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Role of Cell-Surface Lysines in Plasminogen Binding to Cells: Identification of α-Enolase as a Candidate Plasminogen Receptor†

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ABSTRACT: Plasminogen binding to cell surfaces results in enhanced plasminogen activation, localization of the proteolytic activity of plasmin on cell surfaces, and protection of plasmin from α_2-antiplasmin. We sought to characterize candidate plasminogen binding sites on nucleated cells, using the U937 monocytoid cell as a model, specifically focusing on the role of cell-surface proteins with appropriately placed lysine residues as candidate plasminogen receptors. Lysine derivatives with free α-carboxyl groups and peptides with carboxy-terminal lysyl residues were effective inhibitors of plasminogen binding to the cells. One of the peptides, representing the carboxy-terminal 19 amino acids of α_2-antiplasmin, was ~5-fold more effective than others with carboxy-terminal lysines. Thus, in addition to a carboxy-terminal lysyl residue, other structural features of the cell-surface proteins may influence their affinity for plasminogen. Affinity chromatography has been used to isolate candidate plasminogen receptors from U937 cells. A major protein of M_r 54000 was recovered and identified as α-enolase by immunochemical and functional criteria. α-Enolase was present on the cell surface and was capable of binding plasminogen in ligand blotting analyses. Plasminogen binding activity of a molecular weight similar to α-enolase also was present in a variety of other cell types. Carboxypeptidase B treatment of α-enolase abolished its ability to bind plasminogen, consistent with the presence of a C-terminal lysyl residue. Thus, cell-surface proteins with carboxy-terminal lysyl residues appear to function as plasminogen binding sites, and α-enolase has been identified as a prominent representative of this class of receptors.

Cell-surface binding sites for components of the fibrinolytic system, plasminogen and plasminogen activators, provide a mechanism for local regulation of fibrinolysis [reviewed in Miles and Plow (1988) and Blasi (1988)]. The functional consequences of occupancy of cellular plasminogen receptors are currently being elucidated. To date, enhancement of plasminogen activation (Miles & Plow, 1985; Hajjar et al., 1986; Stephens et al., 1989), protection of cell-bound plasmin from α_2-antiplasmin (Plow et al., 1986), localization of plasmin activity (Miles & Plow, 1985; Plow et al., 1986), and promotion of the conversion of single-chain to two-chain urokinase (Ellis et al., 1989; Stephens et al., 1989) are events that have been ascribed to the occupied receptors.

Plasminogen receptors have been detected on most peripheral blood cells as well as on many transformed cell lines (Miles

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& Plow, 1988). The molecular identity of the cellular binding sites for plasminogen has been delineated only for the platelet: resting cells require the presence of the integrin, GPIIb-IIIa, while surface-expressed platelet fibrin enhances plasminogen binding to thrombin-stimulated platelets (Miles et al., 1986). However, these molecules are not present on nucleated cells in sufficient quantities to account for their capacity for plasminogen. Indeed, the high capacity of certain cell types for plasminogen suggests that no single cell-surface entity could mediate all binding and that several cell-surface species which interact with plasminogen with similar affinity must contribute to binding. Gangliosides, cell membrane glycolipids, can bind to plasminogen and, as major cell-surface constituents, are candidate plasminogen binding sites (Miles et al., 1989). In addition, interactions of plasminogen with many proteins are inhibited by lysine and its analogues, and as these lysine derivatives also inhibit plasminogen binding to cells (Miles & Plow, 1988), major cell-surface proteins with appropriately presented lysine residues may serve as candidate receptors. In this study, we have used lysyl derivatives and peptides with lysines situated at various positions to test the hypothesis that cell-surface proteins possessing carboxy-terminal lysines serve as plasminogen binding sites. In addition, we have used affinity chromatography on plasminogen–Sepharose to isolate and to identify α-enolase, a protein with a carboxy-terminal lysine, as a candidate plasminogen receptor and have assessed the role of its carboxy-terminal lysyl residue in the plasminogen binding function of this molecule.

Experimental Procedures

Proteins. Glu-plasminogen[1] was prepared by affinity chromatography on lysine–Sepharose (Deutsch & Mertz, 1970) as previously described from our laboratory (Miles et al., 1988). Lys-Plasminogen (the international reference preparation) was purchased from the National Institute for Biological Standards and Control, South Mims, Potters Bar, Herts, Great Britain. The protein concentrations for both plasminogen forms were determined at 280 nm using an extinction coefficient of 16.8 (Wallen & Wiman, 1972). Radiolabeling of plasminogen was performed by using a modified chloramine-T method as described (Miles & Plow, 1985).

Cells. U937 monocytoid cells were grown in RPMI-1640 containing 5% FCS, penicillin G (100 units/mL), 2 mM L-glutamine, and streptomycin (100 μg/mL). THP-1 cells were grown in the same culture media as the U937 cells but with the addition of 1 mM sodium pyruvate, 0.05 M HEPES, pH 7.35, and 5×10^{-4}% 2-mercaptoethanol. MG63 osteosarcoma cells were grown in DMEM containing 10% FCS. Human umbilical vein endothelial cells (second passage) were cultured as described (Levine et al., 1982) and were provided by Dr. Eugene Levin of the Scripps Clinic and Research Foundation.

Red cells were prepared as described (Miles & Plow, 1987) by centrifugation of blood drawn into ACD over Ficoll–Hypaque [8% Ficoll (Sigma, St. Louis, MO) and 10% Hypaque (Winthrop–Breon, New York, NY), d = 1.3579 g/mL] at 700g for 30 min at 22 °C. The pellets were resuspended to their original volumes in platelet-poor plasma and mixed with 2 parts 3% dextran T-500 (Pharmacia, Uppsala, Sweden). The red cell pellet was allowed to settle, and the top layer, containing granulocytes, was removed. The bottom layer, containing the red cells, was washed 3 times in HBSS.

Ligand Binding Assays. U937 cells (1.5×10^6/mL) were incubated in Hanks' balanced salt solution, 0.05 M HEPES, pH 7.4, containing 0.9 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 0.1% bovine serum albumin [BSA (Calbiochem, La Jolla, CA)] (HBSS), with 0.2 μM ^{125}I-plasminogen and competitors or buffer for 60 min at 37 °C, a time determined to be sufficient for apparent equilibrium to be achieved (Plow et al., 1986), in a volume of 200 μL in 1.5-mL polypropylene snap-cap tubes. Bound ligand was separated from free ligand by layering triplicate 50-μL samples over 300 μL of 20% sucrose in HBSS followed by centrifugation for 2.5 min in a Beckman microfuge (Beckman Instruments, Inc.). The tube tips were severed and counted in an Iso-Data γ counter (Iso-Data, Inc., Palatine, IL). The number of molecules of ligand bound per cell was determined from the specific activity of the radiolabeled ligand. Specific binding was determined as the difference between the total ligand bound and the nonsaturable binding which was determined as the residual binding observed in the presence of a 40-fold molar excess of nonlabeled ligand. The nonsaturable binding, determined by this method ranged from 5 to 9% of total binding.

FACS Analysis of U937 Cells. FACS analysis for cell-surface-expressed enolase was performed as described (Altieri et al., 1988) by first incubating U937 cells (1.5×10^6) with 50 μL of 20% human serum in HBSS for 30 min on ice to block Fc receptors, followed by washing once. Fifty microliters of affinity-purified rabbit IgG (0.04 mg/mL), specific for either the α or the β subunit of the human enolase isozymes (Kato et al., 1981, 1983a,b; Shimizu et al., 1983), or HBSS alone was then added, and incubation was continued for 30 min on ice. FCS was then added as above. The cells were washed again, and 50 μL of fluorescein-conjugated goat anti-rabbit IgG (50 μg/mL) (Tago, Burlingame, CA) was added, and the reaction mixtures were further incubated for 20 min on ice in the dark. After being washed, the cells were resuspended in 0.5 mL of PBS containing 10% FCS.

FACS analysis for expression of plasminogen binding sites on U937 cells used fluorescein-conjugated plasminogen (FITC-plasminogen) prepared and characterized as described (Felez et al., 1990). After pretreatment of the U937 cells (1.5×10^6) with human serum and centrifugation, as above, the cells were resuspended in 50 μL of FITC-plasminogen (0.8 μM), incubated for 30 min on ice in the dark, washed in FCS, and resuspended in 0.5 mL of PBS containing 10% FCS.

The cells were analyzed by flow cytometry in a Becton Dickinson FACS IV/40 (Becton Dickinson Co., Oxnard, CA).

Surface Labeling and Lysing of U937 Cells. U937 cells (3.9×10^9) were washed in HBSS. The cells were suspended in 25 mL of HBSS and treated with 5 mM DFP for 30 min on ice, washed again twice, and resuspended in 16 mL of HBSS. Eight, 2-mL aliquots of cells were then surface-labeled using lactoperoxidase, as described (Skubitz et al., 1983). Briefly, the following reagents were added in order to each aliquot: 3 mCi of $Na^{125}I$; 6 μL of lactoperoxidase at 10 mg/mL in phosphate-buffered saline (PBS) (0.01 M sodium phosphate/0.15 M NaCl, pH 7.3); 10 μL of H_2O_2 (0.06% in H_2O). The reaction was allowed to proceed for 5 min on ice followed by the addition of 10 μL of H_2O_2 and incubation for an additional 5 min. The reaction was terminated by the addition of 0.4 mg/mL L-tyrosine (250 μL) in PBS. The eight reaction

[1] Abbreviations: Glu-plasminogen, native form of plasminogen with N-terminal Glu; Lys-plasminogen, proteolytic derivative of Glu-plasminogen produced by plasmic cleavage with N-terminal Met[68], Lys[77], or Val[78]; BSA, bovine serum albumin; HBSS, Hanks' balanced salt solution (0.05 M HEPES, pH 7.4, containing 0.9 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 0.1% BSA); PBS, 0.15 M NaCl/0.01 M sodium phosphate buffer, pH 7.3; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PBS–BSA, PBS containing 2% BSA; CPB, carboxypeptidase B; 6-AHA, 6-aminohexanoic acid; AcLysMe, N^{α}-acetyl-L-lysine methyl ester; AH, aminohexyl; AP-19, peptide DLKLVPPMEEDYPQFGSPK.

tubes were combined and washed once with 50 mL of HBSS.

The surface-iodinated cells were centrifuged and lysed by resuspending the cell pellet in 5 mL of solubilization buffer (10 mM sodium phosphate, pH 7.3, 75 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 200 mM octyl glucoside, 3 mM PMSF, 90 units/mL trasylol, and 1 μg/mL pepstatin) and incubated for 10 min at 37 °C. The final volume of the cell lysate was 10 mL. The lysate was treated with 5 mM DFP for 30 min on ice followed by dialysis for 1 h at 4 °C vs equilibration buffer (the same as solubilization buffer with the concentration of octyl glucoside reduced to 25 mM) to remove free $Na^{125}I$. The sample was then centrifuged for 15 min at 12500*g* at 4 °C to remove insoluble material as described (Pytela et al., 1985). The final volume of the supernatant was 5.9 mL after the centrifugation and dialysis steps.

Affinity chromatography of surface-labeled U937 cell lysates was performed on four separate occasions, and the protein concentrations and counts applied are given from one representative experiment. Plasminogen or BSA was each coupled to Sepharose at 3 mg of protein/mL of Sepharose. The coupled beads were poured into a column treated with Aquasil (Pierce, Rockford, IL). An aliquot of the lysate obtained above [5.6 mL, 4×10^9 cpm, at 3.7 mg of protein/mL (as determined by the Lowry method (Lowry et al., 1951))] was first applied to a BSA–Sepharose column (6.3 × 2.5 cm, equilibrated with 100 mL of equilibration buffer containing 0.1% KI) to remove material that might nonspecifically absorb either to Sepharose or to unrelated proteins. After entering the column, the lysate was left on the column at 4 °C for 14 h. The column was washed with equilibration buffer at 22 °C (35 mL/h), and the unbound material was pooled on the basis of radioactivity.

The pooled material that did not bind to BSA–Sepharose was then divided in half, and 10.5 mL, containing 1.6×10^9 cpm (2.1 mg/mL), was applied separately to either the plasminogen–Sepharose (7.3 × 2.5 cm) or the BSA–Sepharose column (which had been washed with 9 M urea and reequilibrated). Both columns were washed in equilibration buffer containing 0.1% KI. After the material had entered, the columns were maintained at 4 °C for 14 h and then washed with equilibration buffer at a flow rate of 35 mL/h at 22 °C. After the radioactivity in the column fractions had been reduced to 2–3% of that present in the peak wash fraction, the column was eluted with 0.2 M 6-AHA in equilibration buffer at the same flow rate and temperature.

Protein Sequencing. For the determination of protein sequence, the peak fractions in the 6-AHA eluate were electrophoresed on 16% polyacrylamide–sodium dodecyl sulfate gels (SDS–PAGE) in the system of Laemmli (1970). The gels were stained with Coomassie Blue, and the protein band migrating with an apparent molecular weight of 54 000 was cut out. The gel slice was allowed to dry and then saturated with 100 mg/mL CNBr in 70% formic acid for 24 h at 22 °C in a sealed vial. The gel was lyophilized and rehydrated 4 times to remove residual CNBr. The gel slice was then rehydrated in SDS containing 10 mM dithiothreitol and electrophoresed on a 16% SDS gel run under reducing conditions in the system of Laemmli (1970). The electrophoresed proteins were transferred from the gel to Immobilon membranes (Millipore, Bedford, MA), and protein bands were excised and subjected to NH_2-terminal sequence analysis in an Applied Biosystem Model 475 gas-phase sequenator. Yields of individual residues ranged from 0.3 to 7.8 pmol.

Ligand Blotting. Samples were electrophoresed as above on 10% SDS–PAGE and transferred to Immobilon at 50 mA for 16 h. The transfer buffer was 10 mM CAPS, pH 11.0, and 10% methanol. The Immobilon filters were then incubated with BLOTTO (Johnson et al., 1984) on a shaker for 2 h at 22 °C followed by incubation with 2% BSA in PBS (PBS–BSA). The transfers were incubated with ^{125}I-plasminogen (5–10 nM) for 3–5 h at 22 °C and subsequently washed in PBS–BSA containing 0.5 M NaCl for 1–2 h at 22 °C with frequent changes. The cells that were subjected to ligand blotting were lysed in 1% Triton X-100, 0.05% Tween-80, 50 mM octyl β-D-glucopyranoside, 0.02 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.01 M EDTA, 0.01 M benzamidine, 10 μg/mL soybean trypsin inhibitor, 2 mM PMSF, 0.02% NaN_3, and 5 units/mL trasylol.

Immunoblotting. Immunoblotting was performed by electrophoresis of samples on 10% gels, run in the system of Laemmli (1970), and transfer of the samples at 350 mA for 1.5 h or at 100 mA for 18 h to Immobilon in 10 mM CAPS (pH 11.0)/10% MeOH buffer. The transfers were incubated in BLOTTO, followed by immunospecific rabbit IgG (0.04 mg/mL) in BLOTTO for 1.25 h at 22 °C on a shaker. The transfers were washed 6 times in BLOTTO and then incubated with ^{125}I-labeled goat anti-rabbit IgG (0.5 μg/mL) (Boehringer Mannheim Biochemicals, Indianapolis, IN) for 1.5 h at 22 °C. The transfers were washed 6 times in PBS followed by autoradiography.

Enolase Activity Assay. Enolase activity was measured spectrophotometrically at 240 nm as the conversion of sodium 2-phospho-D-glycerate to phosphoenolpyruvate as described (Baranowski & Wolna, 1975), using rabbit muscle enolase (Sigma) as a standard.

Peptides. Peptides were synthesized on an Applied Biosystems (Foster City, CA) Model 430 peptide synthesizer using *t*-Boc amino acids and peptidylglycine a-amidating monooxygenase resins. For use in binding assays, the peptides were dissolved in PBS, with the pH adjusted to 7.2–7.4, and spun in a microfuge to remove insoluble material.

Reagents. N^{α}-Acetyl-L-lysine, N^{α}-acetyl-L-lysine methyl ester, L-lysine methyl ester, L-lysinamide, N^{α}-acetyl-L-lysinemethylamide, N^{ϵ}-L-lysine, and benzylsuccinic acid were from Sigma. Porcine pancreatic carboxypeptidase B (CPB) was from Calbiochem (La Jolla, CA).

RESULTS

Effects of Lysine Derivatives on Plasminogen Binding to Cells. Lysine and its analogues such as 6-aminohexanoic acid (6-AHA) interfere with the interaction of plasminogen with all cell types tested (Miles & Plow, 1988), suggesting that lysine residues within cell-surface proteins may mediate plasminogen binding to cells. The potency of lysine derivatives may provide insight into the positional requirement of such interactive lysine residues within cell-surface protein(s). Moreover, all kringle-containing fragments of plasminogen, kringles 1–3, kringle 4, and the kringle 5 light-chain region, effectively compete with plasminogen for binding to cells (Miles et al., 1988), and these regions are known to have different recognition specificities for various lysine derivatives (Winn et al., 1980). Thus, although the relative affinities of lysine analogues for plasminogen are well described (Winn et al., 1980), their effects on the interaction of plasminogen with cells cannot be predicted. Therefore, the effects of increasing concentrations of lysine derivatives on Glu-plasminogen binding to U937 cells were tested, and IC_{50} values were determined for each compound (Table I). A free ϵ-amino group was essential for inhibitory potency as its acetylation abolished the activity of lysine (N^{ϵ}-acetyl-L-lysine vs lysine). Derivatization of the α-carboxyl group (i.e., L-lysine methyl ester and L-lysinamide) decreased but did not abolish the effectiveness

Table I: Effectiveness of Lysine Analogues as Inhibitors of Plasminogen Binding to U937 Cells[a]

lysine derivative	IC_{50} (mM)	lysine derivative	IC_{50} (mM)
6-AHA	0.09 ± 0.06	L-lysine methyl ester	4.2 ± 0.5
N^{α}-acetyl-L-lysine	0.29 ± 0.05	N^{α}-acetyl-L-lysinemethylamide	9.2 ± 0.9
lysinc	1.9 ± 0.7		
N^{α}-acctyl-L-lysine methyl ester	2.2 ± 1.6	L-lysinamide	>10
		N^{ϵ}-acetyl-L-lysine	>10

[a] Cells were incubated with 0.2 μM ^{125}I-plasminogen and increasing concentrations of the indicated lysyl derivatives or buffer, and binding was measured after 60 min at 37 °C. IC_{50} values were determined from dose-dependent inhibition curves by linear regression of two to eight determinations for each lysyl derivative.

of lysine. In contrast, acetylation of the α-amino group increased the inhibitory effectiveness of lysine (N^{α}-acetyl-L-lysine) and also increased the effectiveness of compounds derivatized at the α-carboxyl group (i.e., L-lysine methyl ester and N^{α}-acetyl-L-lysine methyl ester). These results are consistent with the requirement for a free ε-amino group, a free α-carboxyl group, and a derivatized α-amino group, such as would occur in a protein with a carboxy-terminal lysine residue for optimal plasminogen recognition by the cells.

The higher affinity of Lys-plasminogen compared to Glu-plasminogen for cells (Miles et al., 1988; Silverstein et al., 1988; Hajjar & Nachman, 1988) might be attributable to the aminohexyl (AH) site which is exposed in Lys-plasminogen but only partially available in Glu-plasminogen (Christensen, 1984). If this were the case, a lysine analogue derivatized at both the α-carboxyl and amino groups, such as N^{α}-acetyl-L-lysine methyl ester (AcLysMe), would have a lower IC_{50} for Lys- than Glu-plasminogen for binding to cells. Furthermore, if Lys-plasminogen interacted with cells exclusively via the AH site, AcLysMe would be a more effective competitor than 6-AHA for Lys-plasminogen binding (Christensen, 1984). To address this possibility, we compared the ability of these two derivatives to inhibit Lys- and Glu-plasminogen binding to U937 cells. The IC_{50} values obtained with AcLysMe were not higher for Glu- compared to Lys-plasminogen (1.02 ± 0.08 vs 1.9 ± 1 mM). In addition, AcLysMe was not more effective than 6-AHA in inhibiting Lys-plasminogen binding. The IC_{50} values for 6-AHA were 0.06 ± 0.01 and 0.15 ± 0.01 mM for ^{125}I-Glu- and ^{125}I-Lys-plasminogen, respectively. (The values with Glu-plasminogen are slightly different than those reported in Table I as they derived from side-by-side comparisons with Lys-plasminogen.) In controls, we verified by SDS–PAGE analysis on 7.5% gels run under reducing conditions in the system of Laemmli (1970) that the added and bound radiolabeled material migrated as expected for either Glu- or Lys-plasminogen, as previously reported for platelets (Miles et al., 1988) and the THP-1 monocytoid cell line (Felez et al., 1990). These results suggest that an interaction of Lys-plasminogen via the AH site is not solely responsible for its increased affinity for cells.

Effects of Peptides with Carboxy-Terminal Lysines on Plasminogen Binding to Cells. If, as the above results suggest, cell-associated proteins with carboxy-terminal lysines could serve as binding sites for both Glu- and Lys-plasminogen, then peptides with carboxy-terminal lysines should be effective competitors of plasminogen binding to cells. Therefore, peptides with C-terminal, N-terminal, and internal lysyl residues were compared for their ability to inhibit Glu-plasminogen binding to U937 cells (Table II). Peptides with a C-terminal alone or C-terminal plus internal lysyl residues effectively inhibited plasminogen binding with similar potencies. Peptides with either internal or amino-terminal lysines, exclusively, were

Table II: Effects of Lysine-Containing Peptides on ^{125}I-Plasminogen Binding to Cells[a]

peptide	inhibn of plasminogen binding (%)
C-terminal	
GSRGSTEDQMAK	85
YAVTGRGDSPASSK	74
C-terminal + internal	
HHLGGAKQAGDVGGYK	86
MAIPPKKNQDK	80
MNEYKLVVGGK	80
internal only	
HHLGGAKQAGDV	2
N-terminal	
KYGGHHLGGAKQRGDV	3
lysine	40

[a] Peptides, at 1 mM, were incorporated into binding assays in which U937 cells (1.5 × 10^6/mL) were incubated for 60 min at 37 °C with 0.2 μM ^{125}I-plasminogen. The data represent the percent inhibition of specific binding and are the average values of triplicates from one to three experiments.

not effective at high concentrations (1 mM), suggesting that the presence of a carboxy-terminal lysine is sufficient for the ability of a peptide to inhibit plasminogen binding. This point is emphasized by comparing the potency of HHLGGAKQAGDVGGYK and KYGGHHLGGAKQRGDV. Although these two peptides are comprised of identical amino acid residues, only the one with a carboxy-terminal lysine is an effective inhibitor.

Not all carboxy-terminal lysyl residues have equal affinity for plasminogen; e.g., the peptide AP-19 (DLKLVPPMEEDYPQFGSPK), corresponding to the 19 carboxy-terminal amino acids of α_2-antiplasmin, and AP-18, the 18 carboxy-terminal residues, are more effective competitors of the interaction between plasmin and α_2-antiplasmin than other carboxy-terminal lysyl peptides or shorter peptides corresponding to this region (Sugiyama et al., 1988; Hortin et al., 1989; Wiman et al., 1989). The AP-19 peptide also was more effective than other carboxy-terminal peptides (see Table II) such as GSRGSTEDQMAK (this peptide is used as a representative one) in inhibiting plasminogen binding. In six experiments, when these two peptides were compared side-by-side, AP-19 was (4.5 ± 1.6)-fold more effective than GSRGSTEDQMAK, similar in effectiveness to 6-AHA, and ~50-fold more effective than free lysine. In four to six experiments, the average IC_{50} for AP-19 was 37 ± 16 μM, 141 ± 40 μM for GSRGSTEDQMAK, 56 ± 31 μM for 6-AHA, and 1740 ± 607 μM for lysine. When AP-19 was extended further toward the amino terminus of α_2-antiplasmin [AP-33 (a gift from Dr. Glen Hortin, Washington University)] (Hortin et al., 1989), the increased potency was maintained but not further increased. This suggests that other structural features of peptides and proteins, in addition to the presence of a carboxy-terminal lysine, may influence inhibitory activity and impact upon the capacity of a cell-surface molecule to serve as a plasminogen binding site.

Role of Carboxy-Terminal Lysines in Plasminogen Binding to Cells. The role of carboxy-terminal lysines in plasminogen binding was further explored by incubating intact U937 cells with increasing concentrations of CPB. After treatment of the cells with CPB and subsequent washing, a dose-dependent decrease in plasminogen binding was observed (Table III). In three experiments, with CPB at 100 units/mL, the inhibition of plasminogen binding ranged from 40 to 67%. DFP treatment, which could inhibit proteases contaminating the CPB preparation but does not effect CPB activity, did not diminish the effect of CPB on plasminogen binding. As additional

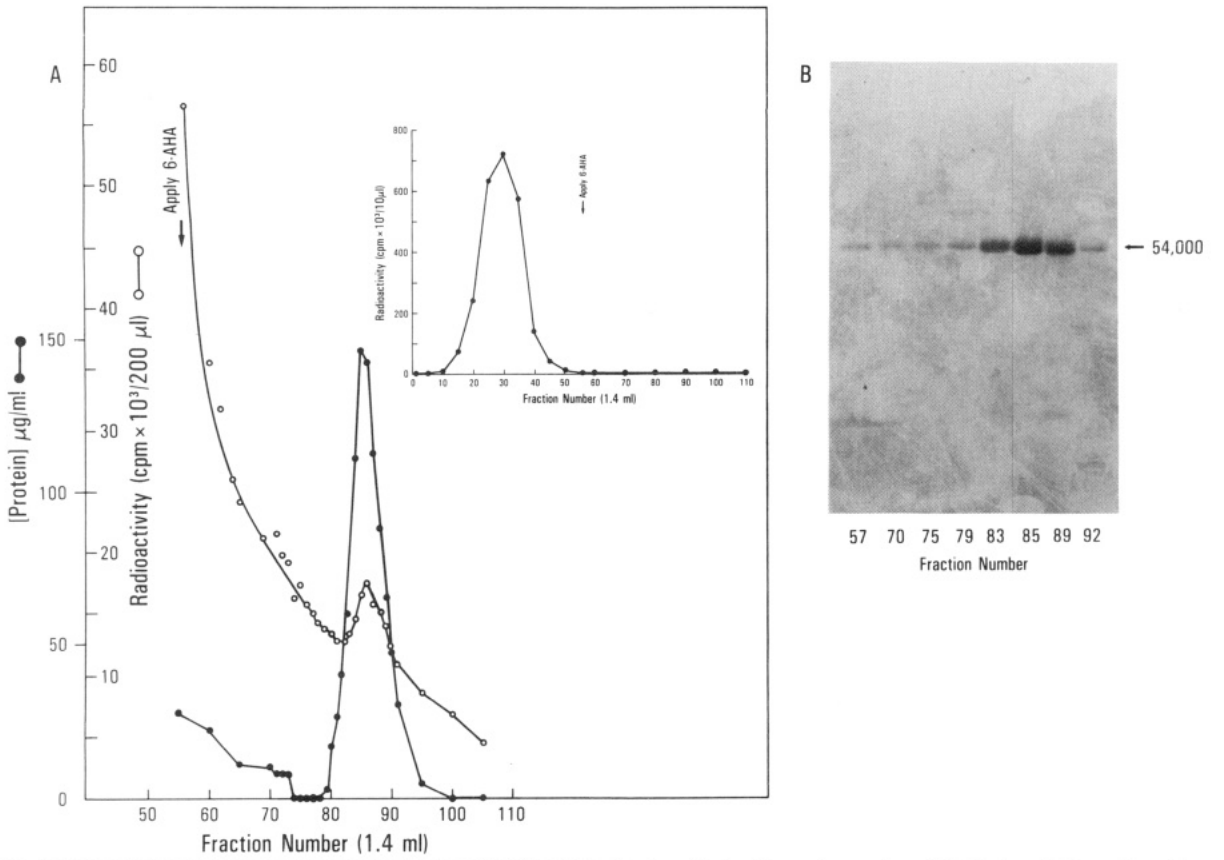

FIGURE 1: Isolation of plasminogen binding proteins from U937 cell lysates by affinity chromatography. (A) Radioactivity and protein profile of the 6-AHA eluate from plasminogen–Sepharose. The inset shows the radioactivity profile of the unbound fraction from the plasminogen–Sepharose column. (B) Electrophoresis of fractions eluted from plasminogen–Sepharose. Fractions eluted by 6-AHA from plasminogen–Sepharose were subjected to SDS–PAGE on 10% gels under nonreducing conditions (Laemmli, 1970) and stained with Coomassie Blue. Numbers correspond to column fraction numbers; 132-μL of column fractions is applied in each lane.

Table III: Effects of Treatment of U937 Cells with Carboxypeptidase B on ^{125}I-Plasminogen Binding[a]

treatment	% inhibn
none	0
carboxypeptidase B (25 units/mL)	8
carboxypeptidase B (50 units/mL)	18
carboxypeptidase B (100 units/mL)	40

[a] Cells (3.4×10^7/mL) were incubated with carboxypeptidase B for 30 min at 37 °C. The cells were washed 4 times with 50 mL of HBSS/0.1% BSA, and binding was assessed as described under Experimental Procedures. Percent inhibition of specific ^{125}I-plasminogen binding is indicated. In the absence of carboxypeptidase B, 3.8×10^6 plasminogen molecules specifically were bound per cell.

controls, cells incubated with CPB in the presence of benzylsuccinic acid, an inhibitor of CPB (McKay et al., 1979), bound plasminogen to the same extent as cells incubated with benzylsuccinic acid alone.

Isolation of a Candidate Plasminogen Receptor by Affinity Chromatography. With these observations in mind, we sought to isolate the major cell-surface plasminogen binding protein(s) and determine the contribution of carboxy-terminal lysines to its receptor function. U937 cells were surface-labeled and solubilized in octyl glucoside, and the detergent extracts were precleared by passage over BSA–Sepharose. The unbound fractions from the BSA column were then applied to plasminogen–Sepharose columns as described under Experimental Procedures. A typical experiment is shown in Figure 1. The profile of radioactivity that did not bind to the plasminogen–Sepharose column is shown in Figure 1A (inset). After the radioactivity in the column fractions had been reduced to 2–3% of that present in the peak wash fraction, the column was eluted with 0.2 M 6-AHA. A peak containing both radioactivity and protein was recovered in the 6-AHA eluate. The eluted radioactivity represented only a small proportion of that (0.12%) in the peak wash fraction (Figure 1A) but was 61% precipitable in 13% trichloroacetic acid (TCA), suggesting that it was protein-associated. In control experiments, 6-AHA did not elute protein or radioactivity from the BSA–Sepharose column, demonstrating a specific interaction with the plasminogen–Sepharose (discussed further below).

The individual fractions eluted with 6-AHA were analyzed on SDS–PAGE under nonreducing conditions and compared with the fraction (no. 57) at which 6-AHA was applied (Figure 1B). A major Coomassie Blue staining protein band of apparent M_r 54 000 was specifically eluted. This 54K band was also detected in fraction 57, but its intensity was low and increased in parallel with the elution of radioactivity. Leaching of small amounts of the 54K protein from the column prior to the application of 6-AHA is consistent with a low-affinity interaction with the immobilized plasminogen.

Autoradiography of the Coomassie Blue stained gel (Figure 2A, lane 1), revealed a major band of radioactivity which was superimposable on the 54K protein band (lane 2). Other more minor radioactive bands at M_r 22 500, 40 500, and 96 500 superimposed upon protein bands that could be detected by silver staining the gel. When subjected to ligand blotting with ^{125}I-plasminogen (lane 3), the major 54K band was reactive and accounted for 89% of the bound radioactivity in the eluted fraction as determined by densitometric scanning of the blots. Ligand blotting also revealed minor plasminogen binding bands. As these bands did not appear to be accessible to surface labeling, they may represent intracellular plasminogen

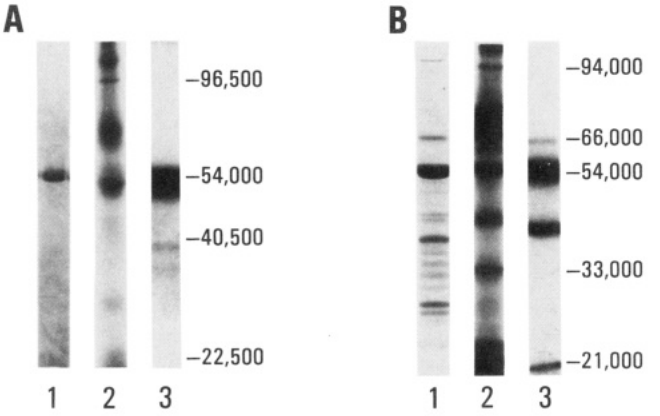

FIGURE 2: Characterization of the 6-AHA eluate from plasminogen–Sepharose by gel electrophoresis and ligand blotting. Gels (10% acrylamide) were run under either nonreducing (panel A) or reducing conditions (panel B) in the buffer system of Laemmli (1970). Lanes are as follows: 1, fraction 85, 132 μL, Coomassie Blue; 2, fraction 85, 132 μL subjected to autoradiography; 3, fraction 86, 17 μL, subjected to ligand blotting with ^{125}I-plasminogen as described under Experimental Procedures.

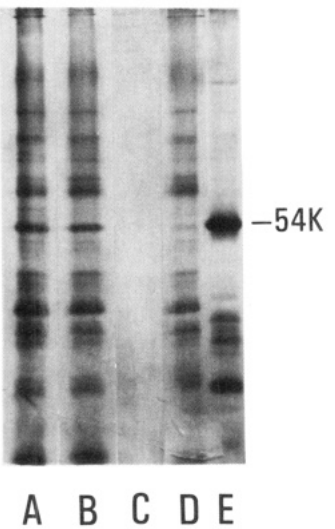

FIGURE 3: Specificity of plasminogen–Sepharose affinity chromatography. Samples were electrophoresed on 10% acrylamide gels (Laemmli, 1970) and silver stained. Lanes are as follows: A, material applied to the plasminogen- and BSA–Sepharose columns, 13 μg; B, the unbound fraction from the BSA–Sepharose column, 13 μg; C, the 6-AHA eluate from the BSA–Sepharose column, 44 μL; D, the unbound fraction from plasminogen–Sepharose, 13 μg; E, the 6-AHA eluate from the plasminogen–Sepharose column, 44 μL.

binding proteins. Plasminogen blotting to all bands, including the 54K, was inhibited by 6-AHA. Gels were also run to assess the characteristics of the 6-AHA-eluted fraction under reducing conditions (Figure 2B). The 54K band did not change its electrophoretic mobility upon reduction (lane 1). Under reducing conditions, additional Coomassie Blue staining protein bands were detected. However, as on the nonreduced gels, only a few bands were radiolabeled by lactoperoxidase-catalyzed iodination. The apparent molecular weights were 21 000, 33 000, 66 000, and 94 000 and in the higher molecular weight region (lane 2). Following reduction, the 54K band remained reactive with ^{125}I-plasminogen by ligand blotting (lane 3).

The specificity of the 54K band for plasminogen binding was analyzed further by comparing its presence in the 6-AHA eluate from BSA- and plasminogen–Sepharose on silver-stained gels (Figure 3). In lane A, the material applied to the plasminogen–Sepharose or to BSA–Sepharose is shown, and each contains a band migrating at 54K. A band with the same molecular weight is present in the unbound fraction of the BSA–Sepharose column (lane B) and is absent in the 6-AHA eluate of this column (lane C). In contrast, the 54K band was depleted from the unbound fraction of the plasminogen–Sepharose column (lane D) compared to other proteins in the starting fraction and to the unbound fraction from BSA–Sepharose and was specifically eluted with 6-AHA (lane E). Although additional protein bands are observed on silver-stained gels, compared to the Coomassie Blue stained gels in Figures 1 and 2, the 54K band remained the major detected protein.

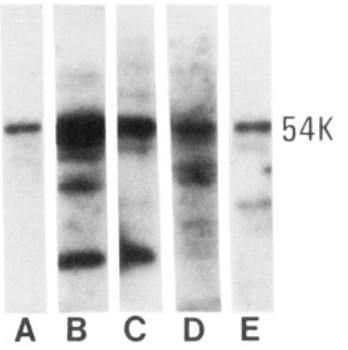

FIGURE 4: Ligand blotting of cell lysates. Cells were lysed as described under Experimental Procedures, and the extracts were subjected to SDS–PAGE on 10% acrylamide gels (Karadi et al., 1988) under reducing conditions. Lanes are as follows: A, plasminogen–Sepharose fraction 87; B, MG63 cells, 5×10^6; C, U937 cells, 1.1×10^7; D, THP-1 cells, 2×10^4; E, endothelial cells, 1×10^4.

Presence of the 54K Plasminogen Binding Protein in Various Cells. Whole lysates of U937 cells were evaluated by ligand blotting with ^{125}I-plasminogen to determine whether the 54K band was a major plasminogen-reactive constituent of these cells. Under reducing conditions, a major band at 54K was reactive (Figure 4, lane C) and had the same electrophoretic mobility as the 6-AHA eluate (lane A). This band represented 84% percent of the total material in the U937 lysate which reacted in ligand blots, as determined by laser densitometric scanning of the autoradiograms of the ligand blots. To assess the presence of this protein in other cell types, MG63 osteosarcoma cells (lane B), THP-1 monocytoid cells (lane D), and human umbilical vein endothelial cells (lane E) were analyzed in parallel, and a major ~54K band was observed. Additional bands were detected in all of these cell types, as well. In the presence of 0.2 M 6-AHA, none of the bands reacted with ^{125}I-plasminogen, indicating that the interaction of all of the bands with plasminogen was dependent upon the lysine binding sites of the ligand. These results suggest that several representative plasminogen binding cell types contain the 54K band as a major plasminogen binding species. In addition, other proteins that can interact with plasminogen are present in the various cell types.

Identification of the 54K Protein as α-Enolase. In order to determine whether the 54K band was a known protein, NH_2-terminal sequencing was performed. In three experiments, the amino terminus was blocked. Therefore, the 54K band was excised from gels and treated with CNBr, followed by electrophoresis and transfer of the bands obtained within the digest to an Immobilon membrane. Individual bands were then excised and subjected to microsequencing analysis as described under Experimental Procedures. Five separate sequences were obtained (Table IV). When aligned by using the PIR and Swiss Protein data bases in the Genbank program, the sequences obtained were found to be identical (with one exception in sequence 1) with three separate regions of the deduced sequence of the α isoform of human enolase (Gial-

Table IV: Alignment of Internal Sequences of the 54K Protein with Human α-Enolase[a]

Fragment #1		I	L	P	V	G	A	A	N	F	R	E	A	M	I[b]	I	R	A	E	V
Enolase	M 168	I	L	P	V	G	A	A	N	F	R	E	A	M	R	I	G	A	E	V
Fragment #2		R	I	G	A	E	V	Y	H											
Fragment #3		R	I	G	A	E	V													
Enolase	M 181	R	I	G	A	E	V	Y	H											
Fragment #4		D	V	A	A	S	E	F	F											
Fragment #5		D	V	A	A	S	E													
Enolase	M 243	D	V	A	A	S	E	F	F											

[a] Amino acid sequences of CNBr fragments derived from the 54K protein (numbered 1–5) are aligned with the cDNA-derived protein sequence of human α-enolase from Giallongo et al. (1986a). Met residues from the published sequence are shown for reference. Fragments 2–5 were obtained from the same 54K preparation, and fragment 1 was from a separate preparation. [b] An R was obtained at 30% of the yield of the I at this position.

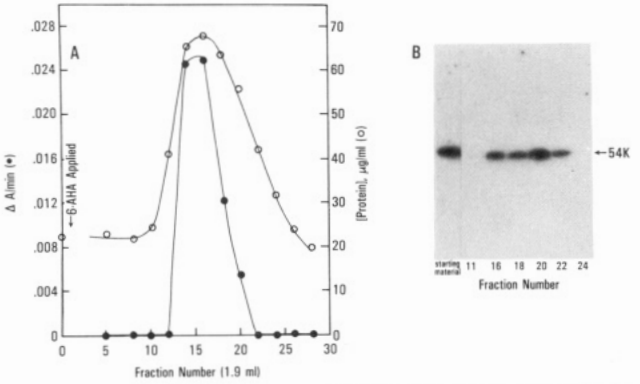

FIGURE 5: (A) Elution of enolase functional activity from plasminogen–Sepharose. 100 μL of column fractions eluted with 6-AHA was added to sodium 2-phospho-D-glycerate, and the rate of substrate cleavage was measured at 240 nm. (●) Rate of substrate cleavage, ΔA/min; (○) protein concentration determined by using the Lowry method (Lowry et al., 1951). (B) Elution of α-enolase immunoreactivity from plasminogen–Sepharose. 50 μL of a 1/9 dilution of the starting material or 50 μL of undiluted column fractions eluted with 6-AHA was electrophoresed on 10% gels run under reducing conditions in the system of Laemmli, transferred to Immobilon, incubated with 0.04 μg/mL rabbit anti-α-enolase, washed, and then incubated with ^{125}I-labeled-goat anti-rabbit IgG. (Panels A and B are from two separate experiments, such that column fraction size and the numbers of the peak fractions are not the same in the two panels.)

longo et al., 1986a). Moreover, each of the corresponding sequences in α-enolase was preceded by a methionine residue, consistent with CNBr cleavage.

To provide further identification of the 54K band as α-enolase, functional and immunochemical assays were performed. Functional activity was determined as the ability of the fractions eluted from the plasminogen–Sepharose column with 6-AHA to catalyze the conversion of sodium 2-phospho-D-glycerate to phosphoenolpyruvate (Baranowski & Wolna, 1975). Enolase enzymatic activity was eluted by 6-AHA from the plasminogen–Sepharose column in parallel with protein (Figure 5, panel A). In the peak fraction, the rate of cleavage of the substrate corresponded to 52 enzyme units of enolase activity per milligram of protein (1 unit of enzyme yields 1 μmol of product/min). This value compares well with the published specific activity of enolase of 84 units/mg after a five-step purification (Baranowski & Wolna, 1975). In controls, incubation of the 6-AHA eluate with 100 μg/mL of an immunopurified IgG fraction of a monospecific

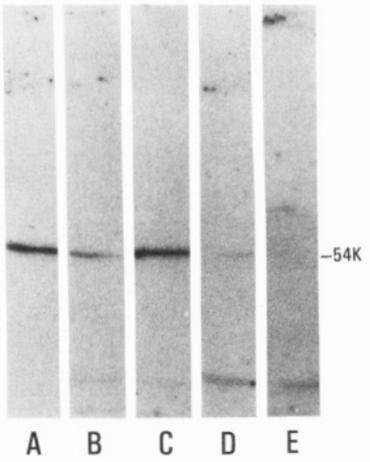

FIGURE 6: Effect of carboxypeptidase B on the ability of the 54K protein to react with ^{125}I-plasminogen in ligand blotting. The peak fraction eluted from plasminogen–Sepharose by 6-AHA was incubated with the indicated concentrations of carboxypeptidase B or buffer for 10 min at 22 °C. Samples were then subjected to SDS–PAGE under reducing conditions on 10% acrylamide gels (Laemmli, 1970), transferred to Immobilon, and subjected to ligand blotting with ^{125}I-plasminogen. Lanes contain the peak fraction plus (A) buffer, (B) CPB, 20 units/mL, (C) CPB, 20 units/mL plus 0.1 M benzylsuccinic acid, and (D) CPB, 100 units/mL. Lane E is 100 units/mL CBP, alone.

rabbit polyclonal antibody to the α form of human enolase (Kato et al., 1981, 1983a; Shimizu et al., 1983) reduced the rate of cleavage of the substrate by 73%. The fractions eluted from the plasminogen–Sepharose column also reacted immunochemically with the anti-α-enolase antibody as assessed by immunoblotting with the antibody (Figure 5, panel B). The intensity of immunoreactive 54K band paralleled both the elution of protein from the column and the ^{125}I-plasminogen reactive material in ligand blots. In controls, when the immunoblotting was performed with the anti-enolase replaced with normal rabbit IgG or buffer, no reaction with the fractions was observed.

Role of the Carboxy-Terminal Lysine in the Plasminogen Binding Function of α-Enolase. α-Enolase is predicted to have a lysyl residue at its carboxy terminus (Giallongo et al., 1986a). In order to test whether this residue is involved in its plasminogen binding function, the peak enolase fraction eluted from the plasminogen–Sepharose column was incubated with increasing concentrations of CPB and then assayed for ligand blotting activity with plasminogen (Figure 6). Treatment of the α-enolase fraction with CPB removed its ability to interact with plasminogen. In the presence of the CPB inhibitor benzylsuccinic acid (McKay et al., 1979), ligand blotting activity was retained. In controls, the migration of the enolase band, as analyzed by Coomassie Blue staining, remained unchanged, indicating that the CPB preparation did not contain contaminating proteases which digested it to lower molecular weight forms.

A 16 amino acid peptide representing the C-terminal sequence, SKAKFAGRNFRNPLAK, of α-enolase was synthesized and tested for the ability to inhibit ^{125}I-plasminogen binding to U937 cells. The α-enolase peptide inhibited binding in a dose-dependent manner, consistent with the presence of the C-terminal lysine, and the IC_{50} was 144 ± 23 μM, similar to that of the representative peptide GSRGSTEDQMAK (see above).

Presence of α-Enolase on the Cell Surface. Enolase isozymes are generally present and function in the cytoplasm, but the γ homodimer also has been localized on cell-surface membranes (Vinores et al., 1986). Therefore, we used FACS

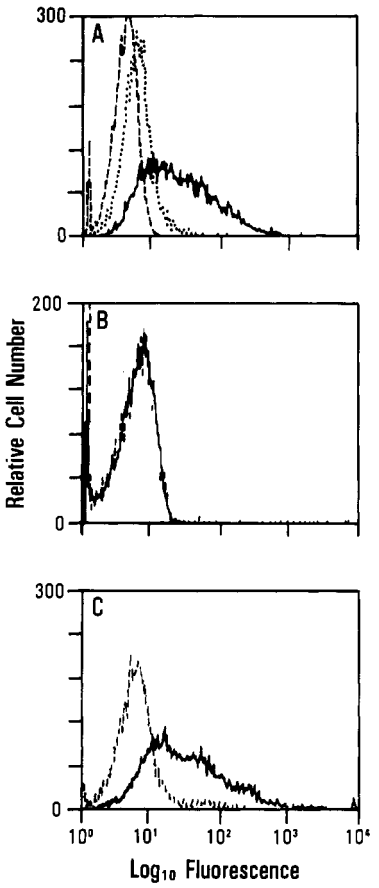

FIGURE 7: FACS analysis of U937 cells for surface expression of α-enolase (A, B) and plasminogen receptor expression (C). U937 cells (A) were incubated with either 0.04 mg/mL anti-human α-enolase (solid line), 0.04 mg/mL anti-human β-enolase (dotted line), or buffer (dashed line) followed by FITC–goat anti-rabbit IgG as described under Experimental Procedures. (B) RBC were incubated with either 0.04 mg/mL anti-α-enolase (solid line) or buffer (dashed line) followed by FITC–goat anti-rabbit IgG. (C) U937 cells were incubated with 0.8 μM FITC–plasminogen in the presence (solid line) or absence (dashed line) of 6-AHA. (Incubation with FITC–plasminogen in the presence of 6-AHA decreased the fluorescence to the same level as that in the absence of FITC–plasminogen.)

analysis to determine whether the α isoform is also present on cell surfaces using U937 cells as a representative cell type. When the cells were incubated with the immunopurified anti-α-enolase IgG, a fluorescent population of cells was clearly detected as compared to cells incubated with an immunopurified IgG specific for the β isoform of enolase (Kato et al., 1983b) or cells treated with fluoresceinated goat anti-rabbit IgG in the absence of a first antibody (Figure 7, panel A). The fluorescence profile indicated that the α-enolase-positive cell population was heterogeneous. Similar heterogeneity also was observed in the profile of a U937 cell population stained with FITC-plasminogen (panel C). As a further control for the specificity of the FACS analysis, red cells also were reacted with the anti-α-enolase, and the staining obtained was the same as the level in the absence of anti-α-enolase (panel B). Furthermore, the presence of FCS in the culture medium did not influence the surface expression of α-enolase as cells cultured in either 5% or 0.5% FCS gave superimposable FACS patterns with the anti-α-enolase antibody (data not shown).

Discussion

This paper provides support for the concept that cell-surface proteins with carboxy-terminal lysyl residues can and do serve as plasminogen binding sites. The following data support this conclusion: (1) lysine analogues with structures corresponding to carboxy-terminal lysines were the most effective lysyl derivatives in inhibiting plasminogen binding to cells; (2) peptides with carboxy-terminal, but not amino or internal, lysyl residues inhibited plasminogen binding to cells; (3) treatment of cell surfaces with CPB reduced the ability of cells to bind plasminogen; (4) a candidate plasminogen receptor of 54K was isolated from U937 cells, and its reactivity with plasminogen was abolished by treatment with CPB.

The effectiveness of the lysine analogues in inhibiting plasminogen binding to cells was similar to the ability of these analogues to bind to plasminogen and to inhibit fibrinolysis; i.e., an intact carboxyl group was required, and the presence of a polar group on the α-amino group enhanced the activity of the derivatives (Skoza et al., 1968; Winn et al., 1980). Thus, the inhibition could be due to an interaction of these compounds with the cell binding site(s) in plasminogen, preventing interaction with a cellular site which is not a carboxy-terminal lysyl residue, such as a ganglioside (Miles et al., 1989). Nonetheless, there is a precedent for a carboxy-terminal lysine-mediated interaction of plasminogen with plasmin-digested fibrin (Christensen, 1985; Tran-Thang et al., 1986) and of plasmin with α_2-antiplasmin (Sugiyama et al., 1988; Hortin et al., 1988; Wiman et al., 1989). In addition, the effects of CPB treatment of the cells and the isolation of a CPB-sensitive plasminogen binding protein are most consistent with an interaction of plasminogen with cell-surface proteins expressing carboxy-terminal lysyl residues. Although CPB also can remove carboxy-terminal arginyl residues, the results are most compatible with an influence on lysine residues, as lysine interacts with plasminogen with much higher affinity than arginine (Winn et al., 1980). Our data on the effects of CPB on plasminogen binding are similar to the observed effects of such treatment on plasmin binding to cells (Camacho et al., 1989) and suggest that these molecular species interact with a common subset of cell-surface proteins even though the affinity of plasmin for cells is proported to be considerably higher (Burtin & Fondaneche, 1988). Proteins with their carboxy termini on the extracellular face, such as α-enolase, or proteolytically processed to yield an extracellular carboxy terminus could serve as plasminogen binding sites. Such proteolytic processing could occur under conditions where cells are stimulated to secrete plasminogen activators resulting in plasmin generation. As in the case of the plasmin receptor (Camacho et al., 1989), plasmic degradation of cell-surface proteins could provide additional plasminogen binding sites.

The inhibition profile by various lysine analogues indicated that, although Lys-plasminogen interacts with platelets and U937 cells with higher affinity than with Glu-plasminogen (Miles et al., 1988; Silverstein et al., 1988), the difference in affinity is probably not due to an interaction via the AH site expressed by Lys-plasminogen. The participation of the AH site in these ligands in the interaction with cells cannot, however, be excluded.

The ability of the peptides from α_2-antiplasmin (i.e., AP-19 and AP-33) to compete with plasminogen for cell binding is compatible with their ability to compete for the interaction of plasmin with α_2-antiplasmin (Sugiyama et al., 1988; Hortin et al., 1989; Wiman et al., 1989). The enhanced effectiveness of these peptides compared to other peptides with carboxy-terminal lysyl residues suggests that although carboxy-terminal lysines can mediate interactions with plasminogen, additional structural features of peptides and proteins can increase affinity. This could explain the higher affinity of plasminogen for cells as compared to carboxy-terminal lysyl peptides and is analogous to the model of the interaction of plasmin with

α_2-antiplasmin peptides (Sugiyama et al., 1988; Hortin et al., 1989; Wiman et al., 1989). The effectiveness of AP-19 and AP-33 may also explain why cell-bound plasmin is protected from inhibition by α_2-antiplasmin (Plow et al., 1986; Miles & Plow, 1988) since the lysine binding sites in plasminogen which are occupied when it binds to the cell surface are those which also mediate its interaction with α_2-antiplasmin. Thus, a specific subset of cell-surface proteins with carboxy-terminal lysines is predicted to mediate plasminogen binding with relatively high affinity (micromolar as opposed to millimolar K_d). Consistent with this interpretation, and in contrast to plasmin binding to cells (Camacho et al., 1989), we have found that trypsin treatment of cells does not enhance plasminogen binding (Plow et al., 1986). Nor have we found that culturing U937 cells in the presence of soybean trypsin inhibitor (1 mg/mL) diminishes the plasminogen binding capacity of the cells.[2]

The structural feature of the AP-19 and AP-33 peptides which enhances their affinity for plasminogen is believed to be the presence of lysyl residues in addition to the one at their carboxy terminus (Sugiyama et al., 1988; Hortin et al., 1989; Wiman et al., 1989). Accordingly, we conducted a computer-assisted search of the NBRF (June 1990) data base for proteins with a C-terminal lysyl residue and a second lysyl residue 12–17 amino acids toward the amino terminus. This search yielded 302 candidate plasminogen binding proteins. Of these, 17 were human proteins, including histidine-rich glycoprotein, a known plasminogen binding protein (Lijnen et al., 1980). Also identified was coagulation factor Xa which we have subsequently shown to be a plasminogen binding protein in ligand blotting experiments.[3] In addition, fimbrial protein of *Pseudomonas aeruginosa* was identified and could potentially be responsible for the plasminogen binding activity of this bacterial strain (Ullberg et al., 1990).

The 54K protein isolated by affinity chromatography on lysine–Sepharose is a representative of the subset of proteins with appropriate carboxy-terminal lysines so as to serve as a high-affinity plasminogen binding site. This protein has been identified as α-enolase based on (1) the agreement of its amino acid sequence with that of human α-enolase, (2) the identical electrophoretic mobility of plasminogen ligand blotting protein and anti-α-enolase immunoreactivity, (3) the elution of enolase enzymatic activity from plasminogen–Sepharose, and (4) the presence of a carboxy-terminal lysine in the enolase sequence, endowing it with the ability to bind plasminogen and also making it susceptible to CPB digestion.

α-Enolase meets the essential criteria to be designated a plasminogen receptor. First, the susceptibility of the 54K protein (α-enolase) to lactoperoxidase-catalyzed surface iodination as well as the reactivity of intact cells with antibodies to α-enolase as assessed by FACS analysis indicates a cell-surface localization. These findings are consistent with reports demonstrating the presence of the γ–γ isozyme on cell-surface membranes of several classes of primary and secondary brain tumor cells (Vinores et al., 1986) as well as on neoplastic and nonneoplastic proliferating Schwann cells (Vinores et al., 1987). Another example of an extracellular localization of an enolase is that turtle τ-crystallin, a major structural lens protein, exhibits extensive amino acid sequence identity with human α-enolase and also exhibits enolase enzymatic activity (Wistow & Piatigorsky, 1987). Second, the ability of the isolated α-enolase to bind to plasminogen in affinity chromatography and in ligand blotting fulfills another necessary criterion for its categorization as a plasminogen receptor. Third, the ability of the peptide corresponding to the carboxy terminus of the peptide to block plasminogen binding also supports a role for α-enolase as a plasminogen receptor. This peptide was less effective than AP-19. Therefore, amino acid residues more upstream and/or secondary structure may be responsible for the high affinity of α-enolase for plasminogen. α-Enolase lacks a typical signal peptide sequence so that the mechanism by which it becomes surface-associated remains uncertain. However, certain proteins lacking consensus signal sequences can occur extracellularly. Representatives of this group include acidic and basic fibroblast growth factors (Jaye et al., 1986; Abraham et al., 1986), IL-1α and -β (Auron et al., 1984; March et al., 1985) and bacterial hemolysin (Felmlee et al., 1985). Nevertheless, on the basis of the Coomassie Blue staining patterns of the total cell lysates, it appears that the bulk of the α-enolase does reside in an intracellular pool.

α-Enolase is up-regulated in lymphocytes in response to concanavalin A and pokeweed mitogen (Giallongo et al., 1986b). Thus, surface-expressed α-enolase could potentially be up-regulated upon cell stimulation and account for the marked increase in the plasminogen binding capacity of nonadherent monocytoid cells upon PMA stimulation (Felez et al., 1990). Plasminogen ligand blotting activity was also present in lysates of a variety of plasminogen binding cells at the molecular weight range of the α-enolase monomer, and enolase is ubiquitous. Surface expression of enolase may contribute to the broad distribution of plasminogen binding sites on cells. Thus, although the participation of other cell-surface proteins is not excluded, the current study implicates α-enolase as a protein which may contribute prominently to the plasminogen binding function of many nucleated cells.

Acknowledgments

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Registry No. 6-AHA, 60-32-2; GSRGSTEDQMAK, 131296-51-0; YAVTGRGDSPASSK, 107140-68-1; HHLGGAKQAGDVGGYK, 131296-52-1; MAIPPKKNQDK, 103951-35-5; MNEYKLVVGGK, 131296-53-2; HHLGGAKQAGDV, 89105-94-2; KYGGHHLGGAKQRGDV, 131296-54-3; N^{α}-acetyl-L-lysine, 1946-82-3; N^{α}-acetyl-L-lysine methyl ester, 6072-02-2; L-lysine methyl ester, 687-64-9; N^{α}-acetyl-L-lysine methylamide, 6367-10-8; L-lysinamide, 32388-19-5; N^{ϵ}-acetyl-L-lysine, 692-04-6; enolase, 9014-08-8; plasminogen, 9001-91-6; lysine, 6899-06-5.

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