Kinetic analysis of the effects of glycosaminoglycans and lipoproteins on urokinase-mediated plasminogen activation

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The glycosaminoglycans (GAGs) heparin, heparan sulphate and chondroitin 6-sulphate stimulate the rate of urokinase activation of human plasminogen. Kinetic analysis of plasminogen activation demonstrates that heparin, heparan sulphate and chondroitin 6-sulphate increased the catalytic rate (K_{cat}) by 5.3-, 3.5- and 2.5-fold respectively. These stimulatory GAGs had no effect on the affinity of urokinase for plasminogen, since the K_m of the reaction is unaltered by the GAGs. The GAGs may enhance the rate of plasminogen activation through an interaction with the catalytic domain of the urokinase, with dissociation constants of approx. 30 nM. Additionally, the lipoproteins, lipoprotein (a) [Lp(a)] and low-density lipoprotein (LDL) inhibit heparin and heparan sulphate stimulation of plasmin formation. Lp(a) is a competitive inhibitor (K_i 20 nM) and LDL is a mixed inhibitor of heparin-enhanced urokinase-mediated plasminogen activation (K_{ic} 24 nM and K_{iu} 60 nM). These inhibition constants correlate with physiological concentrations of these lipoproteins. These data suggest that these GAGs and lipoproteins may play an important role *in vivo* in regulating urokinase-mediated plasmin formation.

INTRODUCTION

Plasmin is the principal enzyme of fibrinolysis. Like other coagulation and fibrinolytic pathway enzymes, plasmin is secreted as an inactive zymogen, plasminogen. The physiological activation of plasminogen is mediated by tissue-type plasminogen activator (t-PA) and urokinase (Collen, 1980; Castellino, 1981). Activation of the fibrinolytic system is regulated by surface components of both macrophage and endothelial cells (Hajjar *et al.*, 1987; Miles & Plow, 1987). Several other vascular components can also regulate plasminogen activation, including extracellular matrix (Silverstein *et al.*, 1985; Stack *et al.*, 1990), Cl^- ions (Urano *et al.*, 1987), α_2 -antiplasmin (Lijnen & Collen, 1986), plasminogen activator inhibitor-1 (Sprengers & Kluft, 1987), lipoprotein (a) [Lp(a)] (Edelberg *et al.*, 1990) and heparin (Markwardt & Klocking, 1977).

The glycosaminoglycan (GAG) heparin enhances the rate of plasmin formation catalysed by both t-PA and urokinase (Andrade-Gordon & Strickland, 1986; Paques *et al.*, 1986). Heparin is found on the vascular surface and basement membrane, where it exerts a well-characterized anticoagulant effect (Marcum & Rosenberg, 1984, 1985; Marcum *et al.*, 1986). In addition, Markwardt & Klocking (1977) proposed that this GAG may also increase fibrinolytic activity. Several recent studies suggest that heparin increases the rate of both t-PA- and urokinase-mediated plasmin formation (Andrade-Gordon & Strickland, 1986; Paques *et al.*, 1986). Further studies have demonstrated that the t-PA N-terminal 'finger' domain is involved in the heparin enhancement of plasminogen activation (Stein *et al.*, 1989; Edelberg & Pizzo, 1990).

Since urokinase does not contain a 'finger' domain, the heparin interaction with this enzyme must differ from the interaction between t-PA and heparin. In addition, Rydzewski *et al.* (1989) demonstrated that the GAG, chondroitin 6-sulphate, increased the rate of urokinase-catalysed plasmin formation, but had no effect on t-PA-mediated plasminogen activation (Andrade-Gordon & Strickland, 1986; Edelberg & Pizzo, 1990). This further suggests that interaction between urokinase and

GAGs is markedly different from the t-PA-heparin interaction. This study describes a kinetic analysis of the effects of heparin, heparan sulphate and chondroitin 6-sulphate on urokinase-mediated plasminogen activation. We have also determined the effect of two related lipoproteins, Lp(a) and low-density lipoprotein (LDL), on this GAG-enhanced urokinase-mediated plasmin formation.

MATERIALS AND METHODS

Reagents

The plasmin substrate D-Val-L-Leu-L-Lys *p*-nitroanilide dihydrochloride (VLK-NH-Np or S-2251) and the urokinase substrate D-Ile-L-Pro-L-Arg *p*-nitroanilide dihydrochloride (IPR-NH-Np or S-2288) were purchased from Helena Laboratories (Beaumont, TX, U.S.A.). All other reagents were of the best grade commercially available.

Glycosaminoglycans

GAGs obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) were: heparin, 155 i.u./ml, molecular mass 17 kDa; heparan sulphate, molecular mass 50 kDa, 10% (w/w) sulphation; chondroitin 4-sulphate, molecular mass 17 kDa; chondroitin 6-sulphate, molecular mass 32 kDa; dermatan sulphate, molecular mass 38 kDa; keratan sulphate, molecular mass 109 kDa. Molecular mass and sulphation data for these preparations were obtained from Sigma Technical Services. Unsulphated chondroitin and decarboxylated heparin, chondroitin 4-sulphate and chondroitin 6-sulphate were kindly given by Dr. Edward Conrad (Glycomed, Alameda, CA, U.S.A.).

Proteins

Human plasminogen glycoform 2 was isolated by affinity chromatography as previously described by Deutsch & Mertz (1970) and modified by Brockway & Castellino (1972) and Gonzalez-Gronow & Robbins (1984). High-molecular-mass two-chain urokinase and low-molecular-mass two-chain urokinase were purchased from Calbiochem (San Diego, CA, U.S.A.).

Abbreviations used: GAG, glycosaminoglycan; Lp(a), lipoprotein (a); LDL, low-density lipoprotein; t-PA, tissue-type plasminogen activator; VLK-NH-Np, D-Val-L-Leu-L-Lys *p*-nitroanilide; IPR-NH-Np, D-Ile-L-Pro-L-Arg *p*-nitroanilide.

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Human fibrinogen was obtained from Kabivitrum (Stockholm, Sweden) and rendered plasminogen-free on lysine-Sepharose. Lp(a) was purified as previously described by Edelberg *et al.* (1989). LDL was obtained from Sigma Chemical Co., and purity was confirmed by SDS/PAGE as previously described (Edelberg *et al.*, 1990).

Determination of heparin, heparan sulphate and chondroitin 6-sulphate kinetic activation constants

Plasminogen was activated with urokinase in a manner analogous to activation by t-PA (Edelberg *et al.*, 1990). Briefly, various concentrations of plasminogen were incubated in 50 mM-Tris/HCl buffer, pH 7.4, with 0.5 nM-urokinase in the presence of the plasmin substrate VLK-NH-Np (300 μ M) and increasing concentrations of heparin, heparan sulphate, dermatan sulphate, keratan sulphate, chondroitin, chondroitin 4-sulphate, chondroitin 6-sulphate or fibrinogen. Similar studies with the low-molecular-mass enzyme were also performed. All kinetic analyses were performed at least in triplicate. The initial rate of plasminogen activation (V_i) was determined by using the equation:

$$V_i = b(1 + K_E/S_0)/\epsilon k_e$$

where b is the initial velocity of substrate hydrolysis derived from a plot of instantaneous rate of substrate cleavage versus time, K_E is the apparent Michaelis constant of VLK-NH-Np hydrolysis by plasmin (0.3 mM), k_e is the catalytic rate constant for plasmin hydrolysis of VLK-NH-Np determined for fully activated plasminogen 2 (2.3×10^3 M/mol of plasmin per s) and ϵ is the molar absorption coefficient of the hydrolysed substrate at 405 nm (10^4 M $^{-1}$ ·cm $^{-1}$) (Erlanger *et al.*, 1961). The kinetic constants for plasminogen activation were determined by double-reciprocal plots of initial rates versus plasminogen concentration in the absence of any GAGs. In the presence of GAGs, the kinetic activation constants were determined by using the equation:

$$V_i = V_m ([S]/K_m + \beta[A][S]/\alpha K_m K_a) / (1 + [S]/K_m + [A]/\alpha K_m + [S][A]/\alpha K_m K_a)$$

α and β are the activation constants that modify K_m and k_{cat} , respectively and K_a is the dissociation constant of the activator for the enzyme (Dixon & Webb, 1979). From a double-reciprocal plot of initial rates of plasmin formation versus plasminogen concentration in the presence of increasing concentration of GAGs, the change (Δ) from control in both abscissa intercepts and slopes were determined for each concentration of GAG (Dixon & Webb, 1979). The constants α , β and K_a were calculated from a double-reciprocal plot of Δ intercept and slope values versus the GAG concentration. In addition, the effect of heparin on urokinase amidolytic activity was determined. Briefly, 0.5 nM-urokinase was incubated with IPR-NH-Np (300 μ M) in the presence of increasing heparin concentration, and the initial rate of substrate hydrolysis measured.

Determination of Lp(a) and LDL kinetic inhibition constants

Plasminogen was activated in a buffer containing 85 nM-heparin, as described above, in the presence of increasing concentrations of Lp(a) or LDL. All kinetic analyses were performed at least in triplicate. The initial rate of plasmin formation was determined as described above. The inhibition constants were determined by using the equation:

$$1/V_i = (K_m + [S])/V_m[S] + \{(K_m/K_{ic}) + ([S]/K_{iu})\}[I]/V_m[S]$$

in which K_{ic} and K_{iu} represent the competitive and uncompetitive inhibition constants respectively (Dixon, 1954; Knight, 1986). Similar experiments were also performed in the absence of any GAGs, and in the presence of heparin, chondroitin 6-sulphate and fibrinogen. In addition, the effect of lipoproteins on urokinase amidolytic activity was determined, as described previously for

t-PA activity (Edelberg *et al.*, 1990). Briefly, 0.5 nM-urokinase was incubated with IPR-NH-Np (300 μ M) in the presence of 85 nM-heparin and increasing concentrations of Lp(a) and LDL, and the initial rate of substrate hydrolysis was measured.

RESULTS

Determination of heparin and heparan sulphate kinetic activation constants

Activation of plasminogen by urokinase was enhanced in the presence of various GAGs (Figs. 1, 2 and 3). The kinetic analysis

Fig. 1. Kinetic analysis of the effect of heparin on plasmin generation

(a) Double-reciprocal plot of plasmin formation (10^{-11} s/mol of plasmin) versus plasminogen concentration (nm^{-1}). Increasing concentrations of plasminogen (40–100 nM) were activated with urokinase (0.5 nM) with increasing heparin concentrations [0 nM ($\square$), 2.5 nM (Δ), 5 nM ($\blacklozenge$), 8 nM ($\blacksquare$), 25 nM ($\diamond$), 100 nM ($\blacktriangle$) and 200 nM ($\square$)] in 50 mM-Tris/HCl buffer, pH 7.4, containing 300 μ M-VLK-NH-Np. Plasmin formation was calculated as described in the Materials and methods section. (b) Double-reciprocal plot of the change (Δ) from control in both vertical-axis intercept (10^{13} s/mol of plasmin) ($\square$) and slope (5×10^6 s/mol of plasmin per nM-plasminogen) ($\blacklozenge$) versus heparin concentration (nm^{-1}). The activation constants were calculated as described in the Materials and methods section.

Fig. 2. Kinetic analysis of the effect of heparan sulphate on plasmin formation

(a) Double-reciprocal plot of plasmin generation (10^{-11} s/mol of plasmin) versus plasminogen concentration (nM^{-1}). Increasing concentrations of plasminogen (40–100 nM) were activated with urokinase (0.5 nM) with increasing heparan sulphate concentrations [0 nM ($\square$), 2.5 nM ($\blacklozenge$), 5 nM ($\triangle$), 10 nM ($\diamond$), 25 nM ($\blacksquare$) and 100 nM ($\blacktriangle$)] in 50 mM-Tris/HCl buffer, pH 7.4, containing 300 μM -VLK-NH-Np. Plasmin formation was calculated as described in the Materials and methods section. (b) Double-reciprocal plot of the change (Δ) from control in both vertical-axis intercept (10^{13} s/mol of plasmin) ($\blacklozenge$) and slope (10^7 s/mol of plasmin per nM-plasminogen) ($\square$) versus heparan sulphate concentration (nM^{-1}). The activation constants were calculated as described in the Materials and methods section.

Fig. 3. Kinetic analysis of the effect of chondroitin 6-sulphate on plasmin formation

(a) Double-reciprocal plot of plasmin generation (10^{-11} s/mol of plasmin) versus plasminogen concentration (nM^{-1}). Increasing concentrations of plasminogen (40–100 nM) were activated with urokinase (0.5 nM) with increasing chondroitin 6-sulphate concentrations [0 nM ($\square$), 2.5 nM ($\blacklozenge$), 5 nM ($\triangle$), 10 nM ($\diamond$), 25 nM ($\blacksquare$), 50 nM ($\square$) and 100 nM ($\blacktriangle$)] in 50 mM-Tris/HCl buffer, pH 7.4, containing 300 μM -VLK-NH-Np. Plasmin formation was calculated as described in the Materials and methods section. (b) Double-reciprocal plot of the change (Δ) from control in both vertical-axis intercept (10^{13} s/mol of plasmin) ($\blacklozenge$) and slope (10^7 s/mol of plasmin per nM-plasminogen) ($\square$) versus chondroitin 6-sulphate concentration (nM^{-1}). The activation constants were calculated as described in the Materials and methods section.

of urokinase-mediated plasminogen activation in the absence of GAGs demonstrated a k_{cat} of 1.2 s^{-1} and a K_m of 60 nM, both of which are within the range of previously published results obtained under a variety of assay conditions (Lucas *et al.*, 1983; Watahiki *et al.*, 1987; Homanberg & Wait, 1990). The addition of heparin, heparan sulphate and chondroitin 6-sulphate to the reaction (Figs. 1a, 2a and 3a respectively) increased the rate of plasminogen activation by 5.3-, 3.5- and 2.5-fold respectively. The replots of the data (Figs. 1b, 2b and 3b) indicate that the dissociation constants (K_a) of the urokinase from the GAGs were

35 nM, 25 nM and 35 nM for heparin, heparan sulphate and chondroitin 6-sulphate respectively. Decarboxylation of the heparin and chondroitin 6-sulphate did not diminish the rate of urokinase-mediated plasmin generation (results not shown). Similar experiments with chondroitin, chondroitin 4-sulphate, dermatan sulphate, keratan sulphate and fibrinogen demonstrated no enhancement of plasminogen activation (results not shown). Experiments employing low-molecular-mass urokinase also demonstrated an increase in plasminogen activation rate with heparin and chondroitin 6-sulphate. Maximum

Fig. 4. Kinetic analysis of the effect of Lp(a) on heparin-enhanced plasmin formation

(a) Dixon plot of the reciprocal velocity of plasmin formation (10^{-11} s/mol of plasmin) versus Lp(a) concentration (nM). Fixed concentrations of plasminogen [40 nM ($\square$), 60 nM ($\blacklozenge$), 80 nM ($\blacksquare$) and 100 nM ($\diamond$)] were activated with urokinase (0.5 nM), with 85 nM-heparin in the presence of Lp(a) (0–40 nM), in 50 mM-Tris/HCl buffer, pH 7.4, containing 300 μM -VLK-NH-Np. The velocity was determined as described in the Materials and methods section. (b) Plot of Dixon slopes [10^{-18} s/mol of plasmin per nM-Lp(a)] (B) versus inverse plasminogen concentration (nM^{-1}). The inhibition constant, K_{ic} , was determined by using the Dixon equation described in the Materials and methods section.

increases were 10- and 3-fold respectively. Urokinase amidolytic activity was independent of the concentration of heparin present, consistent with previous observations (Andrade-Gordon & Strickland, 1986; Paques *et al.*, 1986; Rydzewski *et al.*, 1989). We have observed a similar result with t-PA (Edelberg & Pizzo, 1990).

Determination of Lp(a) and LDL kinetic inhibition constants

Plasminogen was activated by urokinase in the presence of 85 nM-heparin and increasing concentrations of Lp(a) or LDL,

Fig. 5. Kinetic analysis of the effect of LDL on heparin-enhanced plasmin formation

(a) Dixon plot of the reciprocal velocity of plasmin formation (10^{-11} s/mol of plasmin) versus LDL concentration (nM). Fixed concentrations of plasminogen [40 nM ($\square$), 60 nM ($\blacklozenge$), 80 nM ($\blacksquare$) and 100 nM ($\diamond$)] were activated with urokinase (0.5 nM), with 85 nM-heparin in the presence of LDL (0–40 nM), in 50 mM-Tris/HCl buffer, pH 7.4, containing 300 μM -VLK-NH-Np. The velocity was determined as described in the Materials and methods section. (b) Plot of Dixon slopes (10^{-18} s/mol of plasmin per nM-LDL) (B) versus inverse plasminogen concentration (nM^{-1}). The inhibition constants, K_{ic} and K_{iu} , were determined by using the Dixon equation as described in the Materials and methods section.

as described in the Materials and methods section. The addition of either lipoprotein inhibited the rate of urokinase-catalysed plasminogen activation (Figs. 4a and 5a). The Dixon plot in Fig. 4(a) demonstrates that increasing concentrations of Lp(a) inhibit plasmin formation. The replot of the data (Fig. 4b) demonstrates that Lp(a) is a competitive inhibitor with a K_{ic} of 20 nM. Fig. 5(a) demonstrates that LDL also decreased the rate of plasminogen activation. The replot (Fig. 5b) shows that LDL is a mixed inhibitor with a competitive inhibition constant (K_{ic}) of 24 nM and an uncompetitive inhibition constant (K_{iu}) of 64 nM. Similar results were obtained in the presence of heparan sulphate. In addition, experiments either in the absence of stimulatory GAG or in the presence of chondroitin 6-sulphate or fibrinogen resulted

in no inhibition by Lp(a) and LDL. The lipoproteins did not inhibit the amidolytic activity of urokinase, either in the presence or in the absence of stimulatory GAGs.

DISCUSSION

Plasminogen activation is an important component of several physiological and pathophysiological processes. In addition to fibrinolysis, plasmin is involved in tissue remodelling, ovulation, neuronal development, tumorigenesis and metastasis (Dano *et al.*, 1985). Whereas t-PA is active primarily in the vasculature, urokinase is active in both the vasculature and the extravascular space (Dano *et al.*, 1985). Regulation of plasmin formation by plasminogen activators is mediated by several proteins, including fibrin, fibrinogen (Hoyalerts *et al.*, 1982; Ranby *et al.*, 1982; Nieuwenhuizen *et al.*, 1983; De Serrano *et al.*, 1989), collagen, laminin (Stack *et al.*, 1990), thrombospondin, histidine-rich glycoprotein (Silverstein *et al.*, 1985), plasminogen activator inhibitor-1 (Sprengers & Kluft, 1987), α_2 -antiplasmin (Wiman & Collen, 1978) and Lp(a) (Edelberg *et al.*, 1990), as well as by several non-protein components including macrophage- and epithelial-cell binding sites (Hajjar *et al.*, 1987; Miles & Plow, 1987) and GAGs (Andrade-Gordon & Strickland, 1986; Paques *et al.*, 1986; Edelberg & Pizzo, 1990). The focus of recent work has been on the regulation of t-PA by these components, with less research directed towards understanding the regulation of urokinase.

Several recent studies have examined the role of heparin in the enhancement of plasminogen activation by both t-PA and urokinase. Heparin increases the rate of plasmin generation by t-PA and urokinase by 5–30-fold and 3–15-fold respectively (Andrade-Gordon & Strickland, 1986; Paques *et al.*, 1986; Fears, 1988; Rydzewski *et al.*, 1989; Edelberg & Pizzo, 1990). In addition, Fears (1988) reported a stimulation in the rate of urokinase-mediated Glu-plasminogen activation by heparin in the presence of fibrinogen. The above studies demonstrated that the heparin enhancement of t-PA function is due to an increase in the catalytic activity of the enzyme, but did not fully characterize the mechanism of the stimulation of urokinase activity.

Kinetic studies described here demonstrate that heparin stimulates urokinase-mediated plasminogen activation. Furthermore, these experiments demonstrate that heparan sulphate, which is structurally similar to, but less sulphated than, heparin, also increases the rate of plasminogen activation by urokinase. These results are similar to previously reported stimulation of t-PA-catalysed plasminogen activation by heparin and heparan sulphate (Edelberg & Pizzo, 1990). In contrast with t-PA, urokinase activation of plasminogen is also enhanced by chondroitin 6-sulphate, in good agreement with data of Rydzewski *et al.* (1989).

Though the GAG enhancement of both t-PA- and urokinase-mediated plasmin formation is due to an increase in the catalytic rate of the enzymes, the interaction of the GAGs may be markedly different with t-PA and urokinase. Experiments with t-PA, including deletion mutation (Stein *et al.*, 1989) and kinetic modelling studies (Edelberg & Pizzo, 1990), suggest that heparin enhances the catalytic rate of t-PA by binding to the 'finger' domain in the N-terminal region of the activator. The interaction of urokinase with heparin, heparan sulphate and chondroitin 6-sulphate must involve a different binding site, since urokinase lacks a 'finger' domain. In addition, the K_a of GAG binding to urokinase (approx. 30 nM) is 30-fold greater than that of the binding of heparin to t-PA.

Our data demonstrate that the stimulatory GAGs, heparin, heparan sulphate and chondroitin 6-sulphate, enhance the rate

of plasmin generation by increasing the k_{cat} of the urokinase, but they do not affect the K_m of the activation reaction. Furthermore, the activation constants (K_a) of these GAGs for urokinase are similar (25 nM compared with 35 nM), suggesting that the urokinase activity may be enhanced by a common structural feature of the three stimulatory GAGs. These GAGs share two structural similarities, a carboxy group and a sulphate group, at the 6-positions in the altering residues in the disaccharides. GAGs lacking either of these structures (e.g. chondroitin 4-sulphate, dermatan sulphate or keratan sulphate) do not enhance the urokinase-mediated plasminogen activation. However, decarboxylation of the heparin and chondroitin 6-sulphate did not affect the rate of plasmin generation, indicating that the 6-position sulphate group may be critical.

To probe the mechanism of GAG interaction with urokinase, we determined the effect of GAGs on the low-molecular-mass form of the enzyme, which contains only the catalytic domain. Both heparin and chondroitin 6-sulphate increased the rate of plasminogen activation mediated by this enzyme. These results are consistent with studies by Soeda *et al.* (1987, 1989) that suggest that heparin interacts with the isolated catalytic domain of several fibrinolytic serine proteinases. They demonstrated that heparin binds to the catalytic domain of t-PA and stimulates the activity of the proteinase by 3-fold, as compared with the 5–25-fold stimulation measured with the intact enzyme (Andrade-Gordon & Strickland, 1986; Edelberg & Pizzo, 1990). However, Soeda *et al.* (1989) demonstrated that heparin binds to the plasminogen catalytic domain with higher affinity than it binds to native Glu- or Lys-plasminogen. The interaction between GAGs and serine proteinases extends to other proteinases that have only catalytic domains. Marossy (1981) demonstrated that heparin, heparan sulphate and chondroitin 6-sulphate had strong ionic interactions with chymotrypsin and elastase-like enzymes.

The heparin enhancement of urokinase-mediated plasminogen activation may be involved in several physiological and pathophysiological processes. For example, urokinase secreted from endothelial cells (Kjaeldgaard *et al.*, 1986) may bind heparin and heparan sulphate on the endothelial surface (Marcum & Rosenberg, 1984, 1985; Marcum *et al.*, 1986), thereby increasing the local concentration of the activator, as well as enhancing the catalytic rate of plasminogen activation. In addition, heparin produced by embryonic fibroblasts (Woods *et al.*, 1985) may enhance plasminogen activation to stimulate tissue remodelling (Strickland *et al.*, 1976).

Plasmin generation and urokinase synthesis correlate with cell migration and tissue remodelling in ovulation (Beers, 1975; Wang & Leung, 1983; Canipari & Strickland, 1985). Strickland & Beers (1976) proposed that the plasminogen activators are secreted into the follicular fluid, where they convert plasminogen into plasmin, thus initiating the sequence of events that results in follicular rupture. Both heparin and chondroitin 6-sulphate are secreted into ovarian follicles (Mueller *et al.*, 1978; Gebauer *et al.*, 1978; Ax & Ryan, 1979; Yanagishita *et al.*, 1979; Grimek & Ax, 1982). Thus the stimulatory GAGs may regulate the activity of the urokinase in the developing oocytes and thereby regulate the rupture of follicles.

The activation of plasminogen also occurs during cell migration and tissue remodelling which accompany tumorigenesis. Several solid tumours secrete urokinase (Astedt & Holmberg, 1976; Markus *et al.*, 1980; Camiolo *et al.*, 1981; Evers *et al.*, 1982). In addition, several tumour cells produce heparin and heparan sulphate (Hassell *et al.*, 1985; Furwara *et al.*, 1984; Bouziges *et al.*, 1990), suggesting that the production of GAGs may increase the ability of these tumours to metastasize (Reich *et al.*, 1988).

The heparin and heparan sulphate enhancement of urokinase-

mediated plasminogen activation is regulated by lipoproteins in a manner similar to the regulation of heparin-enhanced t-PA-mediated plasminogen activation. Lp(a) contains an apoprotein (a) subunit that has a high degree of sequence similarity to plasminogen. Previous studies demonstrated that Lp(a) acts as a plasminogen analogue by competing for plasminogen-binding sites on cell surfaces, fibrin and heparin (Gonzales-Gronow *et al.*, 1989; Miles *et al.*, 1989; Hajjar *et al.*, 1989; Harpel *et al.*, 1989; Edelberg *et al.*, 1990; Edelberg & Pizzo, 1990). Lp(a) inhibits plasminogen activation by t-PA, but only in the presence of fibrinogen and heparin. In the studies described here, Lp(a) inhibits plasminogen activation by urokinase only in the presence of heparin or heparan sulphate, but not in the presence of chondroitin 6-sulphate or fibrinogen, or in the absence of an enhancer. Lp(a) inhibits by competing with plasminogen for access to the enhancer-bound urokinase, but does not occupy the active site directly. As a competitive inhibitor, Lp(a) may block the access of plasminogen to the heparin- and heparan sulphate-bound urokinase, without competing with urokinase for its binding site on the heparin or heparan sulphate, which kinetically would indicate an uncompetitive inhibitor. This suggests that Lp(a) binds to the heparin and heparan sulphate at a site other than where the urokinase binds. The Lp(a) inhibition constant of 20 nM correlates with a physiological plasma concentration of 8 mg/dl (Rhoads *et al.*, 1986; Dahlen *et al.*, 1986; Murai *et al.*, 1986), suggesting that Lp(a) may regulate the production of plasmin by heparin-enhanced urokinase in the plasma during fibrinolysis.

Our data also indicate that LDL is a mixed inhibitor of urokinase-mediated plasminogen activation, but only in the presence of heparin and heparan sulphate. Similarly to the inhibition observed with Lp(a), LDL blocks the access of plasminogen to the GAG-enhanced urokinase, but also decreases the catalytic rate of the enzyme. This suggests that LDL may bind to a site other than that where urokinase binds to the heparin and may block access of plasminogen to urokinase, as well as to the site at which urokinase binds to the heparin. The resultant effect is to decrease the enhancement in the catalytic rate of the activator. Like Lp(a), the LDL inhibition constants of 24 nM and 64 nM correlate with concentrations below the normal plasma levels of this lipoprotein (9 and 24 mg/dl respectively) (Rhoads *et al.*, 1986). This suggests that both Lp(a) and LDL may be regulators of heparin-enhanced urokinase-mediated fibrinolysis.

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