A Cellular Binding Site for the M_r 55,000 Form of the Human Plasminogen Activator, Urokinase

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ABSTRACT The secretion of plasminogen activators has been implicated in the controlled extracellular proteolysis that accompanies cell migration and tissue remodeling. We found that the human plasminogen activator urokinase (Uk) (M_r 55,000 form) binds rapidly, specifically, and with high affinity to fresh human blood monocytes and to cells of the monocyte line U937. Upon binding, M_r 55,000 Uk was observed to confer high plasminogen activator activity to the cells. Binding of the enzyme did not require a functional catalytic site (located on the B chain of the protein) but did require the noncatalytic A chain of M_r 55,000 Uk, since M_r 33,000 Uk did not bind. These results demonstrate the presence of a membrane receptor for Uk on monocytes and show a hitherto unknown function for the A chain of Uk: binding of secreted enzyme to its receptor results in Uk acting as a membrane protease. This localizes plasminogen activation near the cell surface, an optimal site to facilitate cell migration.

Various physiological and pathological processes, e.g., organogenesis during embryonic development, invasive and metastatic spreading of malignant tumors, and inflammatory reactions, require that certain cell types transgress the normal anatomical boundaries of tissues and migrate in and out of different body compartments. To allow such cellular migrations, mechanisms that provide for the focal degradation of components of the extracellular matrix must be available. Although the enzymatic basis for such degradation is not completely understood, several lines of evidence have suggested that extracellular proteolysis catalyzed by the secretion of plasminogen activators may play an important part in the degradative events necessary for migration of cells in tissues (24).

The plasminogen activator urokinase (Uk),[1] originally identified in human urine as an activator of the fibrinolytic system (27), is a serine protease of tryptic specificity; it converts plasminogen, a zymogen, into plasmin, a neutral protease of broad specificity. Active human Uk consists of two polypeptide chains linked by a disulfide bond, and exists in a high molecular weight (M_r 55,000) and a low molecular weight (M_r 33,000) form; the latter is a proteolytic product of M_r 55,000 Uk lacking most of the A chain but retaining the active site-containing B chain (10). It is now well established that a variety of cell types synthesize and secrete Uk-type plasminogen activators (9); these include cells endowed with migratory and invasive properties, such as monocytes-macrophages (34), polymorphonuclear leukocytes (37), and implanting trophoblast (30), and cells derived from malignant tumors (19).

We describe here the specific binding of M_r 55,000 Uk to the surface of U937 cells, a monocyte-like human cell line (31), and to freshly prepared human peripheral blood monocytes. This interaction provides a means by which Uk-producing cells can express both a secreted and a membrane-associated form of the enzyme. Enzymatic activity of membrane-bound Uk is localized to the vicinity of the cell surface, where it can catalyze the focal lysis of extracellular substrates, and may thereby facilitate monocyte migration.

MATERIALS AND METHODS

Cell Culture: U937 cells were grown in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 5% fetal bovine serum (FBS) (Gibco Laboratories), and cultures were split 1/3 every 4 d.

Monocyte-enriched cultures were prepared from Ficoll-Hypaque–purified human peripheral blood mononuclear cells (3). Mononuclear cells were plated in fibrinogen-coated (10 μg/cm^2) Nunc (Bio AG, Basel, Switzerland) 24-well tissue culture plates (3×10^6 cells in 1 ml RPMI 1640 with 10% FBS that had been heated for 30 min at 56°C [HI-FBS]). 5 h later the wells were washed twice vigorously with RPMI 1640, and the cultures were incubated further for 16 h in RPMI 1640 with 10% HI-FBS.

[1] *Abbreviations used in this paper:* AT-BSA, acid-treated BSA; DFP, diisopropylfluorophosphate; EGF, epidermal growth factor; FBS, fetal bovine serum; TA, tissue activator; Uk, urokinase.

THE JOURNAL OF CELL BIOLOGY · VOLUME 100 JANUARY 1985 86–92

Binding Studies: U937 cells were collected by centrifugation, washed twice in RPMI 1640, and resuspended at 1×10^6 cells/ml in RPMI 1640 containing 1 mg/ml acid-treated (17) BSA (A-7638, Sigma Chemical Co, St Louis, MO) (AT-BSA). Aliquots of 0.3 ml were distributed to Falcon No. 2058 tubes (Falcon Labware, Div., of Becton, Dickinson & Co., Oxnard, CA) and incubated as indicated. At the end of the incubation, the cultures were centrifuged (500 *g*, 5 min, 4°C) and the medium was collected and mixed with an equal volume of 2 × electrophoresis sample buffer (0.1 M Tris-Cl, pH 6.8, with 2% SDS and 20% glycerol). For the experiment of Fig. 1, cells were washed three times with PBS + 1 mg/ml AT-BSA, resuspended in 0.15 ml PBS + AT-BSA, and mixed with an equal volume of 2 × electrophoresis sample buffer. For all other experiments, cells were resuspended in PBS + 3 mg/ml AT-BSA, spun through a layer of 5% sucrose and 2.5 mg/ml AT-BSA in PBS (800 *g*, 5 min, 4°C), and finally lysed in 50 μl 1 × electrophoresis sample buffer.

The monocyte cultures were washed four times with RPMI 1640 and incubated with ^{125}I-Uk in RPMI 1640 + 3 mg/ml AT-BSA. After incubation, the cultures were washed five times with PBS + 3 mg/ml AT-BSA, and the cells were lysed in 1 ml 1 M NaOH.

Radioactivity in the media and cell lysates was determined in a Beckman Gamma 310 counter (Beckman Instruments, Inc., Palo Alto, CA).

Gel Electrophoresis and Autoradiography: Samples (15 μl) were applied to 9% polyacrylamide slab gels in the presence of SDS (15, 34); after electrophoresis the gels were fixed and stained (0.2% Coomassie Blue in 30% methanol and 7.5% acetic acid), destained, and exposed to Kodak XAR-5 film for 16 h at −80°C using DuPont Cronex Par-Speed intensifying screens (E.I. DuPont de Nemours & Co., Newton, CT). Molecular weights were calculated from the position of markers (Pharmacia, Uppsala, Sweden; low molecular weight calibration kit) in the stained gels.

Immunofluorescence: Cultured monocytes-macrophages were washed twice with PBS + 1 mg/ml BSA, incubated for 30 min at 20°C in the presence or absence of M_r 55,000 Uk (1×10^{-8} M in PBS + BSA) or M_r 33,000 Uk (2×10^{-8} M), washed three times with PBS + BSA, incubated for 15 min at 4°C in presence of rabbit anti-human Uk IgG or irrelevant IgG (0.1 mg/ml in PBS + BSA), washed three times with PBS + BSA, incubated 15 min at 4°C in the presence of fluorescein-labeled sheep immunoglobulins anti-rabbit IgG (heavy and light chains) (Institut Pasteur Production, Paris, France) (10 μg/ml in PBS), and finally washed four times with PBS + BSA. Photographs were taken on a Zeiss Photomicroscope 2 with RS3 epifluorescence optics onto Kodak Ektachrome 400 film.

Plasminogen Activator Plaque Assay: U937 cells were incubated with Uk, washed by centrifugation through sucrose as described above under *binding studies*, resuspended in casein-agar-plasminogen medium (36), and plated in Falcon 35-mm tissue culture dishes.

Ficoll-Hypaque–purified mononuclear cells were plated in Falcon 35-mm tissue culture dishes (1.3×10^6 cells in 1.5 ml RPMI 1640 with 10% HI-FBS). After 24 h of incubation, the cultures were washed four times with PBS, kept for 30 min at 37°C in RPMI 1640 + 1 mg/ml AT-BSA with or without added Uk, washed again three times with PBS, and overlayed with the casein-agar-plasminogen medium.

Photographs were taken using dark-ground illumination. The dark plaques represent zones of casein lysis surrounding cells or groups of cells; lysis was not observed when plasminogen was omitted from the assay mixtures.

Preparation of Reagents: M_r 55,000 Uk (high molecular weight) was obtained from the Green Cross Corporation (Osaka, Japan) and ^{125}I-labeled using Iodogen (Pierce Chemical Co, Rockford, IL) and Na-^{125}I (Amersham Ltd., Amersham, England) as described (1), except that spun Sephadex G-50 (Pharmacia, Uppsala, Sweden) columns (18) were used to separate the protein from unreacted ^{125}I. M_r 55,000 ^{125}I-Uk had a specific activity of $\sim 1.9 \times 10^7$ cpm/μg and was at least 80% enzymatically active (8). M_r 33,000 Uk was the generous gift of Serono (Denens, Switzerland), and was ^{125}I-labeled as described for M_r 55,000 Uk; M_r 33,000 ^{125}I-Uk had a specific activity of $\sim 1 \times 10^7$ cpm/μg, showed a single band of radioactivity with an apparent M_r of 33,000 after SDS PAGE, and was at least 80% enzymatically active.

M_r 55,000 and 33,000 unlabeled and ^{125}I-labeled Uk were reacted with diisopropylfluorophosphate (DFP, Merck AG, Darmstadt, Federal Republic of Germany) in 0.5 M $NaPO_4$, pH 7.4, by two additions of 10 mM DFP, for a total of 1 h at 20°C; enzymatic assays (8) of DFP-Uks showed that the enzymes had been inactivated by >95%.

Plasminogen was purified from human plasma by lysine-Sepharose (Pharmacia) affinity chromatography (6). Plasmin was prepared as described (8), and reacted with DFP as described for Uk. Murine epidermal growth factor (EGF) (culture grade) was from Collaborative Research Inc., (Lexington, MA), bovine insulin and human thrombin from Sigma Chemical Co., and human Factor Xa from Boehringer GmbH (Mannheim, Federal Republic of Germany). Human tissue activator (TA), purified from culture medium of HeLa cells, was the kind gift of Dr. W.-D. Schleuning and was ^{125}I-labeled as described for Uk; ^{125}I-TA had a specific activity of $\sim 4 \times 10^7$ cpm/μg, showed a predominant M_r 75,000 radioactive band after SDS PAGE, and in contrast to the unlabeled enzyme, was catalytically inactive (8). Rabbit antibodies to human urinary Uk were also generously provided by Dr W.-D. Schleuning (39); IgG were prepared by Protein A-Sepharose affinity chromatography (12).

RESULTS

The plasminogen activator secreted by human monocytes-macrophages and U937 cells is electrophoretically and immunologically identical to M_r 55,000 Uk (34). To investigate the fate of Uk after secretion, we added ^{125}I-labeled M_r 55,000 enzyme to U937 cells in culture. Cells and media were collected after 30 min of incubation at 37°C, and the cell-associated radioactivity was determined; the samples were also subjected to SDS PAGE under nonreducing conditions, and the gel was analyzed by autoradiography (Fig. 1). A substantial fraction (13.2 ± 0.1%) of the total radioactivity added to the cultures was associated with the cells and migrated with an apparent M_r of 55,000 (lane *2*); in the presence of a 60-fold excess of unlabeled M_r 55,000 Uk, only 1.1 ± 0.2% of the added radioactivity was cell associated (lane *4*). Radioactivity remaining in the medium was quantitatively recovered as two major bands (lane *1*): an M_r 55,000 band, corresponding to the added ^{125}I-Uk, and an M_r 94,000 band, corresponding to a covalent complex formed between Uk and a plasminogen activator-specific inhibitor secreted by U937 cells (34); this complex was not observed in the presence of excess unlabeled Uk (lane *3*), the secreted inhibitor being saturated under these conditions. Cellular binding of ^{125}I-Uk showed only little temperature dependence: after 60 min of

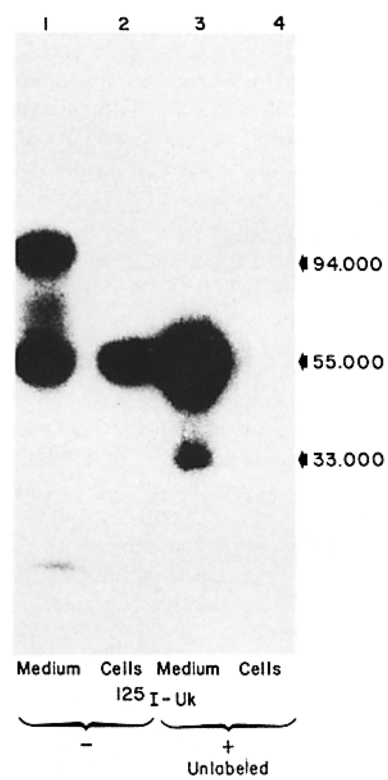

FIGURE 1 Binding of M_r 55,000 ^{125}I-Uk to U937 cells. Lanes *1* and *3*, media; lanes *2* and *4*, cells. Incubation (30 min, 37°C) of M_r 55,000 ^{125}I-Uk (3×10^{-10} M) with U937 cells was performed in the absence (lanes *1* and *2*) or presence (lanes *3* and *4*) of unlabeled M_r 55,000 Uk (1.8×10^{-8} M). Culture media and cell lysates were prepared and subjected to SDS PAGE followed by autoradiography.

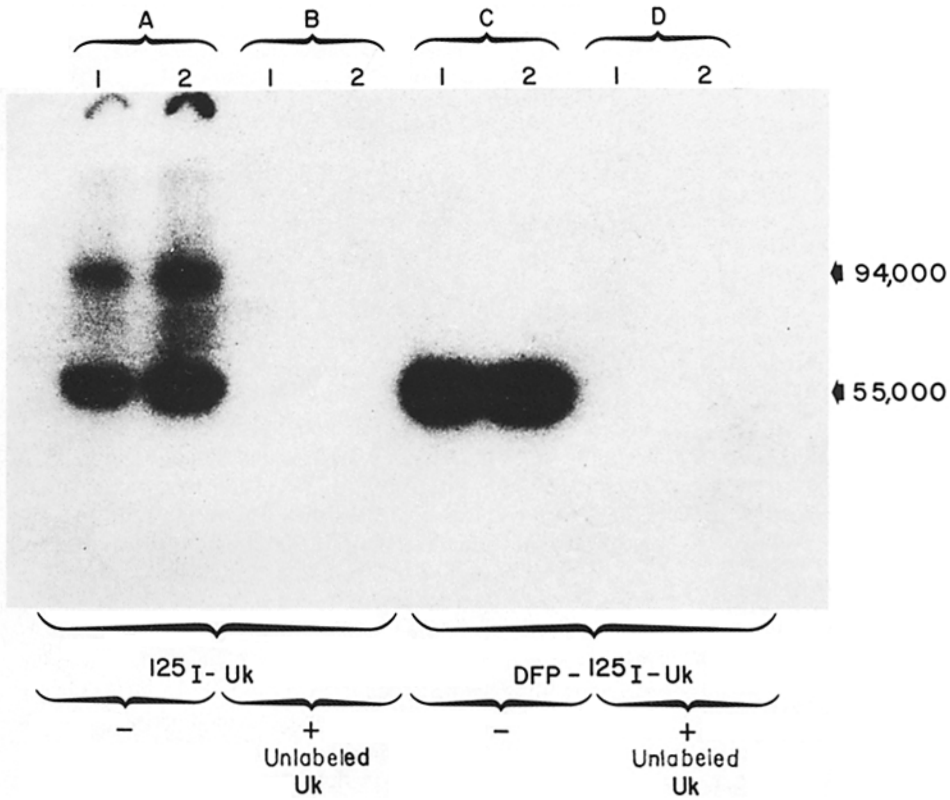

FIGURE 2 Binding of DFP-inactivated M_r 55,000 ^{125}I-Uk to U937 cells. Duplicate cultures (1, 2) of U937 cells were incubated (90 min, 20°C) in the presence of (*A*) M_r 55,000 ^{125}I-Uk (5×10^{-10} M); (*B*) same as *A* plus unlabeled M_r 55,000 Uk (1.6×10^{-8} M); (*C*) M_r 55,000 DFP-^{125}I-Uk (3.5×10^{-10} M); or (*D*) same as C plus unlabeled M_r 55,000 Uk (1.6×10^{-8} M). Both ^{125}I-Uk and DFP-^{125}I-Uk had the same specific activity (1.9×10^{7} cpm/μg). Cell lysates were prepared and subjected to SDS PAGE followed by autoradiography. Cell-bound ^{125}I-Uk represented 9.7 and 10.4% of the total radioactivity in cultures *A1* and *A2*, 0.2 and 0.2% in *B1* and *B2*, 11.4 and 11.7% in *C1* and *C2*, and 0.2 and 0.1% in *D1* and *D2*.

incubation, 15.5 ± 0.6% of the added ^{125}I-Uk was cell associated at 37°C, and 12.3 ± 0.3% at 0°C, indicating that endocytosis of the enzyme was probably not involved. These results suggested the existence of a saturable binding site for free ^{125}I-Uk on the surface of U937 cells, and further experiments were carried out to characterize this interaction.

To investigate the contribution of the enzyme's catalytic site on the cellular binding of Uk, we reacted M_r 55,000 ^{125}I-Uk with DFP, an inhibitor of serine esterases that irreversibly phosphorylates their active site. DFP-inactivated M_r 55,000 ^{125}I-Uk bound to U937 cells as effectively as the active enzyme (Fig. 2, lanes in *A* and *C*), and this binding was also completely prevented in the presence of an excess of unlabeled M_r 55,000 Uk (Fig. 2, lanes in *B* and *D*), or M_r 55,000 DFP-Uk (Table I). We concluded that enzymatic activity was not necessary for cellular binding of Uk, and that the catalytic site was probably not involved in this binding. Since DFP-^{125}I-Uk does not form a covalent complex with the U937-produced plasminogen activator-specific inhibitor, ^{125}I-Uk binding to U937 cells did not appear to be a consequence of covalent complex formation with this ligand. Nevertheless, to avoid the influence of possible secondary interactions between bound Uk and this ligand (such interactions being presumably responsible for the M_r 94,000 radioactive band associated with the cells after longer incubations with M_r 55,000 ^{125}I-Uk [compare Fig. 1, lane *2*, and the lanes in Fig. 2*A*]), we chose to carry out the following experiments using DFP-inactivated ^{125}I-Uk.

Cellular binding of M_r 55,000 DFP-^{125}I-Uk was fast, half-maximal specific binding being achieved in <7 min at 20°C (Fig. 3*A*). We also determined the amount of enzyme bound in the presence of increasing concentrations of M_r 55,000 DFP-^{125}I-Uk (Fig. 3*B*). By Scatchard analysis of these data (Fig. 3*C*), we estimated the number of binding sites per U937 cell at approximately 60,000; the K_d of the interaction was calculated to be on the order of 4×10^{-10} M.

TABLE I

Specificity of the Binding of M_r 55,000 Uk to U937 Cells

	No competitor	DFP-Uk_{55}	DFP-Uk_{33}	TA
DFP-^{125}I-Uk_{55}	5.2 ± 0.2	0.4 ± 0.1	4.8 ± 0.7	4.0 ± 0.4
DFP-^{125}I-Uk_{33}	0.1 ± 0	0.1 ± 0	0.2 ± 0.1	NT
^{125}I-TA	0.9 ± 0.1	0.8 ± 0.1	NT	0.7 ± 0.1

U937 cells were cultured for 90 min at 4°C in the presence of the ligands and competitors as indicated. After incubation the cells were washed and the cell-associated radioactivity (percent of total) was determined (results are expressed as the mean ± SEM of two determinations). Ligands were added at the following concentrations: M_r 55,000 DFP-^{125}I-Uk, 5.4×10^{-10} M; M_r 33,000 DFP-^{125}I-Uk, 1.5×10^{-9} M; ^{125}I-TA, 2.3×10^{-10} M. Competitors: M_r 55,000 DFP-Uk, 2×10^{-8} M; M_r 33,000 DFP-Uk, 4.5×10^{-8} M; TA, 1×10^{-8} M. *NT*, not tested. (Uk_{55} and Uk_{33}, M_r 55,000 and 33,000 Uk, respectively, here and in the following tables.)

UK is synthesized and secreted as a one-chain, M_r 55,000, zymogen (21, 38). Limited proteolysis by plasmin can convert this proUk into the two-chain, M_r 55,000, active enzyme (21, 38), composed of a 158 amino acid A chain and a 253 amino acid B chain, linked by a disulfide bond (11). This Uk form predominates in urine (8). An M_r 33,000 form is also found in urine and cell culture media; it is composed of an intact B chain and a "mini" A chain consisting of the C-terminal 21 amino acids of the A chain (10, 29). We have analyzed the binding of M_r 33,000 DFP-Uk to U937 cells (Table I): we did not detect any specific binding of the ^{125}I-labeled protein; in addition, using a nearly 100-fold molar excess of M_r 33,000 DFP-Uk, we found no inhibition of binding of M_r 55,000 DFP-^{125}I-Uk. These results indicate that Uk binding to U937 cells is a property of the M_r 55,000 form of the enzyme. Since M_r 33,000 Uk differs from M_r 55,000 Uk only by the absence of most of the enzyme's A chain (10), the cellular binding of Uk requires the presence of determinants of the intact A chain.

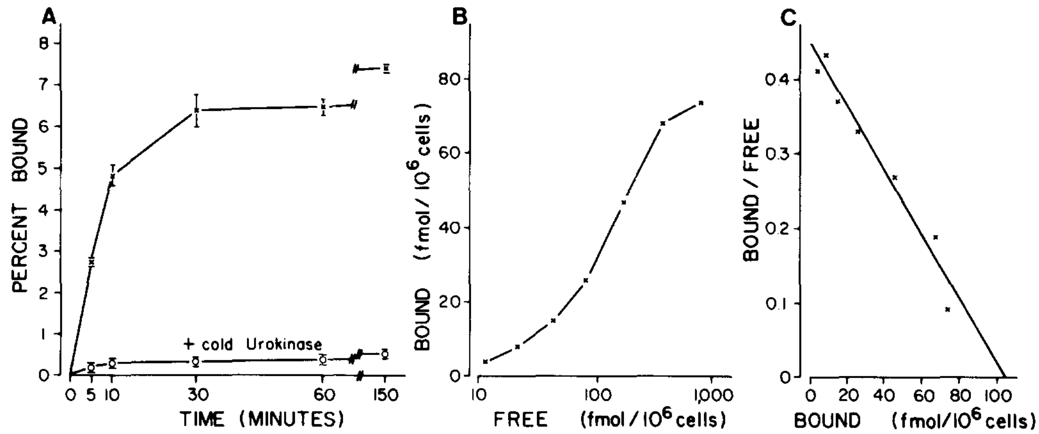

FIGURE 3 Binding of M_r 55,000 DFP-^{125}I-Uk to U937 cells. (*A*) U937 cells were cultured at 20°C for various periods of time in the presence of M_r 55,000 DFP-^{125}I-Uk (6×10^{-10} M), with (O) or without (x) the addition of unlabeled M_r 55,000 DFP-Uk (1.4×10^{-8} M). After incubation the cells were washed, and cell-associated radioactivity was determined. (*B*) U937 cells (2×10^6/ml, 0.3 ml/culture) were incubated for 90 min at 20°C, in the presence of twofold increasing concentrations of M_r 55,000 DFP-^{125}I-Uk (from 2.2×10^{-11} M to 1.4×10^{-9} M). After incubation the cells were washed, and cell-associated radioactivity was determined. Specifically bound Uk was calculated by subtracting the counts per minute bound in the presence of 1.6×10^{-8} M unlabeled M_r 55,000 DFP-Uk. (Nonspecific binding never exceeded 1% of total counts per minute in the cultures, and 8% of total counts per minute bound to the cells.) (*C*) Scatchard analysis of the data presented in *B*.

The amino acid sequence of human Uk has recently been elucidated (11, 29). Two regions of the A chain of Uk show homologies with other proteins: an N-terminal region (amino acids 13 to 43) presents clusters of sequences that are also found in murine EGF and in bovine blood factor X, whereas residues 50 to 132 show 42% of identity with the fifth kringle of human plasminogen (11), and 48% with the putative second kringle of human TA (23). We determined whether other proteins with homologies to the A chain of Uk might compete with M_r 55,000 ^{125}I-Uk for cellular binding (Tables I and II): neither human factor Xa, murine EGF, human plasminogen, DFP-plasmin, nor human TA caused any decrease in the amount of M_r 55,000 DFP-^{125}I-Uk bound to U937 cells. Furthermore, we did not detect any specific binding of ^{125}I-TA. Finally insulin, for which receptors have been described on monocytes (26) and U937 cells (25), and thrombin, recently reported to bind to (7) and be a chemotactic agent for human monocytes (2), were without effect on M_r 55,000 ^{125}I-Uk binding. These results thus suggest that the U937 cellular binding site may be specific for M_r 55,000 Uk.

Specific cellular binding of M_r 55,000 ^{125}I-Uk was also observed when monocyte-enriched cultures prepared from human venous blood mononuclear cells were incubated with DFP-inactivated enzyme (Table III); M_r 33,000 DFP-Uk did not bind to these cells (data not shown), nor did it prevent the binding of M_r 55,000 Uk. Binding is therefore not restricted to the monocyte-like U937 cell line. To visualize Uk bound to cultured monocytes, we used antibodies raised against the human enzyme. Indirect immunofluorescence did not reveal specific staining of untreated cells (Fig. 4*A*), whereas 70% of cells that had been exposed to M_r 55,000 Uk were positive (Fig. 4*B*); binding of M_r 55,000 Uk can thus be demonstrated on most cultured monocytes-macrophages, and it is therefore not the property of a subpopulation or a contaminant in our cultures.

Since the cellular binding of Uk does not require a functional catalytic site, the bound enzyme may be active, and Uk secretion followed by binding to a high-affinity cellular site could result in Uk functioning in part as a cell-bound enzyme. To examine the validity of this hypothesis, we incubated U937 cells and monocytes with M_r 55,000 or 33,000 Uk, washed the cells, and analyzed their plasminogen activator activity by the casein-agar overlay procedure (Fig. 5). After incubation, proteolytic plaques surrounded a few of the U937 cells that had not been exposed to exogenous Uk (Fig. 5*A*); these were due to the cellular synthesis and secretion of Uk. U937 cells that had been exposed to M_r 55,000 Uk produced

TABLE II
Specificity of the Binding of M_r 55,000 Uk to U937 Cells

Added component	Final concentration	Inhibition of binding
	M	%
Uk_{55}	2.5×10^{-8}	93 ± 1
$DFP\text{-}Uk_{55}$	2.5×10^{-8}	92 ± 2
Plasminogen	1×10^{-7}	−6 ± 5
DFP-plasmin	1×10^{-7}	−8 ± 3
Factor Xa	4×10^{-8}	11 ± 1
Thrombin	4×10^{-8}	8 ± 1
EGF	1.7×10^{-6}	−25 ± 13
Insulin	1.7×10^{-6}	8 ± 2

The experimental protocol is the same as for Table I. Percent inhibition was calculated relative to controls containing M_r 55,000 DFP-^{125}I-Uk (3×10^{-10} M) added to the cells in the absence of competitors; under these conditions bound counts per minute represented 11.9 ± 0.8% of total counts per minute added to the cultures.

TABLE III
Binding of M_r 55,000 Uk to Human Monocytes

	No competitor	$DFP\text{-}Uk_{55}$	$DFP\text{-}Uk_{33}$
Monocytes	5.2 ± 0.5	0.5 ± 0	5.2 ± 0.2
Control plates	0.4 ± 0.1	0.5 ± 0	0.4 ± 0.1

Monocytes were cultured for 30 min at 20°C in presence of the ligand (M_r 55,000 DFP-^{125}I-Uk, 5.8×10^{-10} M) and competitors (M_r 55,000 DFP-Uk, 1.7×10^{-8} M; M_r 33,000 DFP-Uk, 2.8×10^{-8} M) as indicated. After incubation the cells were washed, and the cell-associated radioactivity (percent of total) was determined. Control plates were prepared and treated in parallel, except that mononuclear cells were not added.

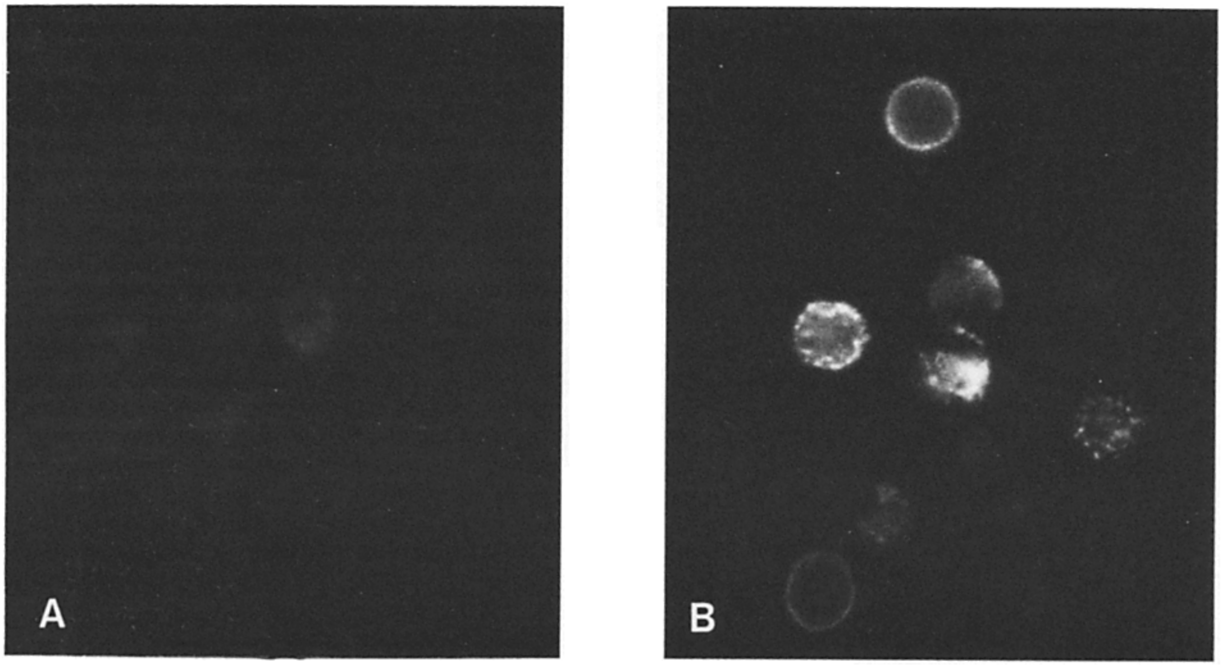

FIGURE 4 Immunofluorescence detection of M_r 55,000 Uk bound to cultured monocytes-macrophages. Cells were incubated in the absence (*A*) or presence (*B*) of M_r 55,000 Uk (1×10^{-8} M), and bound Uk was visualized on live, unfixed cells by indirect immunofluorescence using rabbit antibodies directed against human Uk. Controls included cells incubated with M_r 33,000 Uk and cells incubated with M_r 55,000 Uk followed by irrelevant IgG; fluorescence of control cultures was comparable to that of *A*. × 600.

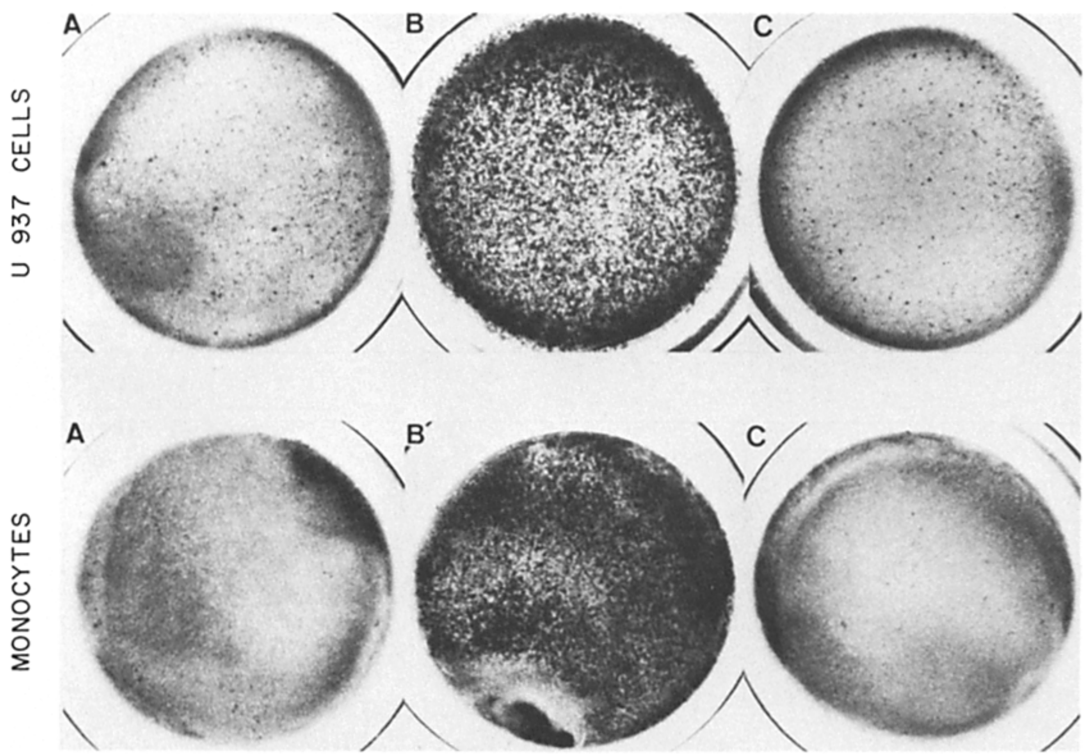

FIGURE 5 Plasminogen activator plaque assay of U937 cells and monocytes. Effect of exogenous M_r 55,000 Uk. U937 cells were incubated for 60 min at 4°C, without (*A*) or with (*B*) M_r 55,000 Uk (1.1×10^{-9} M) or M_r 33,000 Uk (*C*) (1.8×10^{-9} M). The cells were washed, resuspended in casein-agar-plasminogen medium, and plated in tissue culture dishes. Photographs were taken after 3 h of incubation of 37°C. Monocytes were incubated for 30 min at 37°C, without (*A*) or with (*B*) M_r 55,000 Uk (1.2×10^{-9} M) or (*C*) M_r 33,000 Uk (2.4×10^{-9} M). The cells were washed and overlayed with casein-agar-plasminogen medium. Photographs were taken after 40 min of incubation at 37°C. × 1.6.

many more plaques (Fig. 5*B*). These were due to the enzymatic activity of the exogenously added Uk, and not to increased Uk synthesis by treated cells, since cycloheximide (5 μg/ml) did not prevent the increase in plasminogen activator activity after exposure to M_r 55,000 Uk, and since cells that had bound M_r 55,000 DFP-inactivated Uk did not produce more plaques than did control cells (data not shown). Finally, addition of M_r 33,000 Uk did not increase the proteolytic activity of U937 cells (Fig. 5*C*). Similar results were obtained with human monocytes: no lysis was observed in control cultures (Fig. 5*A*) or in cultures that had been exposed to M_r 33,000 Uk (Fig. 5*C*), whereas M_r 55,000 Uk-treated cultures produced many proteolytic plaques (Fig. 5*B*); phase-contrast microscopy confirmed that the zones of lysis surrounded monocytes-macrophages. We concluded that cell-bound M_r 55,000 Uk remains at least in part enzymatically active, and can thereby catalyze plasmin-mediated proteolysis around monocytes and U937 cells.

DISCUSSION

The cellular binding of the plasminogen activator Uk (M_r 55,000 form) provides a mechanism by which the enzyme can function not only as a secreted protease, diffusing away from its site of synthesis, but also in a membrane-associated form, catalyzing focalized plasmin formation and thereby high proteolytic activity in the close environment of the cell. Although the role of plasminogen activator in the biology of mononuclear phagocytes remains a matter of conjecture, enzyme production is thought to be related to the migratory properties of these cells (35). In this context, a membrane-bound plasminogen activator is optimally located to generate proteolytic activity along the path of monocyte migration. In addition, membrane-bound Uk may be less susceptible to the protease inhibitors present in the extracellular fluid (4, 5). Plasminogen activation has been postulated to be essential for the migratory and invasive properties of other Uk-producing cells, such as implanting murine trophoblasts (30) or malignant cells (19, 22); similar Uk-binding sites may thus exist on the surface of these cells as well.

Binding of M_r 55,000 Uk to human monocytes and U937 cells may be related to the fact that these cells themselves produce Uk (34). We propose that during or following secretion, at least some of the (pro?)enzyme binds to the cell surface. In this respect, it is of interest to note that murine macrophages, which synthesize a Uk-type enzyme (in preparation), express a membrane-associated form of plasminogen activator (5, 16, 28). The interaction described here suggests that this membrane-associated form of the enzyme is due to the binding of secreted Uk to a high-affinity cell membrane receptor, rather than to the synthesis and membrane insertion of a distinct species of plasminogen activator. It should also be recalled that Uk and proUk are present in human plasma (32, 39), at a concentration ($1-2 \times 10^{-10}$M) which is on the order of the K_d value determined in this paper for M_r 55,000 Uk binding to U937 cells. One may thus speculate that cells can bind circulating enzyme, or enzyme produced by neighboring cells in the environment of a tissue.

The M_r 33,000 form of Uk did not bind to the cells studied; it differs from M_r 55,000 Uk by the absence of the first 135 amino acids of the 158 amino-acid A chain. This indicates that determinants of the A chain are necessary for binding to occur, although at this time it cannot be taken to imply that the A chain itself does bind to the cellular receptor. To further characterize the cellular binding of M_r 55,000 Uk, it will be of interest to evaluate the binding of proUk, and of various fragments derived from the A chain of the enzyme. In any event, the requirement for the presence of an intact A chain for cellular binding of Uk provides a novel function for this domain of the molecule.

We did not detect any significant competition for binding of M_r 55,000 Uk by other proteins with homologies to the A chain of the enzyme, nor by thrombin, suggesting that the binding site is specific for Uk. Binding of both thrombin and a functional prothrombinase complex (consisting of factors Va and Xa) to human monocytes have also recently been described (7, 33). Fibrin formation as well as fibrinolysis can thus be catalyzed at or near the surface of monocytes-macrophages; membrane binding sites for these seemingly antagonistic reactions appear to be different, and regulation of their expression may control the overall deposition and removal of fibrin around these cells, in particular within inflammatory lesions.

The cellular binding of M_r 55,000 Uk contrasts with the absence of interaction between the other human plasminogen activator, namely TA, and the cells studied in this work. We suggest that M_r 55,000 Uk is a form of plasminogen activator with an affinity for the cell surface, where it catalyzes plasmin formation in the close environment of the cell, in the same way that TA represents a form of plasminogen activator with an affinity for fibrin clots where it catalyzes a high plasmin-mediated thrombolytic activity (20). The determinants that direct these specific extracellular localizations appear to reside in the A chains of both enzymes. The recently described adsorption of TA to cultured fibroblasts (13) suggests that this form of plasminogen activator also can become cell associated. However, in contrast with the cellular binding of Uk described here, binding of TA was due to the formation of an SDS-resistant complex between the enzyme and a cellular ligand; furthermore, TA adsorption to fibroblasts was slow as compared with that of Uk to U937 cells, and appeared to be involved in the clearance of the enzyme.

Finally, the interaction of Uk with U937 cells and monocytes could be considered as that of a hormone with its membrane receptor. The region within the A chain of M_r 55,000 Uk which is homologous to murine EGF has been described as a "growth factor domain" (11). Our studies show that EGF does not compete for the Uk binding site; nevertheless, we intend to search for possible "trophic" effects of M_r 55,000 Uk on monocytes and other cells. Increased levels of Uk have been observed in malignant tumors (19), and autocrine effects (14) may be responsible for some aspects of the malignant phenotype.

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