fold lower and in the other 7.8-fold higher) with those determined by more conventional approaches. Differences in the conditions of analysis, such as pH and ionic strength, could be responsible for the observed differences in values. Our immunochemical data suggest that dissociation constants for lysine relative to 6-aminohexanoic acid are 23.5- and 13.5-fold higher for EDP II and Glu-plasminogen, respectively. Markwardt (4) found that AMCA was 10-fold more potent than 6-aminohexanoic acid as an antifibrinolytic agent, and, with both $^{125}$I-EDP II and $^{125}$I-Glu-plasminogen, an 8.8-fold lower concentration of AMCA was required to produce the same inhibition as 6-aminohexanoic acid.

In further immunochemical analyses, it was found that only the plasminogen derivatives containing EDP II (the plasmin heavy chain, plasmin, and plasminogen) were reactive with
anti-EDP II, whereas derivatives lacking the EDP II region in their structure (EDP I, EDP III, and the plasmin light chain) were unreactive. Thus, despite the extensive structural homologies between the five plasminogen kringles (15), they were effectively discriminated by the antibody. This implies that the low affinity lysine binding sites are nonidentical and differences in their functional activities may be considered. The conversion of plasminogen to plasmin did not alter the accessibility to the EDP II region to anti-EDP II; however, complex formation between plasmin and α2-antiplasmin increased the exposure of the EDP II region. We have previously shown that the conformation of free α2-antiplasmin is modulated by complex formation with plasmin (34), and the present observation suggests that the conformation of plasmin is also altered. At high concentrations (10^-5 M), fibrinogen and α2-antiplasmin did not inhibit the binding of EDP II by anti-EDP II. This implies that the affinity of the lysine binding site of EDP II for these proteins is less than 10^5 M^-1, and is consistent with kinetic analysis suggesting that the high affinity lysine binding site participates primarily in these intermolecular interactions (11). In addition, the lysine binding site within EDP II also appeared to be unoccupied in plasma, suggesting that potential interactions of plasminogen with other plasma proteins such as the histidine-rich glycoprotein (35) are not mediated by this low affinity site.

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