The interaction of streptokinase·plasminogen activator complex, tissue-type plasminogen activator, urokinase and their acylated derivatives with fibrin and cyanogen bromide digest of fibrinogen

Relationship to fibrinolytic potency *in vitro*

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The effects of purified soluble fibrin and of fibrinogen fragments (fibrin mimic) on the activation of Lys-plasminogen (i.e. plasminogen residues 77–790) to plasmin by streptokinase·plasminogen activator complex and by tissue-type plasminogen activator were studied. Dissociation constants of both activators were estimated to lie in the range 90–160 nM (fibrin) and 16–60 nM (CNBr-cleavage fragments of fibrinogen). The kinetic mechanism for both types of activator comprised non-essential enzyme activation via a Rapid Equilibrium Ordered Bireactant sequence. In order to relate the fibrin affinity of plasminogen activators to their fibrinolytic potency, the rate of lysis of supported human plasma clots formed in the presence of unmodified or active-centre-acylated precursors of plasminogen activators was studied as a function of the concentration of enzyme derivative. The concentrations of unmodified enzyme giving 50% lysis/h in this assay were 0.9, 2.0 and 11.0 nM for tissue-type plasminogen activator, streptokinase·plasmin(ogen) and urokinase respectively. However, the potencies of active-centre-acylated derivatives of these enzymes suggested that acylated-tissue plasminogen activator and streptokinase·plasminogen complexes of comparable hydrolytic stability were of comparable potency. Both types of acyl-enzyme were significantly more potent than acyl-urokinases.

INTRODUCTION

The binding to fibrin of the components of the fibrinolytic system forms an essential part of any model of selective physiological fibrinolysis (Wiman & Collen, 1978; Collen, 1980). The interaction of plasminogen with fibrin has been the subject of many studies and the affinity of different plasminogen forms for various types of fibrin has been reported (Suenson & Thorsen, 1981; Lucas *et al.*, 1983; Bok & Mangel, 1985). Dissociation constants (K_d) for Glu-plasminogen of 24–42 μ M were found, whereas those for Lys-plasminogen ranged from 0.32 to 7.9 μ M. Plasminogen activators, which are trypsin-like serine proteinases catalysing the cleavage of the Arg-560–Val-561 bond in human plasminogen, may undergo several types of interaction with fibrin, the nature and strength of which may influence the performance of such enzymes as thrombolytic agents. Hoylaerts *et al.* (1982) estimated a K_d for tissue-type plasminogen activator (t-PA) binding to formed polymeric fibrin 20–160 nM according to whether Lys-plasminogen or Glu-plasminogen was present to form a ternary complex with t-PA and fibrin. A similar study based on the kinetics of plasminogen activation by urokinase (u-PA) in the presence of CNBr-cleavage fragments of fibrinogen suggested that u-PA underwent much weaker interactions with fibrin-related structures (Lijnen *et al.*, 1984). Although the complexing of streptokinase with plasminogen has been reported to increase the affinity of the latter

protein for fibrin (Cederholm-Williams, 1981), a quantitative comparison of the binding of the streptokinase·plasminogen complex (SK·Plg) and t-PA in the presence of excess plasminogen has not been made. Kinetic and thrombus-binding studies in this laboratory with the acyl-enzyme *p*-anisoyl-plasminogen·streptokinase complex (BRL 26921, APSAC; Smith *et al.*, 1981) have suggested that the undegraded SK·Plg complex can bind to fibrin with an affinity that may be of significance for the clinical use of this fibrinolytic pro-drug (Fears *et al.*, 1985a,b). We therefore undertook the present comparison of SK·Plg and t-PA using a fibrin interaction assay based upon fibrin stimulation of plasminogen activation in a purified system. We then compared the potencies of free and active-centre-acylated forms of these enzymes, together with u-PA, in a clot-lysis assay *in vitro*. These comparisons were aimed at clarifying the relationship between the affinity for fibrin and the lytic potency of plasminogen activators with therapeutic potential.

MATERIALS AND METHODS

Materials

Human two-chain urokinase (M_r 54000; specific activity 100000 units/mg; urinary origin) was obtained from Serono (Freiburg, W. Germany). Recombinant tissue-type plasminogen activator was purified to homo-

Abbreviations used: Glu-plasminogen, human plasminogen with *N*-terminal glutamic acid (residues 1–790); Lys-plasminogen, human plasminogen with *N*-terminal lysine (residues 77–790); SK, streptokinase; SK·Plg, streptokinase complexed with human Lys-plasminogen; u-PA, urinary-type plasminogen activator; t-PA, tissue-type plasminogen activator; PAGE, polyacrylamide-gel electrophoresis; PDAEB, 4-*N*-(2-*N'*-[3-(2-pyridyldithio)-propionyl]aminoethyl)aminobenzoyl.

geneity from the culture supernatant of a genetically manipulated human cell line (Browne *et al.*, 1985) and had M_r 63 000 by SDS/PAGE/fibrin-overlay autoradiography (Granelli-Piperno & Reich, 1978). Human thrombin (1000 NIH units/mg of protein) was obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). ^{125}I -fibrinogen was a product of Amersham International (Amersham, Bucks., U.K.) and was repurified as described previously (Dupe *et al.*, 1981). 4-Anisoyl-Lys-plasminogen-streptokinase complex (BRL 26921) was prepared as described elsewhere (Smith *et al.*, 1982). Active-site-acylated derivatives of u-PA and t-PA were prepared by treating the activators (6–9 μM) in 0.05 M-sodium phosphate buffer, pH 7.4, containing 0.1 M-NaCl and 0.01 % Tween 80 with the appropriate 4-amidinophenyl esters (1.0 mM) at 25 °C for 60 min. Acyl-enzymes were freed of excess acylating agent by gel filtration into the same buffer on small Sephadex G-25 columns (Pharmacia, Milton Keynes, Bucks., U.K.). Acyl-enzymes were stored in solution for short periods at –70 °C. Deacylation rate constants were determined as described previously (Smith *et al.*, 1985). The preparation of CNBr-cleavage fragments of fibrinogen was adapted from the procedure of Verheijen *et al.* (1982) as follows. Human fibrinogen (grade L; Kabivitrum, Stockholm, Sweden) (1.0 g) was treated with CNBr (1.3 g) in 70 % (v/v) formic acid (100 ml) at 18 °C for 17 h. Unused CNBr and formic acid were removed by dialysis against deionized water for 24 h and the product was freeze-dried. Analysis by SDS/PAGE (10 % separating gel) in non-reducing conditions disclosed six protein-stainable degradation products at apparent M_r values 88 000, 70 000, 38 000, 36 000 and two less than 20 000. The total yield of fragments was 90 %. Fragments were dissolved in 0.05 M-NaOH to give a stock solution of 30 mg/ml, which was then diluted to 5 mg/ml with 0.05 M-Tris/HCl buffer, pH 7.4, containing 0.1 M-NaCl. Small portions were stored at –40 °C at which temperature plasminogen-activation-enhancing activity was stable (R. Fears & R. Standring, unpublished work). The sources of other materials and the preparation of soluble fibrin oligomers have been described previously (Fears *et al.*, 1985b).

Active-site titration

The operational molarity of t-PA and u-PA was determined by active-site titration with the chromogenic inverse substrate 4-anisic acid 4'-amidino-2'-nitrophenyl ester (Smith *et al.*, 1985; Smith, 1986). This compound reacts with the enzymes, releasing 4-amidino-2-nitrophenol which has ϵ_{405} 5028 $\text{M}^{-1}\cdot\text{cm}^{-1}$ in 20 mM-Tris/HCl buffer, pH 7.4, containing 0.5 M-L-arginine, 0.5 M-NaCl and 0.01 % Tween 80 at 25 °C. SK·Plg was titrated with 4-nitrophenyl 4'-guanidinobenzoate during complex-formation (Chase & Shaw, 1967; McClintock & Bell, 1971).

Plasminogen activation

The method was the same as that described previously (Fears *et al.*, 1985b) except that t-PA was diluted with the phosphate buffer described above. The concentration of activator added was 0.3 nM for t-PA and 0.1 nM for streptokinase. Kinetic constants were averages of quadruplicate determinations for a range of concentrations of Lys-plasminogen ($0.2\text{--}10 \times K_m$ for SK and $0.2\text{--}5 \times K_m$ for t-PA) and enhancing protein (0.01–0.5 μM).

In previous studies the assay had been found to be reproducible. For example, with t-PA the basal K_m was estimated to be $17.5 \pm 1.5 \mu\text{M}$ ($n = 5$), which decreased to $0.044 \pm 0.003 \mu\text{M}$ in the presence of a digested fibrin clot (Fears *et al.*, 1985b), and the basal K_m for streptokinase was $0.33 \pm 0.07 \mu\text{M}$ ($n = 5$), decreasing to $0.036 \pm 0.008 \mu\text{M}$.

Clot-lysis assay

Enzymes or acyl-enzymes were diluted from stock solutions into ice-cold portions (0.6 ml) of pooled citrated human plasma (fibrinogen content 7.3 μM) containing 6 kBq of ^{125}I -human fibrinogen/ml. For each enzyme concentration, groups of five deactivated micro-fibre-glass filter discs (1.0 cm, type GF/D; Whatman, Maidstone, Kent, U.K.) were treated with human thrombin (10 μl of 100 NIH units/ml in 0.05 M-sodium phosphate buffer, pH 7.4, containing 0.1 M-NaCl, 0.01 % Tween 80 and 10 mg of human serum albumin/ml) followed by plasma containing the agent (0.1 ml). The plasma was allowed to clot for 10 min at ambient temperature (20–24 °C) and the discs were then washed by gentle rotation in phosphate/albumin buffer (20 ml at 4 °C for 15 min). The discs were squeezed free of buffer with forceps and transferred to vials, and the ^{125}I content was determined by γ -radiation counting (Bioscint; Panax, Ruislip, Middx., U.K.). They were added to unlabelled unsupplemented human plasma (0.9 ml) and incubated at 37 °C for 1 h.

Discs were freed of adhering plasma by squeezing with forceps and the residual ^{125}I content was determined. With each set of determinations (three to five concentrations), a control group of five discs with no enzyme added to the labelled plasma was included.

Data processing

Lysis-rate data were fitted to a general form of the Langmuir Adsorption Isotherm {lysis rate = $V/(1 + [E]_{50\%} \cdot [\text{agent}])$ where $[E]_{50\%}$ is the concentration of agent giving a half-maximal lysis rate} by non-linear regression analysis on a Digital VAX 11/750 computer with RS/1 software (Bolt, Beranek and Newman, Cambridge, MA, U.S.A.).

RESULTS AND DISCUSSION

Plasminogen activation

For the activation of Lys-plasminogen by streptokinase (as the activator complex with plasminogen formed *in situ*), the decrease in K_m (fibrin enhancement) was proportional to the concentration of soluble fibrin present (Fig. 1a). V_{max} was unaffected and the requirement for fibrin disappeared when the substrate concentration was high. If the experimental data were replotted as the reciprocals of initial velocity and fibrin concentration (Fig. 1b), the fibrin dissociation constant could be determined (Table 1). Results from the double-reciprocal plots were consistent with a kinetic mechanism involving non-essential enzyme activation via a Rapid Equilibrium Ordered Bireactant sequence, and the competitive activation by fibrin is one means of distinguishing this mechanism from Rapid Equilibrium Random and Steady State Ordered reactions (Segal, 1975). Although K_m can be determined from the limiting plot in Fig. 1(a), it was more practical to calculate K_m from Fig. 2, which

Fig. 1. (a) Lineweaver–Burk plots for activation of Lys-plasminogen by streptokinase in the presence of various concentrations of soluble fibrin, and (b) double-reciprocal plots relating initial rates of activation to fibrin concentration

(a) Concentrations of soluble fibrin used were 0.02 μM (●), 0.05 μM (○), 0.1 μM (■) and 0.2 μM (△). The initial rates of activation at infinite concentration of fibrin (□) were obtained from the ordinate intercepts in (b). Inset: replot of slopes versus reciprocal fibrin concentration. (b) Concentrations of Lys-plasminogen used were 0.01 μM (●), 0.02 μM (■), 0.03 μM (△), 0.05 μM (○) and 0.2 μM (▲). The rate at infinite plasminogen concentration (□) was obtained from the ordinate intercept in (a). Inset: replot of slopes versus reciprocal substrate concentration.

Table 1. Kinetics of Lys-plasminogen activation by streptokinase and tissue plasminogen activator

Each experiment comprised quadruplicate assays for a range of concentrations of substrate and enhancing protein as described in the Materials and methods section.

Activator	Enhancing protein	Kinetic constant (nM)			
		From double-reciprocal plots		From plot of $K_{m(\text{app.})}$ versus reciprocal of enhancer concentration	
		K_m	K_t	K_m	K_t
SK·Plg	Soluble fibrin	30	91	27	125
	CNBr-cleavage fibrinogen fragments	43	11	45	16
t-PA	Soluble fibrin	50	145	30	154
	CNBr-cleavage fibrinogen fragments	20	—	24	57

also provided confirmation of the value for K_t (Table 1). Another method of deriving K_t is shown in Fig. 1(a) inset. This yielded a value of 125 nM (Table 1). Fig. 1(b) inset shows that the slope of the double-reciprocal plot (Fig. 1b) was a linear function, passing through the origin, of the reciprocal of the substrate concentration and confirmed that, when the latter was infinitely high, the slope of $1/[S]$ versus $1/[F]$ was zero. Thus the

reaction was ordered with fibrin adding before plasminogen substrate according to the following scheme:

where E represents enzyme, F represents fibrin and S represents plasminogen. A similar obligate sequence has been proposed for the fibrin enhancement of t-PA

Fig. 2. Dependence of apparent K_m on fibrin concentration

Experimental values for $K_{m(app.)}$ were obtained from Fig. 1(a).

activity (Hoylaerts *et al.*, 1982) and for the moderate stimulation of streptokinase activity by intact fibrinogen or fibrinogen fragment (Chibber *et al.*, 1985). We have shown previously (Fears *et al.*, 1985b) that streptokinase·plasmin(ogen) activation of plasminogen is much less responsive to fibrinogen than to fibrin.

The molecular events in the formation of plasminogen activator activity within mixtures of streptokinase and plasminogen comprise the following (McClintock & Bell, 1971; Brockway & Castellino, 1974):

It has been proposed (Chibber *et al.*, 1985) that there is more than one conformational arrangement of the streptokinase·plasminogen activator complex and that only an early species can be stimulated, at least by fibrinogen. It should be noted that, unless the active centre within the plasminogen moiety of the complex is inactivated by acylation, proteolytic cleavage within the streptokinase component occurs (Brockway & Castellino, 1974; Markus *et al.*, 1976). It is likely, at least initially, that the deacylated form of APSAC/BRL 26921 (which is prepared by acylative trapping of the nascent streptokinase·plasminogen complex) will be intact SK·Plg, responsive to fibrin stimulation.

The use of soluble fibrin in this and previous studies (Fears *et al.*, 1985b) permitted kinetic investigations that were not possible with solid-phase fibrin. However, the quantification and characterization of such preparations is laborious, the preparations cannot be stored for reasonable periods and it proved difficult to obtain reproducible products with different batches of fibrinogen and thrombin. The soluble cofactor, CNBr-digested fibrinogen, has also been found to be useful as a fibrin mimic in kinetic studies with t-PA (Zamarron *et al.*, 1984); we have also used this cofactor in the study of streptokinase.

The activation of Lys-plasminogen by SK·Plg was enhanced by the CNBr-cleavage fibrinogen fragment

Table 2. Clot-lysis potencies of fibrinolytic enzymes and their acylated precursors

Each potency value was determined from a concentration-response curve fitted to at least eight data points, each the mean of five clot-lysis determinations, as described in the Materials and methods section. Deacylation of u-PA and t-PA derivatives was performed in 0.05 M-sodium phosphate buffer, pH 7.4, containing 0.1 M-NaCl and 0.01 % Tween 80 at 37 °C. Deacylation of acyl-SK·Plg complexes was carried out in 0.1 M-Tris/HCl buffer, pH 7.4, containing 0.14 M-NaCl and 20 % (v/v) glycerol at 37 °C. Deacylation half-life values are means for at least two determinations.

Activator	Acyl group	Half-life for deacylation (min)	$[E]_{50\%}$ (nM)
u-PA	None	—	11.4
	Cyclohexylideneacetyl	40	19
	Cyclohex-1-enecarbonyl	56	20
	1-Methylcyclohexylidene-acetyl	81	31
	4-Aminobenzoyl	191	97
t-PA	4-Guanidinobenzoyl	274	105
	None	—	0.9
	4-Anisoyl	32	3.1
	PDAEB	63	4.6
	4- <i>NN</i> -Dimethylamino-benzoyl	240	11.3
SK·Plg	4-Aminobenzoyl	565	15.8
	None	—	2.0
	4-Anisoyl	39	7.1
	4-Guanidinobenzoyl	191	14.5
	4-Aminobenzoyl	1050	27.1

population used (see the Materials and methods section). K_m at infinite enhancer concentration was similar to that derived with soluble fibrin (Table 1, individual plots not shown). The reaction mechanism also appeared to be similar (plots not shown), but K_t was somewhat lower (Table 1). This apparent discrepancy in K_t between the two types of cofactor preparation may be an artefact: at nominally equivalent concentrations of protein, digestion with CNBr may uncover the greater number of binding sites. The generation of such binding sites in soluble fibrin may be inefficient both because of incomplete polymerization of fibrin monomers and because of overt precipitation of fibrin strands in which binding-site accessibility is restricted.

In agreement with previous results (Hoylaerts *et al.*, 1982; Zamarron *et al.*, 1984; Fears *et al.*, 1985b), the K_m of t-PA for plasminogen activation was markedly decreased by both soluble fibrin and by CNBr-cleavage fibrinogen fragments (Table 1). It was not possible to derive K_t for the CNBr-cleavage fragments by using double-reciprocal plots because of slight concomitant changes in V_{max} , but, in general, K_t for t-PA was rather higher than for SK·Plg (Table 1, individual plots not shown). The reaction mechanism was similar. The relative fibrin affinities implied by these K_t values were reflected in the magnitude of the second-order rate constants k_{cat}/K_m , measurements of the relative catalytic efficiencies of plasminogen activation that, in the presence of fibrin, were calculated to be $2030 \mu M^{-1} \cdot \text{min}^{-1}$ (SK·Plg) and $1690 \mu M^{-1} \cdot \text{min}^{-1}$ (t-PA). For comparison, high- M_r

urokinase has a very large K_t (Lijnen *et al.*, 1984) and a small second-order rate constant in the presence of fibrin: $70 \mu\text{M}^{-1} \cdot \text{min}^{-1}$ (R. Fears, unpublished work).

Clot lysis

The clot-lysis system used in this study consisted of a small (100 μl) human plasma clot induced by addition of citrated plasma containing ^{125}I -labelled human fibrinogen and the fibrinolytic enzyme to a filter-disc support impregnated with human thrombin. After clotting, serum and unbound enzyme were removed by washing at 4°C and the lysis rate was measured radiochemically during subsequent incubation at 37°C in citrated human plasma containing no fibrinolytic agent. By incorporating the fibrinolytic enzymes into forming fibrin, diffusion barriers to exogenous lytic agents were eliminated.

The dependence of lysis rate upon the concentrations of both unmodified enzymes and their acyl derivatives was found to be sigmoidal in form and could be fitted by computerized non-linear-regression analysis to the generalized form of the Langmuir Adsorption Isotherm (Van den Brink, 1977). Such analysis yielded values for the maximum lysis rate (V) and for the concentration of enzyme giving half-maximal lysis rate ($[E]_{50\%}$). The former was not correlated with the nature of the enzyme used. This finding is consistent with fibrinolysis by different types of plasminogen activator proceeding through a common intermediate (fibrin-bound plasmin), as in the following scheme:

where AE is an acyl-enzyme, k_d is the pseudo-first-order deacylation rate constant of the acyl-enzyme and the other terms have the meanings noted above.

The average value of the maximum lysis rate was found to be $64.0 \pm 0.28 \text{ \%}/\text{h}$ (mean $\pm$ S.E.M., $n = 15$), corresponding to a rate of release of ^{125}I -fibrin degradation products of $0.76 \text{ nM}/\text{s}$. (The initial fibrin concentration within the clots was approx. $4.3 \mu\text{M}$, as calculated from the fibrinogen content of the plasma and the proportion of ^{125}I -fibrinogen tracer taken up during clotting.)

Values of $[E]_{50\%}$ for the three unmodified enzymes and various acylated precursors are summarized in Table 2. $[E]_{50\%}$ is a measure of fibrinolytic potency in this assay and cannot be equated with the thermodynamic dissociation constant K_t , because the rapid processes of plasminogen binding, plasminogen activation on fibrin and fibrin breakdown prevent a true equilibrium between fibrin and the (acyl)-enzyme being established. Unmodified t-PA appeared to be slightly more potent than streptokinase, and both agents were significantly more active than two-chain high- M_r urokinase. Streptokinase was added to plasma in a form uncomplexed with plasminogen. In this case, therefore, the plasminogen activator species formed initially in plasma would be

native SK $\cdot$ [Glu¹]Plg complex, but degradation within the SK moiety to give a $37000\text{-}M_r$ fragment and conversion of the Glu-plasminogen into Lys-plasmin are known to occur very rapidly in plasma (Brockway & Castellino, 1974; Markus *et al.*, 1976). The active species throughout most of the period of fibrinolysis is likely to have been the streptokinase ($37000\text{-}M_r$)-Lys-plasmin complex. It was therefore of interest to examine the effect of stabilizing the structure of the native streptokinase-plasminogen complex by active-centre acylation. In addition, the use of inactive precursors of the fibrinolytic enzymes decreases the extent of proteolytic modification of fibrin before the lysis rate is measured and permits a better equilibration of plasminogen activator and fibrin. In order to make valid comparisons between the different activator classes, it was essential to use acyl-enzymes with equivalent deacylation rates. It was necessary to use slightly different conditions for determining the deacylation rates of acyl-enzymes derived from t-PA and u-PA on the one hand and SK $\cdot$ Plg on the other. The former were deacylated in a phosphate buffer (Table 2), but the deacylation products of acyl-plasminogen-streptokinase complexes were found to be unstable in this buffer system at 37°C . In order to obtain accurate deacylation rate constants for these complexes from activity-regeneration measurements, it was necessary to use a buffer containing 20% (v/v) glycerol. At this concentration, glycerol was found to increase the deacylation rate constants of t-PA and u-PA derivatives approximately 3-fold when compared with rates in phosphate buffer. Radiochemical determination of the deacylation of 4-anisoyl-plasminogen-streptokinase complex (BRL 26921) in human plasma with the use of a tritiated 4-anisoyl function confirmed that a correction factor of this magnitude needed to be applied to measurements in glycerol-containing buffer (M. J. Hibbs, unpublished work). Therefore the relevant stability of BRL 26921 lies between that of the heterobifunctional acyl-enzyme PDAEB-t-PA (half-life for deacylation 63 min) and that of 4-*NN*-dimethylaminobenzoyl-t-PA ($t_{1/2}$ 240 min) and is similar to that of 4-aminobenzoyl-u-PA ($t_{1/2}$ 140 min). 4-Guanidinobenzoyl-plasminogen-streptokinase was similar in stability to 4-aminobenzoyl-t-PA.

The above comparisons indicated (Table 2) that the potencies of acyl-plasminogen-streptokinase complexes were similar to those of acyl-t-PAs of comparable stability and that comparable derivatives of both enzymes were at least 5-fold more potent than derivatives of two-chain u-PA. The relationship between the potency ($[E]_{50\%}^{-1}$) and the deacylation rate constant (corrected for solvent effects) is shown in Fig. 3. It is noteworthy that fibrinolytic activity was demonstrable with very slowly deacylating derivatives of t-PA and SK $\cdot$ Plg but was much less marked with similar derivatives of u-PA. This phenomenon may be explained by high-affinity fibrin binding of the first two classes of plasminogen activator followed by sufficient deacylation during the period of incubation of the clot in plasma to generate a small amount of fibrin-bound active enzyme, which rapidly activated a much larger amount of fibrin-bound plasminogen. In the limit of slow deacylation, when equilibration between acyl-enzyme and fibrin is possible, the apparent $[E]_{50\%}$ values for acyl-t-PA and acyl-plasminogen-streptokinase complex were similar at about 20–30 nM. The limiting $[E]_{50\%}$ for acyl-u-PA could not be determined accurately but was probably greater than 500 nM. These values are

Fig. 3. Relationship of the reciprocal of the concentration giving half-maximal lysis rate to the pseudo-first-order deacylation rate constant for a series of acylated SK·Plg complexes (●) and acylated u-PA (△) or t-PA (○) derivatives

Deacylation rate constants and $[E]_{50\%}$ values were taken from Table 2.

consistent with the values of K_m for plasminogen in the presence of fibrin that were obtained from plasminogen activation kinetics.

The present study suggests that, provided that it is presented to fibrin in an undegraded form, streptokinase·plasminogen complex can display an affinity for fibrin that is not only significantly greater than that shown by plasminogen forms or two-chain urokinase but which is also comparable with that of t-PA. This conclusion contrasts with the widely held view that streptokinase·plasminogen does not bind specifically to fibrin (Collen & Verstraete, 1983; Fox *et al.*, 1984). That conclusion derived partly from the structural resemblance of the complex to the weakly binding plasminogen and partly from clinical experience with streptokinase itself. Our results suggest that, in agreement with the conclusions of Cederholm-Williams (1981), the binding of streptokinase to plasminogen confers substantial extra fibrin affinity upon the latter protein. The precise structural basis for an increase in binding energy to the level apparently similar to that provided by the 'kringle' and 'finger' domains of t-PA (Pennica *et al.*, 1983; Banyai *et al.*, 1983) is unknown. An interaction between the streptokinase moiety and fibrin-bound plasminogen or a streptokinase-induced conformation change in the plasminogen kringles are both plausible hypotheses.

In clinical use, streptokinase probably undergoes rapid internal proteolytic degradation (Markus *et al.*, 1976), and the activator complex is also inactivated and removed from the circulation by plasma proteinase inhibitors, particularly α_2 -macroglobulin (Gonias *et al.*, 1982). All of these processes may be delayed by the provision of the complex in an undegraded pro-drug form. The data presented here suggest that improved fibrin affinity contributes to the potency and hence to the emerging clinical usefulness of BRL 26921 (Been *et al.*, 1985).

We thank the staff of the Beecham Biological Development and Cell Products Units and the Thrombosis Project Molecular Biology Group for the provision of BRL 26921 and

recombinant t-PA. We are also grateful to Dr. R. Standing for preparing and characterizing the CNBr-cleavage fibrinogen fragments, to Dr. S. B. Kalindjian for enzyme acylating agents, to Dr A. J. Garman and Mr M. J. Hibbs for deacylation rate studies, to Mrs J. Martin for technical assistance, and to Dr H. Ferres for support and advice.

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