Biochimica et Biophysica Acta, 567 (1979) 472—481

BBA 68692

ENZYMIC PROPERTIES OF THE NEO-PLASMIN-Val-442 (MINIPLASMIN)

ULLA CHRISTENSEN [a], LARS SOTTRUP-JENSEN [b], STAFFAN MAGNUSSON [b], TORBEN E. PETERSEN [b] and INGE CLEMMENSEN [c]

[a] *Chemical Laboratory IV, University of Copenhagen, Universiteitsparken 5, DK-2100 Copenhagen,* [b] *Department of Molecular Biology, University of Aarhus, Aarhus, and* [c] *Department of Clinical Chemistry, Hvidovre Hospital, Hvidovre (Denmark)*

(Received September 26th, 1978)

Key words: Plasmin; Neo-plasmin-Val-442; Miniplasmin; α_2-Antiplasmin

Summary

The enzymic properties of the activated plasminogen fragment (Val-442-Arg-560; S-S-bridged to Val-561-Asn-790) also called miniplasmin or neo-plasmin-Val-442, were studied. This neo-plasmin was prepared by urokinase catalysed conversion of the corresponding fragment of plasminogen (Val-442-Asn-790) produced by specific limited proteolysis of native plasminogen by porcine pancreatic elastase and purified by chromatography on L-lysine-Sepharose 4B.

The kinetic parameters of hydrolysis of a number of synthetic substrates by plasmin and by 'miniplasmin', respectively, were found to be alike. Furthermore the inhibition by 6-aminohexanoic acid and the pH-dependence of the hydrolysis of Bz-Arg-OEt were identical for the two enzymes. It is concluded that the catalytic site of 'miniplasmin' is very similar to that of plasmin.

The interaction of 'miniplasmin' with α_2-antiplasmin was studied in the presence and in the absence of 6-aminohexanoic acid. The reaction scheme which accounts satisfactorily for the reaction of plasmin with the inhibitor also fits the reaction of 'miniplasmin' with the inhibitor. However, it was found that 'miniplasmin' initially reacts with the inhibitor less readily than does plasmin, but that the rate of the second reaction step is equal to that of the corresponding step in the inhibitor-plasmin reaction. The hypothesis that site(s) other than the catalytic site of plasmin located at the NH_2-terminal part of the heavy chain (residues 77—441) primarily determine the association rate of plasmin and α_2-antiplasmin is supported.

Abbreviations used: Bz-Arg-OEt, α-*N*-benzoyl-L-arginine ethyl ester; D-Val-Leu-Lys-pNA, D-valyl-L-leucyl-L-lysine 4-nitroanilide; Bz-Phe-Val-Arg-pNA, α-*N*-benzoyl-L-phenylanalyl-L-valyl-L-arginine 4-nitroanilide.

Introduction

Recently Sottrup-Jensen et al. [1] prepared and isolated a fragment of human plasminogen, which consists of the COOH-terminal part, Val-442-Asn-790, of plasminogen, M_r 38 100. This fragment was called neo-plasminogen-Val-442 or 'miniplasminogen'. It does not require 6-aminohexanoic acid or similar compounds for elution from lysine-Sepharose-columns, indicating that it binds lysine only weakly or not at all. Urokinase catalyzes its conversion to an active enzyme by cleaving the same peptide bond, Arg-560-Val-561, which is cleaved in urokinase catalyzed activation of native plasminogen. The product is called neo-plasmin-Val-442 or 'miniplasmin' and consists of two polypeptide chains. One is the COOH-terminal 119-residue fragment of the original heavy chain of plasmin (residues 442—560). This heavy chain fragment is connected by the two disulfide bridges Cys-557 to Cys-565 and Cys-547 to Cys-665 to the light chain (residues 561—790), identical to the light chain of normal plasmin.

The present work describes the results of a number of kinetic experiments designed to investigate the enzymic properties of 'miniplasmin' and to compare them with those of plasmin. Particular attention was paid to the interaction of the two plasmins with α_2-antiplasmin.

Materials

The compounds D-Val-Leu-Lys-pNA, Bz-Phe-Val-Arg-pNA and 6-aminohexanoic acid were provided as gifts from Kabi Vitrium A/S, Copenhagen, Denmark. Bz-Arg-OEt was obtained from Calbiochem, La Jolla, CA, U.S.A. and 4′-nitrophenyl-4-guanidino-benzoate · HCl from Merck, Darmstadt, F.R.G. Kunitz trypsin inhibitor (Trasylol) was purchased from Bayer, Leverkusen, F.R.G. and had an activity of 10 000 Kallikrein inhibitory units/ml. Urokinase (EC 3.4.99.26) was obtained from Leo A/S, Copenhagen, Denmark and had a specific activity of 10 000 Ploug units/mg.

Human plasminogen was purified as previously described [2]. Human plasmin was prepared from plasminogen with NH_2-terminal glutamic acid on a column of urokinase-substituted Sepharose 4B [3] prepared essentially according to Wiman [4] and the eluate was concentrated by ultrafiltration.

The primary inhibitor of plasmin, α_2-antiplasmin, was purified from human plasma as previously described [5].

'Miniplasminogen' was prepared by limited specific proteolysis catalyzed by porcine pancreatic elastase of plasminogen with NH_2-terminal glutamic acid and was isolated as previously described [1].

'Miniplasmin' was prepared by urokinase catalyzed conversion of 'miniplasminogen'. Lyophilized 'miniplasminogen' was dissolved (approx. 1.5 mg/ml) in a buffer, 0.05 M Tris-HCl, 0.2 M NaCl, pH 7.8, 4°C and the solution was desalted on a column of Sephadex G-25 (16 mm × 40 cm) equilibrated with the same buffer. In reaction mixtures consisting of 50 Ploug U/ml urokinase (approx. 10 nM), 10—20 μM 'miniplasminogen' (desalted), 0.05 M Tris-HCl, 0.2 M NaCl and 25% (v/v) glycerol, pH 7.8, the results of the active site titrations of aliquots removed from the reaction mixtures at different times showed that the 'miniplasminogen'-'miniplasmin' conversion was complete

after 5 h at 25°C or after 22 h at 4°C and that the concentration of 'miniplasmin' remained constant for at least a further 24 h at 25°C. After appropriate incubation as described such reaction mixtures were frozen at −20°C and were later used as 'miniplasmin' stock solutions. When reaction mixtures of urokinase and 'miniplasminogen' were incubated with no glycerol present, the content of 'miniplasmin' decreased significantly within a few hours at 25°C after the maximal amount was formed. Obviously glycerol stabilizes 'miniplasmin' as it does plasmin.

'Miniplasmin' stock solutions showed only two bands on dodecylsulphate-polyacrylamide gel electrophoresis after reduction (M_r 25 100 and 13 000, respectively) [1].

Methods

All kinetic experiments were run at least three times at 25°C. Most reaction mixtures were prepared in 0.05 M Tris-HCl, 0.1 M NaCl, pH 7.8.

'Miniplasmin', plasmin and trypsin concentrations were determined by titration with 4′-nitrophenyl-4-guanidinobenzoate as described by Chase and Shaw [6].

The kinetic parameters of enzyme-catalyzed hydrolysis of the synthetic substrates were determined essentially as described earlier [3]. Each pair of kinetic parameters was calculated from at least 24 initial velocities, v, measured at 8 substrate concentrations covering in general the range $0.2 \times K_m$—$10 \times K_m$. The results were fitted to Eqn. 1.

$$v/e_0 = k_c/(K_m/s + 1) \quad (1)$$

using a least square method [7]. Enzyme concentrations in the reaction mixtures were 0.1—0.2 μM.

The reaction of α_2-antiplasmin with 'miniplasmin'

The kinetic measurements were made in a manner analogous to some described previously [5,8]. 'Miniplasmin' stock solutions were appropriately diluted by adding buffer or, when experiments were to be performed in the presence of 6-aminohexanoic acid, buffer containing the desired amount of 6-aminohexanoic acid so that equilibrium between 'miniplasmin' and 6-aminohexanoic acid was attained prior to incubation [1—2 h] with inhibitor. Then the desired amounts of 'miniplasmin' and inhibitor were incubated (total volume 1450 μl) and after the chosen incubation time (t) 50 μl 9.0 mM D-Val-Leu-Lys-pNA was added, the reaction mixture was poured into a 1 cm semimicro cuvette and the reaction was followed by measuring the appearance of 4-nitroaniline at $\lambda = 410$ nm. The initial velocities were taken as the slope of (the tangents to) the recorded curves at zero time. A Beckman model 35 spectrophotometer was used. Measurements were made at 0, 1, 12.5 and 25 mM concentrations of 6-aminohexanoic acid with total concentration of 'miniplasmin', $e_0 = 45$ nM at concentrations of α_2-antiplasmin, i_0, in the range 0—60 nM. The concentrations of α_2-antiplasmin solutions were determined as described previously [8,5]. The concentration of 'miniplasmin'-inhibitor complex(es) formed in a given incubation mixture at time, t, is given by $e_0(1 - v/$

v_0), where v is the initial velocity obtained in the reaction mixture in question, and v_0 is the initial velocity obtained with no inhibitor present, but at the same concentrations of substrate and of 'miniplasmin', e_0, at which v is determined. It is assumed, and the results confirm it, that the dissociation of 'miniplasmin'-inhibitor complex(es) is so slow that when the substrate is added, only the amount of 'miniplasmin' free to react at that time is determined.

Titration of 'mimiplasmin' with α_2-antiplasmin was performed by measuring the fraction of 'miniplasmin' inhibited after incubation for 900 s at a fixed 'miniplasmin' concentration and at various inhibitor concentrations as described above and determining the equivalence point as described by Ganrot [9]. Titration with Trasylol was performed in a similar manner. Titrations were thus performed in solutions containing less than 0.1% v/v of glycerol.

Results

Titration of 'miniplasmin'. The 'miniplasmin' preparations obtained in this study showed the same value of normality when titrated with 4'-nitrophenyl-4-guanidinobenzoate [6], Trasylol [9] and α_2-antiplasmin [8] (Table I). The preparations contained approx. 15% of inactive 'miniplasmin' as seen from the ratio of the concentration determined by titration and that determined by amino acid analysis. The fraction of 'miniplasmin' inhibited by α_2-antiplasmin under these experimental conditions, of which t = 900 s is the most important, was found to be $1-(i_0/e_0)$, if $(i_0/e_0) < 1$, and 1, if $(i_0/e_0) \geqslant 1$, so that even near the equivalence point no dissociation of enzyme-inhibitor complex(es) occurred.

Catalytic activity of 'mimiplasmin'. Table II summarizes the results obtained in comparison of the catalytic activities of 'miniplasmin' and plasmin. Towards typical synthetic plasmin-substrates 'miniplasmin' shows activities very similar to those of plasmin. The kinetic parameters are identical for 'miniplasmin' and plasmin catalyzed hydrolysis of Bz-Arg-OEt and of D-Val-Leu-Lys-pNA; pH 7.8, 25°C, whereas some difference is seen with Bz-Phe-Val-Arg-pNA as the substrate. Table III illustrates the pH-dependence of the kinetic parameters of 'miniplasmin' catalysed hydrolysis of Bz-Arg-OEt; no significant differences are found between plasmin and 'miniplasmin'. Fig. 1 illustrates the effect of 6-aminohexanoic acid on 'miniplasmin'. 6-Aminohexanoic acid is seen to be a competitive inhibitor of 'miniplasmin' with an inhibition constant, K_i = 58

TABLE I

THE CONCENTRATION OF "MINIPLASMIN" DETERMINED BY THREE DIFFERENT TITRATION METHODS AND FROM THE AMINO ACID ANALYSIS OF TWO "MINIPLASMIN" PREPARATIONS

Method	Concentration of "miniplasmin" (μM)	
	Sample 1	Sample 2
4'-Nitrophenyl-4-guanidino-benzoate [6]	10.1	21.7
Trasylol [9]	10.2	21.9
α_2-Antiplasmin [8]	10.2	22.0
Amino acid analysis	11.9	25.8

TABLE II

KINETIC PARAMETERS OF "MINIPLASMIN" AND PLASMIN AT 25°C

Enzyme	Substrate	K_m (mM)	k_c (s^{-1})
Plasmin	Bz-Arg-OEt	0.19 ± 0.01	12.0 ± 0.2
"Miniplasmin"	Bz-Arg-OEt	0.18 ± 0.02	12.6 ± 0.5
Plasmin	D-Val-Leu-Lys-pNA	0.12 ± 0.01	11.7 ± 0.3
"Miniplasmin"	D-Val-Leu-Lys-pNA	0.13 ± 0.01	10.8 ± 0.5
Plasmin *	Bz-Phe-Val-Arg-pNA	0.9 ± 0.1	10 ± 1.0
"Miniplasmin" *	Bz-Phe-Val-Arg-pNA	1.2 ± 0.1	12.8 ± 0.9

* These experiments were performed at pH 7.6.

mM, pH 7.8, 25°C, identical to that obtained with inhibition of plasmin [10]. An activation effect corresponding to that, which 6-aminohexanoic acid has on plasmin (K = 0.3 mM) [10] is not observed.

Kinetics of the reaction between 'miniplasmin' and α_2-antiplasmin. Table IV shows some results obtained for the reaction of the inhibitor and 'miniplasmin'. Of the following three reaction schemes

$$E + I \xrightarrow{k_1(I)} C\,, \tag{I}$$

$$E + I \underset{k_{-1}(II)}{\overset{k_1(II)}{\rightleftharpoons}} C\,, \tag{II}$$

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \overset{k_2}{\rightarrow} C_2 \tag{III}$$

only Scheme III is compatible with the reaction of 'miniplasmin' and the inhibitor. The values of the rate constant of Scheme I, k_1(I), calculated from the rate equation of Scheme I by using the experimental values of the concentrations of reactants after incubation for t seconds (Table IV) clearly show that Scheme I is not a valid model for the reaction of 'miniplasmin' and the inhibitor. The reasons why Scheme II is not a valid model for this reaction were given above.

Analysing Scheme III, where C_1 and C_2 are two different 'miniplasmin'-inhibitor complexes with the concentrations c_1 and c_2, E is 'miniplasmin' with

TABLE III

KINETIC PARAMETERS OF "MINIPLASMIN" AND PLASMIN CATALYSED HYDROLYSIS OF Bz-Arg-OEt AT VARIOUS pH-VALUES, 25°C

Buffers: 0.05 M Tris · HCl, 0.1 M NaCl.

pH	"Miniplasmin"		Plasmin	
	K_m (mM)	k_c (s^{-1})	K_m (mM)	k_c (s^{-1})
6.8	0.16 ± 0.03	9.3 ± 0.5	0.20 ± 0.02	9.5 ± 0.3
7.2	0.16 ± 0.03	10.9 ± 0.6	0.19 ± 0.02	11.8 ± 0.3
7.8	0.18 ± 0.02	12.6 ± 0.5	0.19 ± 0.01	12.0 ± 0.2
8.1	0.21 ± 0.02	12.4 ± 0.4	0.28 ± 0.01	12.1 ± 0.2
8.4	0.42 ± 0.04	13.2 ± 0.6	0.40 ± 0.03	12.7 ± 0.5

TABLE IV

INHIBITION OF "MINIPLASMIN" BY α_2-ANTIPLASMIN IN THE PRESENCE AND ABSENCE OF 6-AMINOHEXANOIC ACID

e/e_0 is the experimental value of the fraction of miniplasmin free to react after incubation of miniplasmin at an initial concentration e_0 = 45 nM and inhibitor at a concentration, i_0 = 60 nM. $k_1(I)$ is the value of the rate constant of reaction Scheme I calculated from the experimental e/e_0-value using the equation

$$k_1(I) = t^{-1}(i_0 - e_0)^{-1} \log_e[e_0(e + i_0 - e_0)/(i_0 e)].$$

(t, e/e_0) values equal to those found with no 6-aminohexanoic acid present were obtained in a series of experiment performed at 1.0 mM concentration of 6-aminohexanoic acid.

0 mM 6-aminohexanoic acid			12.5 mM 6-aminohexanoic acid		
Incubation time (s)	e/e_0	$10^{-6}k_1(I)$ ($M^{-1} \cdot s^{-1}$)	Incubation time (s)	e/e_0	$10^{-6}k_1(I)$ ($M^{-1} \cdot s^{-1}$)
10	0.648	0.85	10	0.674	0.76
15	0.514	0.94	30	0.487	0.52
30	0.401	0.71	60	0.362	0.41
45	0.313	0.60	120	0.254	0.31
60	0.269	0.57	240	0.124	0.28
90	0.220	0.47	360	0.062	0.29
120	0.172	0.44	480	0.030	0.31
150	0.148	0.40	600	0.016	0.31
180	0.132	0.36			
210	0.110	0.35			
240	0.096	0.34			
360	0.050	0.32			
600	0.013	0.33			

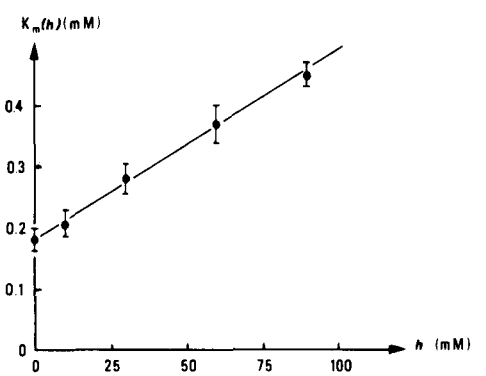

Fig. 1. The apparent Michaelis constant of 'miniplasmin' catalysed hydrolysis of Bz-Arg-OEt in the presence of 6-aminohexanoic acid ($K_m(h)$ mM) plotted against the concentration of 6-aminohexanoic acid (h mM). The catalytic constant was independent of h, the mean value of those obtained was $k_c(h)$ = 12.4 ± 0.4 s^{-1}. Experimental conditions: buffer 0.05 M Tris-HCl, 0.1 M NaCl, pH 7.8, 25°C. $K_m(h)$ and $k_c(h)$ and their standard errors were determined by fitting to Eqn. 1 the results of 24 initial velocity measurements for which the concentrations of 6-aminohexanoic acid was held constant and the substrate concentration was varied in the range of 0.1—3 mM [3].

the concentration e (initially e_0), and I is the inhibitor with the concentration i (initially i_0), and assuming that the first reaction step is fast and the second slow, so that $K = k_{-1}/k_1 = e \cdot i/c_1$ eventually holds, $-dc_1/dt = k_2 c_1$ and $c_1 = e \cdot (i_0 - e_0 + e)/K$. Thus:

$$-\log_e[(e(i_0 - e_0 + e))/e_0^2] = k_2 t - \log_e[(e_{eq}(i_0 - e_0 + e_{eq}))/e_0^2] , \quad (2)$$

where e_{eq} is determined by

$$K = (e_{eq}(i_0 - e_0 + e_{eq}))/(e_0 - e_{eq}) .$$

Fig. 2 shows a plot of

$$-\log_e[(e(i_0 - e_0 + e))/e_0^2]$$

against t for one series of (t, e) measurements. Table V shows the values of K and k_2 determined from such plots, and the values of k_1 and k_{-1} estimated. The value of k_1 was estimated from k_1(I)-values obtained for $t \to 0$.

The effect of 6-aminohexanoic acid on the reaction between 'miniplasmin' and the inhibitor was studied. Some results are illustrated in Fig. 2 and Tables IV and V. It is seen that 6-aminohexanoic acid inhibits the first step of the

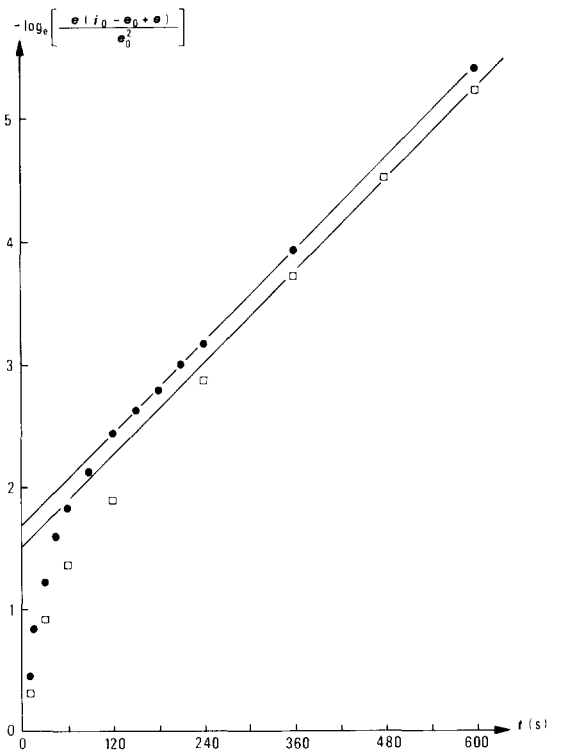

Fig. 2. Time-dependence of the interaction of 'miniplasmin' with α_2-antiplasmin. The values of $-\log_e[(e/e_0)(i_0/e_0 - 1 + e/e_0)]$ are plotted against time, t (s) (Eqn. 2). e/e_0 is the experimental value of the fraction of 'miniplasmin' free to react after incubation t seconds of inhibitor at an initial concentration, i_0 = 60 nM and 'miniplasmin' at a concentration e_0 = 45 nm. 6-aminohexanoic acid concentrations were 0 (●——●) and 12.5 mM (□——□). The lines shown are those obtained from least squares fits to Eqn. 2 of values determined at $t > 120$ s (●) and at $t > 240$ s (□) respectively.

TABLE V

INTERACTION OF "MINIPLASMIN" WITH α_2-ANTIPLASMIN. VALUES OF THE EQUILIBRIUM CONSTANT OF THE FIRST REACTION STEP AND OF THE RATE CONSTANTS (REACTION SCHEME III)

$K = k_{-1}/k_1$; k_2; k_1 were determined as described in the text. The inhibition constant of 6-aminohexanoic acid for "miniplasmin" is K_i = 58 mM. Therefore, the theoretical values of K'/K are: $K'_{12.5}/K$ = 1.22 and $K'_{25.0}/K$ = 1.43.

Concentration of 6-aminohexanoic acid, h (mM)	K (nM)	K' (nM)	K'/K	$10^3 k_2$ (s^{-1})	$10^{-6} k_1$ ($M^{-1} \cdot s^{-1}$)	$10^3 k_{-1}$ (s^{-1})
0	12.1	—	1.00	6.25	0.9	11
12.5	—	14.9	1.23	6.25	0.8	12
25.0	—	17.5	1.45	6.50	0.6	11

inhibitor-'miniplasmin' reaction, but not the second. Since 6-aminohexanoic acid and 'miniplasmin' form a complex with a dissociation constant, K_i = 58 mM (Fig. 1) it is expected that K' and k'_1, calculated from the initial velocities measured with 6-aminohexanoic acid present in the reaction mixtures in equilibrium with miniplasmin, depend on the concentrations of 6-aminohexanoic acid h [8] thus:

$$K' = K(1 + h/K_i) \quad \text{and} \quad k'_1 = k_1/(1 + h/K_i) ,$$

which are the results obtained. The effect of 6-aminohexanoic acid on the inhibitor-'miniplasmin' reaction satisfies the definition of a linear competitive inhibition of 'miniplasmin', no other effect was observed.

Discussion

The objective of the present study was to investigate the enzymic properties of neo-plasmin-Val-442 (miniplasmin) and to compare them with those of plasmin. As judged from the kinetic parameters of 'miniplasmin' catalysed hydrolysis of synthetic substrates the specificity and the mechanism of catalysis are very similar. 'Miniplasmin' catalyzes the hydrolysis of arginyl and lysyl bonds. Thus, it has the same side-chain specificity as does plasmin. Identical kinetic parameters are found for 'miniplasmin' and plasmin towards substrates covering the subsites S_3-S_2-S_1-S'_1 of the active site, and also with regard to competitive inhibition by 6-aminohexanoic acid. In the pH range 5.8—9.0 the kinetic parameters of plasmin are influenced by two groups with pK values 6.5 and 8.4 respectively [3]. In the pH range 6.8—8.4 'miniplasmin' shows the same pH dependence as does plasmin indicating that the catalytic site of 'miniplasmin' closely resembles that of plasmin and the pancreatic serine proteinases. In a recent paper Wohl et al. [11] reported that the isolated and partially reduced light chain of plasmin (Val-561-Asn-790, M_r 25 100) possesses some catalytic activity, which is, however, significantly less than that of plasmin. The 'miniplasmin' is the smallest fragment so far described which retains full plasmin activity, proving that residues 1—441 are not required for plasmin activity. Whether the polypeptide chain Val-442-Arg-560 of pasmin

really is important for the catalytic activity can not be decided. The great loss of activity seen by Wohl et al. [11] may be due to reduction of disulfide bridges of their plasmin light chain preparation, such reduction is apparent from their reported high C_m Cys content.

The reaction between α_2-antiplasmin and 'miniplasmin' follows a reaction scheme (III) (Fig. 2), which also, as has been shown in previous papers [5,8], accounts satisfactorily for the reaction between α_2-antiplasmin and plasmin. The inhibitory effects of L-lysine and 6-aminohexanoic acid on the reaction between the inhibitor and plasmin occur at concentrations too low to be accounted for by assuming only competition for the active site of plasmin between the amino acid and the inhibitor [5,8]. The effects are best explained by assuming such a competition for other sites in plasmin. Since the presence of these amino acids showed no effects on the second step of the inhibitor-plasmin reaction, it was concluded that other sites of plasmin are important participants only in the first step of this reaction. The formation of the intermediate enzyme-inhibitor complex proceeds at a slower rate and the complex dissociates more readily when plasmin is replaced by 'mimiplasmin' in the reaction with the inhibitor (Table V). The effect of 6-aminohexanoic acid on the reaction between the inhibitor and 'miniplasmin' can be described as competition for the active site of 'miniplasmin' between the amino acid and the inhibitor and no other effect is observed. Markus et al. [12,13] have recently shown that plasminogen possesses a single strong site (K_D = 9 μM) and five weaker ones (K_D = 5 mM) which bind 6-aminohexanoic acid, and they suggested that the five 'kringle' structures might have one weak binding site each. The reaction of α_2-antiplasmin with plasmin is affected by 6-aminohexanoic acid in a manner corresponding to interactions at the weak binding sites, K_D = 5 mM [5]. The results presented here indicate that 'miniplasmin' has lost that (those) other site(s) of plasmin which appear to indirectly accelerate the interaction of α_2-antiplasmin with the serine proteinase part of plasmin. Of course this does not exclude the possibility that 'miniplasmin' has a weak binding site, which may bind 6-aminohexanoic acid but does not interact with α_2-antiplasmin.

Wiman et al. [14] have published a paper reporting on the kinetics of the reaction between α_2-antiplasmin and 'miniplasmin' at pH 7.3. These authors also conclude that the reaction may be represented by reaction Scheme III. They found values of k_1 30 to 60 times less than and a value of K 10 times greater than those of the reaction of α_2-antiplasmin with plasmin. Some influence of 6-aminohexanoic acid was also reported, but the effect was not analysed.

Acknowledgement

L.S.J., S.M. and T.E.P. acknowledge the support of the National Heart, Lung and Blood Institute, NIH, grant no. HL-16238. Bethesda, MD, U.S.A.

References

1 Sottrup-Jensen, L., Claeys, H., Zajdel, M., Petersen, T.E. and Magnusson, S. (1977) in Progress in Chemical Fibrinolysis and thromboltsis (Davidson, J.F., Samama, M. and Desnoyers, P., eds.), Vol 3, pp. 139—209, Raven Press, New York

2 Christensen, U. (1977) Biochim. Biophys. Acta 481, 638—647
3 Christensen, U. (1975) Biochim. Biophys. Acta 397, 459—467
4 Wiman, B. and Wallén, P. (1973) Eur. J. Biochem. 36, 25—31
5 Christensen, U. and Clemmensen, I. (1978) Biochem. J. 175, 635—641
6 Chase, J. and Shaw, E. (1969) Biochemistry 8, 2212—2224
7 Cleland, W.W. (1967) Adv. Enzymol. 29, 1—32
8 Christensen, U. and Clemmensen, I. (1977) Biochem. J. 163, 389—391
9 Ganrot, P.O. (1967) Acta Chem. Scand. 21, 595—601
10 Christensen, U. (1978) Biochim. Biophys. Acta. 526, 194—201
11 Wohl, R.C., Arzadon, L., Summaria, L. and Robbins, K.C. (1977) J. Biol. Chem. 252, 1141—1147
12 Markus, G., DePasquale, J.L. and Wissler, F.C. (1978) J. Biol. Chem. 253, 728—732
13 Markus, G., Evers, J.L. and Hobika, G.H. (1978) J. Biol. Chem. 253, 733—739
14 Wiman, B., Boman, L. and Collen, D. (1978) Eur. J. Biochem. 87, 143—146