Functional Analysis of the Cellular Receptor for Urokinase in Plasminogen Activation

RECEPTOR BINDING HAS NO INFLUENCE ON THE ZYMOGENIC NATURE OF PRO-UKINASE*

(Received for publication, November 27, 1995, and in revised form, February 20, 1996)

Vincent Ellis†
From the Thrombosis Research Institute, Manresa Rd., London SW3 6LR, United Kingdom

Plasminogen activation catalyzed by the urokinase-type plasminogen activator (uPA) constitutes a reciprocalzymogen activation system, as plasmin can efficiently activate pro-uPA, the single-chain zymogenic form of the protease. We have previously shown that the overall efficiency of this plasminogen activation system is greatly enhanced by its assembly on the cell surface, involving binding of pro-uPA to its cellular binding site uPAR, and the concurrent cellular binding of plasminogen. We have now studied the effect of a recombinant soluble form of uPAR (residues 1–277) on the proteolytic reactions of this system. In contrast to the increased efficiencies of plasminogen activation and pro-uPA activation observed with cell-surface uPAR, soluble uPAR had an inhibitory effect on both of these individual reactions. Soluble uPAR also caused no increase in the low, but discernible, intrinsic activity of pro-uPA. Consistent with the observations on the isolated reactions, the overall activity of the pro-uPA-mediated plasminogen activation system was significantly inhibited. These observations confirm the previous interpretation of the observations made with cell-surface uPAR that the mechanism of the enhanced plasmin generation is due to the catalytically favorable interaction of uPAR-bound uPA/pro-uPA with cell-bound plasminogen/plasmin, rather than direct effects on the properties of uPA or pro-uPA on binding to uPAR.

Serine protease zymogens possess weak but intrinsic enzymatic activity generally several orders of magnitude less than that of the proteolytically activated enzyme (1). This activity was first demonstrated in studies of the stoichiometric reaction of active site-directed inhibitors such as DFP with chymotrypsinogen and trypsinogen to form stable covalent adducts but at reaction rates $10^{-5}$ to $10^{-3}$ times slower than those of the enzyme (2). The plasminogen activator zymogen pro-uPA is no exception, generally being regarded as a relatively inactive zymogen which has some degree of intrinsic activity against both plasminogen and low molecular weight peptide substrates (3–9) and has recently been demonstrated to react slowly with DFP (10, 11). However, quantifying the exact magnitude of this intrinsic activity has been complicated by the extreme sensitivity of pro-uPA to proteolytic activation. This activation to two-chain protease by limited proteolysis of the Lys158-Ile159 bond can be catalyzed by both trypsin-like serine proteases (12–14), including plasmin (3, 4), and also certain non-serine proteases (15–17). The action of plasmin results in a reciprocal zymogen activation system and a consequently amplified generation of plasmin (5, 18).

A variety of approaches has been taken in attempting to overcome the effect of feedback activation by plasmin. These include assay of plasminogen activation in the presence of plasmin substrates at high concentrations to act as competitive inhibitors of plasmin activity, various assays of plasminogen activation in the presence of the plasmin inhibitor aprotinin, and the use of recombinant pro-uPA mutated at the P$_{1}$ site Lys$_{158}$. Although having varying degrees of efficiency in overcoming this problem none of these approaches, including the Lys$_{158}$ mutants, exclude the involvement of two-chain uPA generated by trace amounts of proteases other than plasmin. Such “exogenous” proteolytic activity is extremely difficult to eradicate and may arise from a number of sources, including the pro-uPA preparation itself (19). It should be noted that the technically difficult problem of obtaining “virgin” zymogen and the determination of its activity is also encountered with other serine proteases, e.g. coagulation factor VII (20).

We have shown that binding of pro-uPA to its specific high-affinity cell surface receptor uPAR, in addition to providing a facile mechanism for confining uPA to the cell surface (21), greatly enhances plasmin generation (22). This is accomplished by increased efficiencies of both plasminogen activation and the reciprocal activation of pro-uPA by plasmin (22, 23). We have shown that the fundamental mechanism responsible for these effects is the specific approximation of the reactants on the cell surface, rather than the interaction of pro-uPA/uPAR per se. (i) They are dependent on the concurrent binding of plasminogen/plasmin to the cells (22, 23). (ii) A recombinant chimeric mutant of pro-uPA directly anchored to the cell surface via a glycolipid moiety activates plasminogen with overall characteristics corresponding to those of the uPAR-bound system and with comparable kinetics (24, 25). (iii) An anti-uPA monoclonal antibody, by forming a ternary complex between antibody, pro-uPA/uPAR, and plasminogen/plasmin, potentiates plasminogen activation by mimicking the enzyme kinetic effects that occur on assembly of the uPAR-bound system (10). Accordingly, both the antibody and the cell surface act as specific templates for the catalytically favorable assembly of the protease/zymogen components, a mechanism that is also fundamental to the various cell-associated reactions of the blood coagulation cascade (26).

We have also shown by kinetic simulation, based on the data obtained using the anti-uPA...
monoclonal antibody, that the intrinsic activity of pro-uPA is sufficient to efficiently initiate plasminogen activation when the zymogen components are assembled on a suitable template (10). Therefore, although we have previously speculated that the intrinsic activity of pro-uPA may be modulated by binding to accessory components (21), there being precedents for such effects, e.g. the induction of an active site in plasminogen upon binding to the bacterial protein streptokinase (27), it is not necessary to invoke such an induction mechanism to rationalize the initiation process.

Two recent reports have, however, suggested that the interaction between pro-uPA and uPAR, both in the plasma membrane bound (11) and soluble recombinant forms (28), leads to a direct increase in the intrinsic enzymatic activity of pro-uPA, i.e. in the absence of cleavage at the activation site, and that this is responsible for the initiation of uPAR-dependent plasmin generation. Due to the large discrepancy in the interpretation of these and our own data, we have directly studied the effect of soluble recombinant uPAR on the various protodetical reactions of this reciprocal zymogen activation system. Consistent with the interpretation of our previous observations, we find that uPAR causes no direct potentiation of any of these reactions.

EXPERIMENTAL PROCEDURES

Proteins and Reagents—Human uPA, pro-uPA, Glu-plasminogen, plasmin, and PAI-1 were all as described previously (23, 29). Soluble, truncated uPA (residues 1–277) was purified by immunoaffinity chromatography of the conditioned media from transfected Chinese hamster ovary cells as described (30) and was the kind gift of Dr. Michael Ploug (Finsen Laboratory, Copenhagen). The integrity of the ligand binding sites of s-uPAR, pro-uPA, and uPA was confirmed by competitive binding assay as described (30), giving apparent equilibrium binding constants coinciding with those previously determined. Porcine pancreatic kallikrein and Tween 80 were obtained from Sigma. The plasmin-specific chromogenic substrate H-Val-Leu-Lys-NA (S-2251) was obtained from Chromogenix AB, Mölndal, Sweden, and the plasmin- and uPA-specific fluorogenic substrates H-Val-Leu-Lys-AMC and glutaryl-Gly-Arg-AMC were from Bachem AG, Bubendorf, Switzerland.

Chromogenic Assays for Plasminogen Activation—Pro-uPA and s-uPAR at concentrations shown in the figure legends, were mixed and incubated for 5 min in a volume of 180 μl in the wells of standard uncoated polystyrene 96-well microtiter plates (Falcon) prior to addition of 20 μl of a solution containing H-Val-Leu-Lys-NA (to a final concentration of 0.45 mM) and varying concentrations of Glu-plasminogen. Substrate hydrolysis was followed at 405 nm in a Molecular Devices Thermomax plate reader at 60-s intervals. All assays were performed at 37 °C, and solutions were equilibrated at this temperature before use. Unless otherwise stated, assays and dilutions were made in 0.01 M sodium phosphate, pH 7.4, 2.7 mM KCl, 0.137 M NaCl containing 0.01% Tween 80 (PBS/Tween). In experiments where ionic strength was varied, 0.05 and 0.3 mM NaCl were substituted in this buffer.

Plasmin generation was determined by dAbs over each 60-s measuring interval and converted to plasmin by reference to a linear standard curve made with active site-titrated plasmin. In assays where substantial amounts of plasmin were generated, analysis was only made of measurements with an A570 of less than 0.200 to eliminate artifacts due to reduced substrate concentration (less than 5% substrate conversion).

When the effect of solid-phase immobilization of the components was determined, this was achieved by incubating either pro-uPA or uPA alone or in equimolar mixtures with s-uPAR in 96-well microtiter plates for 2 h at 37 °C in the absence of TWEEN 80. The wells were then washed 6 times in PBS/Tween and plasmin activation measured as described above.

Fluorogenic Assays for Plasminogen Activation—These were performed essentially using the conditions described for the chromogenic assay, with the exception that the total reaction volume was adjusted to 850 μl, and the measurements were made in polyvinylene semi-microfluorimeter cuvettes. Fluorescence intensity was measured in a Perkin-Elmer LS-5B spectrophuorimeter using excitation and emission wavelengths of 370 and 470 nm, respectively, with 5-nm slit widths.

Assay of Pro-uPA Activation—Pro-uPA (100 nM) in the presence or absence of a varying molar excess of s-uPAR (up to 4-fold) was incubated with either plasmin (1–100 μM) or kallikrein (100 μM) and the uPA-specific fluorogenic substrate glutaryl-Gly-Arg-AMC (0.5 mM) or Glu-plasminogen (9 μM) in PBS/Tween at 37 °C. Fluorescence intensity was monitored continuously and dF/dt converted to [uPA] by reference to standard curves. At 50 min, aprotinin (33 μg/ml) was added to each incubation and measurement of the generation of uPA activity continued (solid symbols). This concentration of aprotinin was shown to have no effect on uPA activity in a control experiment. The inset shows the rate of generation of uPA activity (expressed as pmn−1) plotted against s-uPAR concentration in the presence (●) and absence (○) of aprotinin.

RESULTS

Effect of s-uPAR on uPA/Pro-uPA Amidoactive Activity—Complex formation between uPA and s-uPAR was found to have no effect on the activity of the protease against the low molecular weight fluorogenic substrate glutaryl-Gly-Arg-AMC. Similarly formation of the pro-uPA-s-uPAR complex using equimolar amounts of the proteins (2 nM)2 caused no detectable change in the low level of intrinsic activity of the zymogen, which was 0.03% that of two-chain uPA. However, in the presence of an increasing molar excess of s-uPAR a time-dependent increase in amidoactive activity was observed (Fig. 1). This increase could be completely abolished by low concentrations of aprotinin, demonstrating its origin as protease impurity in the s-uPAR preparation rather than a consequence of complex formation. Estimated on the basis of an activity equivalent to that of plasmin, this impurity represents approximately 8 pm protease activity in the stock s-uPAR preparation (40 μM) or 0.02 × 10−3%.

2 Under these conditions greater than 90% of the pro-uPA will be complexed, calculated from experimentally determined equilibrium binding constants.
contaminating pro-uPA activating activity is only detected due to the sensitivity of the fluorogenic assay used, and it can be calculated that with equimolar concentrations of pro-uPA and s-uPAR (2 nM) and determination of the rate of plasmin generation using the fluorogenic peptide substrate H-o-Val-Leu-Lys-AMC (0.2 mM) and expressed here as the rate of change in arbitrary fluorescence units. Mean values and S.D. of 3 separate experiments are shown. The $K_m$ for plasminogen both in the presence and absence of s-uPAR was calculated to be 20 nM. It should be noted that we have previously shown the $K_m$ for the uPA:s-uPAR interaction to be 0.1 nM (30) and that under these conditions the majority of uPA will be complexed.

It has previously been reported that pro-uPA is able to specifically incorporate radiolabeled DFP (10, 11), reflecting the partial availability of the active site. Complex formation with s-uPAR caused no increase in the incorporation of $[^{35}C]DFP$ into pro-uPA, detected by fluorography of reduced SDS gels, although an increased incorporation into the B-chain of two-chain uPA was detected reflecting the proteolytic activation of pro-uPA could contribute significantly to the initiation of plasminogen activation.

Effect of s-uPAR on the Proteolytic Activation of Pro-uPA—Consistent with observations that we have previously made with glycolipid-anchored uPAR purified from phorbol 12-myristate 13-acetate-stimulated U937 cells (23), s-uPAR was found to cause a slight inhibition (15%) of uPA-catalyzed plasminogen activation. As shown in Fig. 2, this was due to a decrease in the $k_{cat}$ for plasminogen activation rather than an effect on $K_m$. This is possibly due to a slight steric interference with plasminogen binding to the uPA:s-uPAR complex. A similar degree of inhibition was observed in the presence of varying concentrations of 6-AHA (up to 10 mM), suggesting that it was not due to a lysine binding-site-dependent interaction between plasminogen and s-uPAR (or uPA).

Effect of s-uPAR on the Proteolytic Activation of Pro-uPA—The effect of complex formation on the activation of pro-uPA by plasmin was determined over a range of concentrations of both complex and plasmin. Under all conditions the pro-uPA:s-uPAR complex was activated at approximately 50% of the rate of uncomplexed pro-uPA (Table I). This protection from activation was not limited to the action of plasmin as pro-uPA activation by kallikrein was similarly affected. These effects were titratable with respect to s-uPAR concentration, demonstrating that this inhibition was dependent on complex formation rather than the presence of an inhibitory activity in the s-uPAR preparation.

Effect of Solid-phase Immobilization on Plasminogen Activation—Previous studies of the effect of s-uPAR on the two principal reactions in the reciprocal zymogen activation system consisting of pro-uPA and plasminogen described above would suggest that s-uPAR would have an overall inhibitory effect on plasmin generation. Fig. 3 shows this to be the case, at varying concentrations of plasminogen (panels A and B versus C and D) and with or without 60-min preincubation of pro-uPA and s-uPAR (panels A and C versus B and D).

Fitting the data in the absence of s-uPAR to a kinetic model showed that these data are consistent with the pro-uPA intrinsic activity determined by amidolytic assay and the known kinetic constants for both plasminogen and pro-uPA activation (10). In the presence of s-uPAR this was also the case with the exception of the pro-uPA activation kinetics which were approximately 7-fold slower than those in the absence of s-uPAR, compared with the 2-fold determined directly. The cause of this effect is not known, but it persisted under a variety of reaction conditions, e.g. varying ionic strength and in the presence of 6-AHA. However the overall inhibitory effect was a direct result of complex formation, as increased molar ratios of s-uPAR had no further effect and the inhibition was abolished in the presence of the peptide uPAR binding-antagonist AEPMPHS-LNF SQLYWYT (31).

To determine whether despite the overall inhibition of plasminogen activation by s-uPAR there might be an initial enhancing effect which is concealed by the rapid amplification of plasmin generation in the absence of s-uPAR, these experiments were repeated using a fluorogenic substrate for plasmin giving a greater than 100-fold increase in sensitivity and thus allowing the earliest events of plasminogen activation to be observed (Fig. 4). These data show that at early time points, when plasmin generation is undetectable in the chromogenic assay, plasmin is easily detected using this assay system, but nevertheless at no time point does its generation in the presence of s-uPAR exceed that in its absence. This effect was maintained over a wide range of plasminogen concentrations, with preincubation of the complex, and also at varying ionic strengths and in the presence of 6-AHA.

Effect of s-uPAR on protelytic activation of pro-uPA

Pro-uPA (100 nM) was incubated with either plasmin (10 pM) or kallikrein (100 pM). Pro-uPA generation was measured continuously with the fluorogenic substrate glutaryl-Gly-Arg-AMC. Data shown are the mean ± S.D. of triplicate experiments. The activation rates relative to those in the absence of s-uPAR are shown in brackets. Pro-uPA generation was undetectable in the absence of added protease under these experimental conditions. ND, not determined.

| Molar ratio, s-uPAR:pro-uPA | Pro-uPA activation |
|---------------------------|--------------------|
|                           | Plasmin $^a$       | Kallikrein         |
| s-uPAR                    | 7.81 ± 0.30 (100%) | 12.0 ± 0.25 (100%) |
| 1:1                       | 3.91 ± 0.22 (50%)  | 6.09 ± 0.32 (56%)  |
| 2:1                       | 3.87 ± 0.15 (49%)  | 6.34 ± 0.31 (53%)  |
| 4:1                       | 4.01 ± 0.21 (51%)  | ND                 |

$^a$ Similar degrees of inhibition were also observed using 1 and 100 pM plasmin.
increasing surface density of adsorbed protein generally leads to less denaturing conditions for the molecules subsequently bound (32). To establish whether such effects were involved in the contradictory conclusions of this and the previous study, varying concentrations of either pro-uPA or pro-uPA-s-uPAR complex were coated onto the surface of plastic microtiter plates, extensively washed, and plasminogen activation determined. At high coating concentrations (40-4000 nM), the characteristic of plasmin generation by pro-uPA were essentially the same as seen under solution phase conditions, i.e. a lag phase in plasmin generation which was prolonged in the presence of s-uPAR (Fig. 5A). Therefore also under these conditions no enhancement of plasminogen activation by s-uPAR was evident.

At the lowest coating concentrations at which activity was detectable (4 nM), plasminogen activation was faster in the presence of s-uPAR (Fig. 5B). However, when the experiments were repeated with uPA instead of pro-uPA, the same phenomenon was observed (Fig. 5D); plasmin generation being faster in the presence of s-uPAR compared with the reduction seen both at the higher concentration (Fig. 5C) and in the solution phase. The apparent enhancement is therefore not specific for pro-uPA, the most likely cause being preferential adsorption or less denaturation of the complexes compared with free uPA/pro-uPA at these low concentrations where the higher protein mass of the complexes may be significant.

Inhibition of Complexes by PAI-1—We have previously shown that uPA bound to uPAR on U937 cells is efficiently inhibited by PAI-1 with an association rate constant 57% that for free uPA (4.5 × 10⁸ M⁻¹ s⁻¹ compared with 7.9 × 10⁷ M⁻¹ s⁻¹) (29). As shown in Fig. 6 (upper panel) formation of the uPA-s-uPAR complex has a similar effect on uPA inhibition. Therefore, the steric hindrance that was speculated to be responsible for the effect of cell-associated uPAR is duplicated by s-uPAR, suggesting that the effects of s-uPAR observed here on plasminogen activation and pro-uPA activation are also relevant to the cell-bound system. Due to the high concentration of pro-uPA needed to detect its intrinsic activity, it was not possible to kinetically analyze its inhibition by PAI-1. Despite this lack of quantitation, PAI-1 was observed to inhibit the activity of pro-uPA both in the presence and absence of s-uPAR (Fig. 6, lower panel). The relatively high level of residual activity in this experiment was not due to a proportion of pro-uPA being refractory to inhibition, as the residual activity was progressively reduced with increasing concentrations of PAI-1. Rather it is interpreted as...
an approximately 200-fold decreased reciprocal activation of pro-uPA, with the former being due to increased efficiencies of both plasminogen activation and the large overall enhancement in the generation of plasmin (22).

These effects are all dependent on the concurrent cellular binding of plasminogen/plasmin, and therefore we have inferred that they are not a result of uPAR binding per se, but due to the catalytically favorable assembly of the protease/zymogen components on the appropriate template. This is consistent with the known domain structure of uPA, as NMR studies have shown that each of the domains of uPA has a high degree of dynamic independence and with no interdomain contacts (34, 35). Therefore, binding of uPA, via its NH₂-terminal epidermal growth factor-like module, would not be expected to influence the function of the COOH-terminal catalytic domain of the enzyme.\(^3\)

Furthermore uPAR has been shown to be a dispensable component in this system as direct anchorage of uPA to the cell membrane via a recombinantly introduced COOH-terminal glycolipid anchor leads to plasmin generation with kinetic characteristics comparable with those of the uPAR-bound system (24, 25).

The data here support the contention that uPAR is not directly involved in the potentiation either of plasminogen activation or pro-uPA activation and that it does not alter the zymogenicity of pro-uPA, as the very initial phase of plasminogen activation when the reaction is catalyzed by the low, but finite, intrinsic activity of pro-uPA is also unaffected. Rather the effects observed on each of these reactions are of an inhibitory nature, leading to a quite marked overall inhibition of the reciprocal zymogen activation system in the presence of s-uPAR. Although it may seem that these inhibitory effects are at variance with the independence of the catalytic domain of uPA discussed above, they may easily be explained by a small degree of steric hindrance to the binding of macromolecular ligands, i.e. plasmin or plasminogen, to the uPA/pro-uPA-s-uPAR complex.\(^4\) Therefore the cell surface assembly of this system not only potentiates these reactions but also overcomes the inherent inhibitory effects displayed in the purified system. This may be due to purely kinetic effects or the intermolecular constraints leading to the steric hindrance being relieved on the cell surface by a somewhat different orientational relationship between uPA and uPAR.

Although the conclusions that can be drawn from the data presented here are completely in accord with those from our previous studies on cell-associated uPAR, they are in contradiction to those of Higazi et al. (28). These investigators observed that s-uPAR was able to enhance both the amidolytic and plasminogen activator activity of pro-uPA but not those of fully active two-chain protease. The effect on plasminogen activation was particularly dramatic, with a 850-fold increase in \(k_{cat}/K_m\). Such large discrepancies are clearly not easy to rationalize, particularly when the inhibitory effects that we observe were maintained over a wide range of experimental conditions. However, as pro-uPA is extremely susceptible to proteolytic activation and a relatively promiscuous substrate with respect to the range of activating proteases, we contend that nonspecific proteolytic activation of pro-uPA is responsible for the s-uPAR-mediated induction in activity observed by these investigators. The s-uPAR preparation used in the present study was shown to contain such a proteolytic activity, which although present in exceedingly low amounts would be sufficient to cause significant activation under certain experimental conditions, such as the prolonged preincubations used in the former study. The role of protein adsorption in the former study also cannot be ignored, as the experiments were performed in the absence of surfactants or carrier protein. As demonstrated here deliberate immobilization of relatively high concentrations of pro-uPA or pro-uPA-s-uPAR onto microtiter wells results in a kinetically enhanced plasmin generation compared with the solution phase reactions, and at low protein concentrations complexes were preferentially adsorbed compared with uncomplexed uPA/pro-uPA. These effects may augment the effects of direct proteolytic activation, such that s-uPAR would appear to markedly enhance the initial phase of plasmin generation. In these circumstances efforts to suppress the activation of pro-uPA by generated plasmin (by using high concentrations of peptide substrate as a competitive inhibitor) will not be effective in preventing this exogenous activation of pro-uPA.\(^5\) Therefore we can only conclude that the observations of Higazi et al. (28) are due to proteolytic activation of pro-uPA.

\[^3\]As the overall topology of neither the complex nor uPAR is known, it cannot be explicitly excluded that interactions with the catalytic domain of uPA occur, although such interactions have not been demonstrated.

\[^4\]It should be noted that the interaction of the complex with peptide substrates for uPA was unaltered.

\[^5\]It should be noted that substitution with the substrate H-o-norleucyl-hexahydrotryosyl-lysine-pNA, as used in the study of Higazi et al. (28) and which has an approximately 10-fold lower \(K_m\) for plasmin than the substrates used in the present study, had no effect on the degree of the inhibitory effect of s-uPAR.
exacerbated by the experimental conditions.

The significance of the issues addressed here is primarily in relation to the initiation of uPAR-mediated plasminogen activation. The physiological mechanism of this initiation remains unknown, but it may be speculated that, despite the abundance of proteases able to activate pro-uPA, it involves the intrinsic activity of pro-uPA. The data presented here demonstrate that there is no induction of the intrinsic activity of pro-uPA on binding to s-uPAR. Nevertheless, plasminogen activation is rapidly initiated on the cell surface, although in this complex milieu it is difficult to exclude the possibility that cellular proteases are involved in the direct activation of pro-uPA. However, the observation that a specific anti-uPA monoclonal antibody also causes the rapid initiation of plasminogen activation, by mimicking the enzyme kinetic effects seen on the cell surface (10), demonstrates that efficient initiation can occur in the absence of exogenous proteolytic activity. Kinetic simulation using the experimentally derived constants for the individual proteolytic reactions confirmed that this initiation could be completely accounted for by the unmodulated intrinsic activity of pro-uPA (10), the enhancement being a consequence of the kinetically favorable conjunction of pro-uPA and plasminogen on the antibody or cell surface. This model helps explain the finding of Manchanda and Schwartz (11) that the fibrinolytic activity of a non-plasmin-activable mutant of pro-uPA is enhanced 100-fold on binding to monocyte-associated uPAR.

The magnitude of this apparent modulation of pro-uPA intrinsic activity is equivalent to the increase we have observed in the catalytic efficiency of two-chain uPA on binding to cell-associated uPAR (0.029–2.23 M·s⁻¹) (23). This increase is not due to any alteration in the intrinsic catalytic properties of uPA but to the favorable interaction with cell-associated plasminogen. Therefore, the activities of both pro-uPA and uPA are potentiated in parallel.

From a teleological viewpoint a mechanism whereby a significant amount of activity is directly induced in pro-uPA on interaction with uPAR is untenable, as this mechanism is tantamount to constitutive activation of the system. In tissues in which uPA immunoreactivity has been found in association with cell membranes, the protein when extracted has proved to be pro-uPA (36), suggesting that the system is quiescent and not constitutively active. This mechanism would also afford no protection of pro-uPA from inactivation by PAI-1, and again complexes with PAI-1 are not extracted from tissues. Despite this latter consideration it does appear that the intrinsic activity of pro-uPA can be inhibited by PAI-1. Although Higazi et al. (28) showed no inhibition of pro-uPA by PAI-1 consistent with earlier reports of the lack of binding of PAI-1 to pro-uPA (37), an inhibitory interaction between these two proteins has recently been reported (38). We also find that PAI-1 can inhibit the intrinsic activity of pro-uPA, although the characteristics of this inhibition are not in accordance with the general model usually ascribed to serpin activity (39), displaying a high degree of reversibility as also suggested by the previous report (38). Thus, the inhibition of pro-uPA activity by PAI-1 is not really in contradiction to the earlier observations as there appears to be no stable complex formation. The mechanism of the reaction of serine protease zymogens with both serpins and substrates is an area worthy of further investigation particularly for those zymogens, such as pro-uPA, in which the intrinsic activity does seem to be of biological relevance.

Acknowledgments—I would like to thank Dr. Michael Ploug for providing recombinant soluble uPAR and Dr. Michael Scully for critical reading of this manuscript.

REFERENCES

1. Neurath, H., and Walsh, K. A. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3825–3832
2. Morgan, P. H., Robinson, N. C., Walsh, K. A., and Neurath, H. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 3312–3316
3. Higazi, A. A.-R., Cohen, R. L., Henkin, J., Kniss, D., Schwartz, B. S., and Cines, D. B. (1995) J. Biol. Chem. 270, 1297–1298
4. Manchanda, N., and Schwartz, B. S. (1991) Biochemistry 30, 979–986
5. Petersen, L. C., Lund, L. R., Nielsen, L. S., and Kragh, L. (1988) J. Biol. Chem. 263, 11189–11195
6. Manchanda, N., and Schwartz, B. S. (1991) J. Biol. Chem. 266, 14580–14584
7. Brunner, G., Simon, M. M., and Kramer, M. D. (1990) FEBS Lett. 260, 141–144
8. Ichinose, A., Fujikawa, K., and Suyama, T. (1986) J. Biol. Chem. 261, 3486–3489
9. Stack, M. S., and Johnson, D. A. (1994) J. Biol. Chem. 269, 9416–9419
10. Marcotte, P. A., and Henkin, J. (1993) Biophys. Chem. Acta 1161, 105–112
11. Goretzki, L., Schmitt, M., Mann, K., Calvete, J., Chucholowski, N., Kramer, M., Günzler, W. A., Jänicke, F., and Graeff, H. (1992) FEBS Lett. 297, 112–118
12. Kobayashi, H., Schmitt, M., Goretzki, L., Chucholowski, N., Calvete, J., Kramer, M., Günzler, W. A., Jänicke, F., and Graeff, H. (1991) J. Biol. Chem. 266, 15147–15152
13. Collen, D., Zamarron, C., Lijnen, H. R., and Hoylaerts, M. (1986) J. Biol. Chem. 261, 1259–1266
14. Huisman, S. S. (1991) Biochemistry 30, 5797–5805
15. Williams, E. B., Krishnaswamy, S., and Mann, K. G. (1989) J. Biol. Chem. 264, 7536–7545
16. Ellis, V., Behrendt, N., and Danø, K. (1992) Ann. N. Y. Acad. Sci. 667, 13–31
17. Ellis, V., Pyke, C., Eriksen, J., Solberg, H., and Danø, K. (1992) J. Biol. Chem. 267, 14580–14584
18. Brunner, G., Simon, M. M., and Kramer, M. D. (1990) FEBS Lett. 260, 141–144
19. Ichinose, A., Fujikawa, K., and Suyama, T. (1986) J. Biol. Chem. 261, 3486–3489
20. Williams, E. B., Krishnaswamy, S., and Mann, K. G. (1989) J. Biol. Chem. 264, 7536–7545
21. Huisman, S. S. (1991) Biochemistry 30, 5797–5805
22. Williams, E. B., Krishnaswamy, S., and Mann, K. G. (1989) J. Biol. Chem. 264, 7536–7545
23. Ellis, V., Behrendt, N., and Danø, K. (1991) J. Biol. Chem. 266, 12752–12758
24. Lee, S. W., Ellis, V., and Dichek, D. A. (1994) J. Biol. Chem. 269, 2411–2418
25. Ellis, V., Lee, S. W., Dichek, D. A., and Danø, K. (1994) Fibrinolyis 8, Suppl. I, 1–1
26. Mann, K. G., Nesheim, M. E., Church, W. R., Haley, P., and Krishnaswamy, S. (1990) Blood 76, 1–16
27. Reddy, K. N. N., and Markus, G. (1972) J. Biol. Chem. 247, 1683–1691
28. Higazi, A. A.-R., Cohen, R. L., Henkin, J., Kniss, D., Schwartz, B. S., and Cines, D. B. (1995) J. Biol. Chem. 270, 17375–17380
29. Ellis, V., Wun, T.-C., Behrendt, N., Rönne, E., and Danø, K. (1990) J. Biol. Chem. 265, 9904–9908
30. Ploug, M., Ellis, V., and Danø, K. (1994) Biochemistry 33, 8991–8997
31. Goodson, R. J., Doyle, M. V., Kaufman, S. E., and Rosenberg, S. N. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7129–7133
32. Longstaff, C., and Gaffney, P. J. (1991) Biochemistry 30, 979–986
33. Higazi, A. A.-R., Cohen, R. L., Henkin, J., and Keswick, S. (1994) Biochemistry 33, 4847–4854
34. Goretzki, L., Schmitt, M., Kramer, M., Günzler, W. A., Jänicke, F., and Graeff, H. (1991) J. Biol. Chem. 266, 15147–15152
35. Kieberg, V., Andreasen, P. A., Grandahl-Hansen, J., Nielsen, L. S., Skriver, L., and Danø, K. (1985) FEBS Lett. 182, 441–445
36. Andreasen, P. A., Nielsen, L. S., Kristensen, P., Grandahl-Hansen, J., Skriver, L., and Danø, K. (1986) J. Biol. Chem. 261, 7644–7651
37. Manchanda, N., and Schwartz, B. S. (1995) J. Biol. Chem. 270, 20032–20035
38. Travis, J., and Salvesen, G. S. (1983) Annu. Rev. Biochem. 52, 655–709
Functional Analysis of the Cellular Receptor for Urokinase in Plasminogen Activation: RECEPTOR BINDING HAS NO INFLUENCE ON THE ZYMOSOMATIC NATURE OF PRO-UROKINASE

Vincent Ellis

J. Biol. Chem. 1996, 271:14779-14784.
doi: 10.1074/jbc.271.25.14779

Access the most updated version of this article at http://www.jbc.org/content/271/25/14779

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 38 references, 24 of which can be accessed free at http://www.jbc.org/content/271/25/14779.full.html#ref-list-1