*Proc. Natl. Acad. Sci. USA*
Vol. 82, pp. 4939–4943, August 1985
Biochemistry

# Differentiation-enhanced binding of the amino-terminal fragment of human urokinase plasminogen activator to a specific receptor on U937 monocytes

**(cell migration/phorbol ester/proteolysis)**

M. PATRIZIA STOPPELLI*†, ANGELO CORTI‡, ADOLFO SOFFIENTINI‡, GIOVANNI CASSANI‡, FRANCESCO BLASI†§, AND RICHARD K. ASSOIAN*

*Laboratory of Chemoprevention, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205; †International Institute of Genetics and Biophysics, Consiglio Nazionale delle Ricerche, v. Marconi 10, 80123 Naples, Italy; ‡Lepetit Spa Laboratories, v. Durando 38, Milan, Italy

*Communicated by Christian B. Anfinsen, April 5, 1985*

ABSTRACT **The purified amino-terminal fragment (ATF) of human urokinase plasminogen activator (residues 1–135), which is not required for activation of plasminogen, binds with high affinity to specific plasma membrane receptors on U937 monocytes. Intact urokinase efficiently competes for $^{125}$I-labeled ATF binding; 50% competition occurs with 1 nM urokinase. A large part of receptor-bound urokinase remains on the cell surface for at least 2 hr at 37°C. Differentiation of U937 monocytes into macrophage-like cells specifically increases ATF binding 10- to 20-fold. These results suggest an important role for urokinase in monocyte/macrophage biology: the native enzyme binds to the cells with the amino-terminal domain; the catalytic, carboxyl-terminal domain remains exposed on the cell surface to stimulate localized proteolysis and facilitate cell migration.**

Urokinase, one of two plasminogen activators, is a serine protease that activates plasminogen by converting it to plasmin. Consequently, urokinase can regulate a variety of events that require extracellular proteolysis, such as cell migration, tissue remodeling, and involution (1, 2). In tumor cells, cell migration may be essential to manifest the malignant phenotypes of invasiveness and metastasis (3). In agreement with this model, many transformed cell lines secrete relatively large amounts of urokinase (reviewed in ref. 4).

The physiological function of monocytes requires cell migration. These cells must leave the blood stream, pass through the endothelial wall and basement membrane, enter the neighboring tissue, differentiate into macrophages, and participate in phagocytosis (5). Plasminogen activators may facilitate the early stages of this cascade by contributing to the dissolution of basement membrane and the breaking of intercellular bridges (1, 6). This possibility has been strengthened by the recent report of Vassalli *et al.* (7), who showed that urokinase binds specifically to freshly isolated blood monocytes and to the U937 monocyte line.

Urokinase (8) is synthesized as a single-chain prepropolypeptide (9), secreted as an inactive single-chain prourokinase zymogen (411 residues) (10–12), and activated by proteolysis, which removes lysine-158 (10) to generate a two-chain urokinase molecule (residues 1–157 and 159–411; $M_r$ = 50,000) (13, 14). This form of active urokinase is referred to as high molecular weight (HMW) urokinase. Another active form of the enzyme (the $M_r$ 33,000 urokinase) contains only the carboxyl-terminal two-thirds of HMW urokinase (residues 136–157 and 159–411) (13). Thus, the amino-terminal portion of HMW urokinase (residues 1–135) is not required for catalytic activity.

We have prepared specific fragments of urokinase (the amino-terminal peptide, which lacks proteolytic activity, and the carboxyl-terminal peptide, which contains the catalytic domain) and have examined the binding of these fragments to urokinase receptors. We report that (*i*) the purified amino-terminal fragment of urokinase (ATF; residues 1–135) is totally sufficient to bind specifically to the urokinase receptor on U937 monocytes; (*ii*) the receptor-bound ligand remains on the cell surface, possibly to stimulate localized proteolysis through the catalytic, carboxyl-terminal domain; and (*iii*) differentiation of the monocytes into macrophage-like cells results in a 10- to 20-fold increase in ATF binding.

## MATERIALS AND METHODS

**Materials.** Human urinary urokinase was purified to homogeneity at Lepetit Spa Laboratories (specific activity, 120,000 international units/mg). Epidermal growth factor was purified from male mouse submaxillary glands similarly to the procedure described (15). $^{125}$I-labeled insulin was purchased from New England Nuclear. Phorbol 12-myristate 13-acetate (PMA; LC Service, Woburn, MA) was dissolved at 1 mg/ml in dimethyl sulfoxide and diluted to working concentrations with RPMI 1640 medium (GIBCO) containing 10% heat-inactivated fetal bovine serum (Biofluids, Rockville, MD). Purified tissue plasminogen activator (80,000 international units/mg) was the gift of Keith Marotti (Upjohn).

**Purification of ATF.** Purified HMW urokinase (30 mg in 1 ml) was incubated for 8 hr in 50 mM sodium phosphate buffer, pH 8/0.2 M NaCl. Reaction products were separated by gel filtration at a flow rate of 3 ml/hr on a column (1.5 × 100 cm) of Sephadex G-100 (superfine) equilibrated with 50 mM sodium phosphate buffer, pH 8.0/0.2 M NaCl. Fractions containing ATF were pooled and directly subjected to ion-exchange chromatography using a fast protein liquid chromatography (FPLC) apparatus (Pharmacia) and a Mono S HR5/5 column equilibrated with 50 mM sodium acetate buffer, pH 4.8. A sodium chloride gradient (0–1.0 M in 35 min) was used to elute bound proteins. FPLC purified ATF to homogeneity with an overall recovery of 1.4 mg.

**Radioiodination.** ATF and HMW urokinase were radioiodinated as described (16) using 1 nmol of carrier-free Na$^{125}$I (Amersham), 2 nmol of chloramine-T, and 0.1 nmol of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HMW urokinase, high molecular weight urokinase; $M_r$ 33,000 urokinase, active urokinase containing residues 136–157 and 159–411; ATF, amino-terminal fragment of urokinase containing residues 1–135 ($M_r$, 17,000); PMA, phorbol 12-myristate 13-acetate; FPLC, fast protein liquid chromatography.

§To whom reprint requests should be addressed.

protein. Radiolabeled proteins were purified by gel filtration and stored as described (17). The specific activity of the labeled proteins ranged from 50 to 120 $\mu$Ci/$\mu$g (1 Ci = 37 GBq).

**Receptor Binding.** U937 monocytes (18) (grown in RPMI 1640 medium/10% heat-inactivated fetal bovine serum to a density of $10^6$ cells per ml) were collected by centrifugation and suspended ($5 \times 10^6$ cells per ml) in serum-free RPMI 1640. After incubation (1 hr at 37°C in 5% $CO_2$/95% air), 1-ml portions of the suspension were distributed to 1.5-ml Eppendorf tubes and the tubes were centrifuged at $1000 \times g$ for 5 min. The cell pellet was suspended in 0.2 ml of binding buffer (RPMI 1640/50 mM Hepes, pH 7.4, containing bovine serum albumin at 1 mg/ml) containing ligand and rocked gently for 1 hr at 23°C (see figure legends for details of individual experiments). Cell-associated radioactivity was determined as described (19). Nonspecific binding was measured in the presence of 0.5 nM unlabeled HMW urokinase or ATF. Ligands were quantitated by amino acid analysis after hydrolyzing aliquots of stock solutions in 6 M HCl (24 hr, 110°C).

**Phorbol Ester-Induced Differentiation.** U937 monocytes ($0.5 \times 10^6$/ml) were differentiated to macrophage-like cells by incubation in medium containing 150 nM PMA (20). A 4-day treatment resulted in a totally adherent cell population that actively phagocytized latex beads (21). About 30% of the differentiated cells internalized latex during a 1-hr incubation at 37°C. About 90% of the differentiated cells were surrounded by a crown of beads. Control cells did not show these effects. Binding of $^{125}$I-labeled ATF or $^{125}$I-labeled insulin to untreated and PMA-treated cells was carried out as described above, except that $0.5 \times 10^6$ cells were used per tube and cells (control and differentiated) were incubated with labeled ligand at 4°C for 150 min. Adherent cells were collected with a rubber policeman and dispersed to a single-cell suspension by repeated pipetting.

## RESULTS

**Preparation of Urokinase Fragments.** Incubation of HMW urokinase at pH 8 results in the cleavage of native enzyme at position 135 and production of specific amino- and carboxyl-terminal fragments (Fig. 1*A Inset*). These reaction products were separated by gel filtration (Fig. 1*A*). The column fractions were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis (22) and assayed for enzymatic activity (23). Peaks 1 and 2 from the gel filtration column contained,

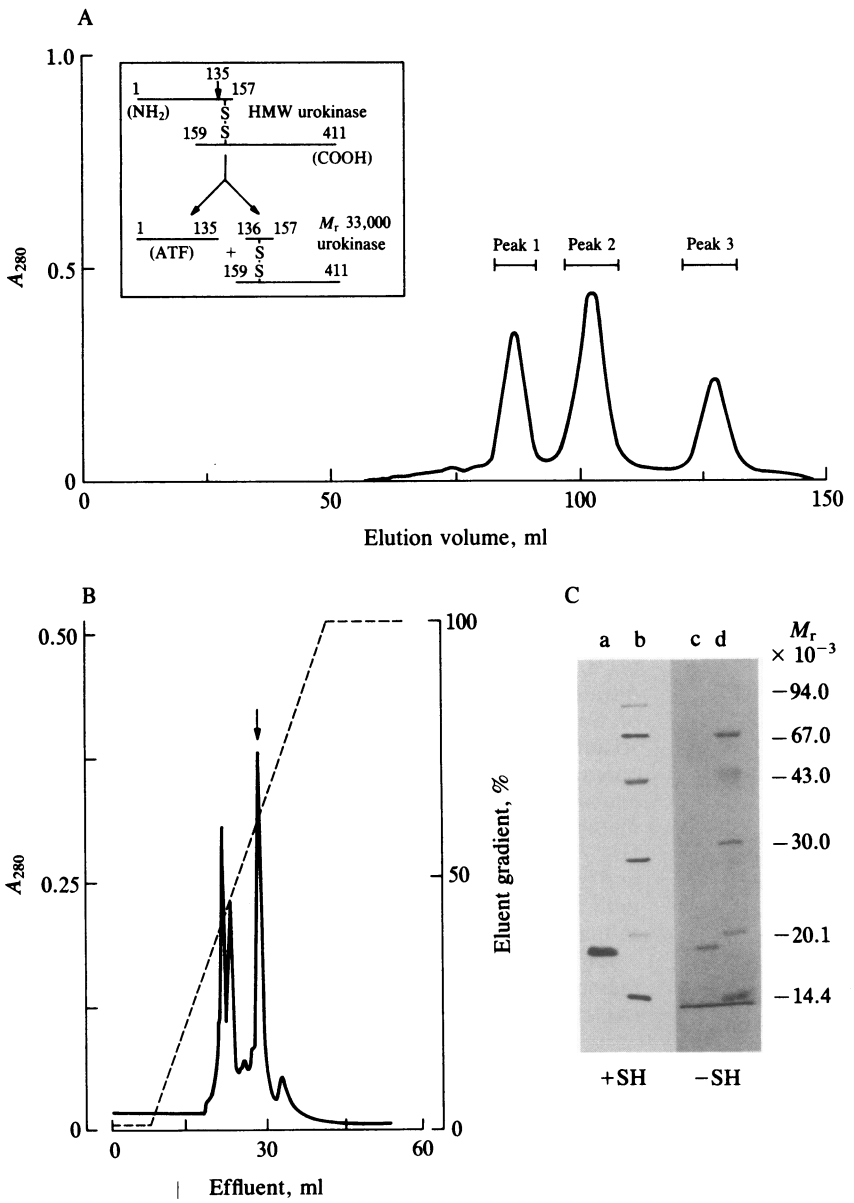


FIG. 1. Purification of ATF. (*A*) Human urokinase was cleaved by incubation at pH 8, and the reaction products were gel-filtered on Sephadex G-100. (*Inset*) Products obtained from the pH 8 incubation. (*B*) Profile obtained by FPLC of the material in peak 3 from the G-100 column. The arrow indicates the fraction containing pure ATF as determined by 12.5% NaDodSO$_4$/polyacrylamide gel electrophoresis (22) followed by staining with Coomassie blue (*C*). Lanes: a and c, migration of purified ATF; b and d, molecular weight standards determined in the presence (lanes a and b) or absence (lanes c and d) of 2-mercaptoethanol (22). Molecular weight standards were from Pharmacia.

respectively, HMW urokinase and a fully active $M_r$ 33,000 urokinase (170,000 international units/mg) corresponding to the carboxyl-terminal urokinase fragment. Peak 3 contained two peptides ($M_r$, 17,000) that lacked enzymatic activity (data not shown).

The major component in peak 3 was purified to homogeneity by FPLC (Fig. 1*B*). The FPLC fraction indicated by the arrow (Fig. 1*B*) contained a single $M_r$ 17,000 peptide as determined by $NaDodSO_4$ gel electrophoresis under both reducing and nonreducing conditions (Fig. 1*C*). The amino acid composition of this peptide (Table 1) was in complete agreement with that predicted from the primary sequence of residues 1–135 in human urokinase (14). In addition, greater than 90% of the $M_r$ 17,000 peptide was immunoprecipitated by a monoclonal antibody specific for the amino-terminal region of HMW urokinase (data not shown). The carboxyl-terminal fragment of urokinase ($M_r$ 33,000 urokinase) was obtained from peak 2 of the gel filtration column and used without further purification.

**Specific Binding of ATF to U937 Monocytes.** Competition assays were used to examine the domains of urokinase responsible for receptor binding in U937 cells. The results of an experiment in which radioiodinated HMW urokinase was bound to cells and the two urokinase fragments, ATF and the $M_r$ 33,000 urokinase, were used as unlabeled competitors, are shown in Fig. 2*A*. In agreement with the results of Vassalli *et al.* (7), $M_r$ 33,000 urokinase does not compete for $^{125}$I-labeled urokinase binding. In addition, we find that ATF binds to the urokinase receptor with an affinity similar to or slightly greater than that of the native enzyme: 50% of maximal competition occurs with 0.2 nM ATF and 0.5 nM HMW urokinase.

The complementary competition assay, in which $^{125}$I-labeled ATF was used as the probe and ATF or HMW urokinase was used as unlabeled competitor, was also carried out. [Binding of $^{125}$I-labeled ATF to U937 cells is time- and temperature-dependent (Fig. 2*B Inset*): 50% of apparent steady-state binding is attained within 5 min at 37°C, 7 min at 23°C, and 20 min at 4°C.] The results show (Fig. 2*B*), again, that the relative affinity of ATF for the urokinase receptor (50% competition at 0.4 nM) is similar to, or slightly greater than, that of HMW urokinase (50% competition at 1 nM). Taken together, these data show that ATF contains all the binding determinants present in native urokinase; the catalytic carboxyl-terminal portion of the enzyme has no role in receptor binding. Note that the affinities of both ATF and HMW urokinase are within the range of plasma urokinase concentration (24).

Table 1. Amino acid composition of purified ATF

| Amino acid | Residues per mol protein | |
|---|---|---|
| | Found | Expected |
| Asx | 17.4 | 18 |
| Thr | 5.7 | 6 |
| Ser | 8.2 | 8 |
| Glx | 12.2 | 11 |
| Pro | 7.1 | 7 |
| Gly | 12.5 | 12 |
| Ala | 5.4 | 5 |
| Cys | 12.3 | 12 |
| Val | 8.6 | 7 |
| Met | 1.8 | 2 |
| Ile | 2.0 | 2 |
| Leu | 8.7 | 9 |
| Tyr | 5.3 | 6 |
| Phe | 2.9 | 3 |
| His | 7.1 | 8 |
| Lys | 8.3 | 9 |
| Arg | 6.4 | 7 |
| Trp | ND | 3 |

ATF (5 μg) was hydrolyzed *in vacuo* with constant-boiling HCl (24 hr at 110°C) containing a trace amount of phenol. Analysis was performed using ninhydrin for detection. Half-cystine content (Cys) was determined after performic acid oxidation. Valine and isoleucine content was determined after 72 hr of hydrolysis. Tryptophan content was not determined (ND). The composition was determined by comparison with the known primary sequence of human urokinase (14).

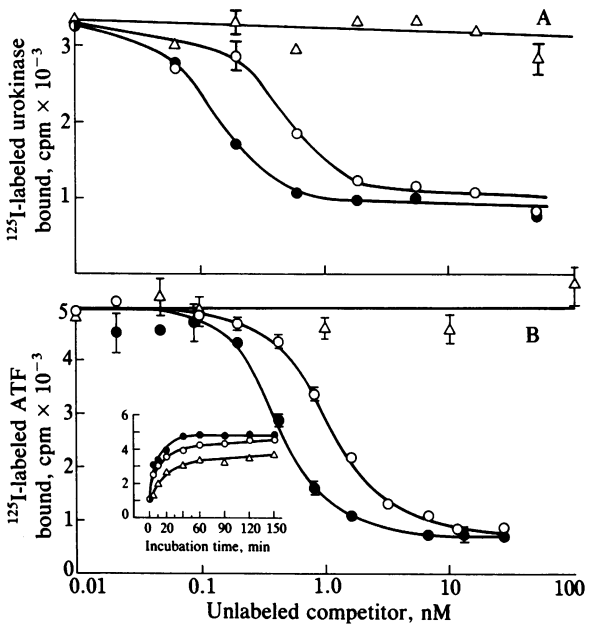


FIG. 2. Specific binding of ATF to U937 cells. Binding of ATF and HMW urokinase to U937 monocytes was as determined by radioreceptor competition assays. (*A*) Competition between $^{125}$I-labeled HMW urokinase ($1.5 \times 10^5$ cpm per tube) and unlabeled ATF (●), the $M_r$ 33,000 urokinase (△), or HMW urokinase (○) for binding to the urokinase receptor on U937 cells. (*B*) Results of an experiment in which $^{125}$I-labeled ATF was used as the probe ($3 \times 10^4$ cpm per tube) and HMW urokinase (○), ATF (●), or epidermal growth factor (△) was used as unlabeled competitor. Data are shown as means of duplicate determinations; ranges (when observed) are indicated by error bars. (*Inset*) Time-dependent binding of $^{125}$I-labeled ATF to U937 cells at 4°C (△), 23°C (○), and 37°C (●).

Although ATF is structurally related to epidermal growth factor (14), this protein did not compete with $^{125}$I-labeled ATF for receptor binding (Fig. 2*B*). In agreement with the results of Vassalli *et al.* (7), insulin, tissue plasminogen activator, thrombin, and coagulation factors IX and X do not compete for $^{125}$I-labeled ATF binding (not shown).

To examine the fate of ATF subsequent to receptor binding, $^{125}$I-labeled ATF was incubated with U937 cells at 4°C to prepare a ligand–receptor complex, the unbound label was removed, and the cells were then incubated (2 hr at 37°C) in fresh medium without additional ligand. Under conditions similar to these, the majority of insulin–receptor complexes on U937 cells are internalized, the ligand is degraded, and trichloroacetic acid-soluble products are released into the medium during the 37°C incubation (25). Interestingly, cell-associated $^{125}$I-labeled ATF was degraded poorly by the U937 cells; only 20% of the cell-associated radioactivity appeared in the medium as trichloroacetic acid-soluble products during incubation for 2 hr at 37°C (Fig. 3).

$^{125}$I-labeled ATF–receptor complexes, or $^{125}$I-labeled HMW urokinase–receptor complexes, were also treated with trypsin to determine the degree of ligand internalization (Table 2). Consistent with many ligand–receptor interactions,

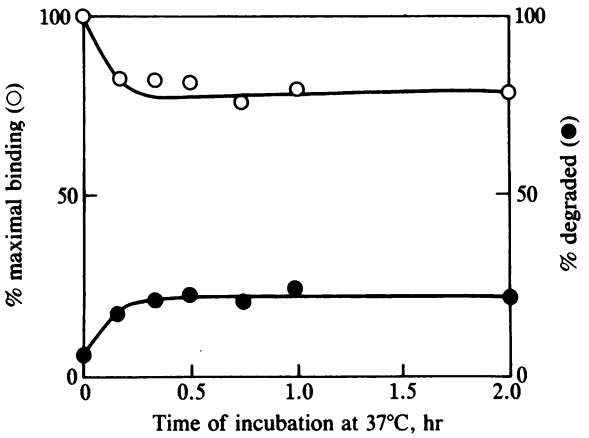


FIG. 3. Fate of receptor-bound ATF. U937 cells were incubated with $^{125}$I-labeled ATF (0.3 × $10^5$ cpm) for 2.5 hr at 4°C to form cell-surface ligand–receptor complexes. The cells were washed twice (by repeated suspension in 1-ml portions of binding buffer followed by mild centrifugation) to remove unbound label. These "pre-loaded" cells were suspended in binding buffer (0.2 ml) at 37°C to allow internalization and degradation of the ligand. At the indicated times, cells were centrifuged, the medium was collected, and the radioactivity (○) in the pellet was determined. Cell-mediated degradation of ligand to low molecular weight products (●) was determined by making the collected cell media 10% in trichloroacetic acid, incubating the solution at 4°C, and centrifuging the resulting suspension at 10,000 × *g* to prepare trichloroacetic acid-soluble and -precipitable fractions. Maximal binding of $^{125}$I-labeled ATF (cpm bound after the 4°C incubation) was about 800 cpm.

the radioactivity associated with the U937 monocytes after the 4°C incubation remained on the cell surface as determined by its susceptibility to treatment with trypsin. Even after incubation of the cells for 2 hr at 37°C, the majority of cell-associated ATF and HMW urokinase remained sensitive to trypsin. Taken together, the results of Fig. 3 and Table 2 indicate that most of ATF and HMW urokinase associated with U937 cells remains exposed on the plasma membrane. Note that the percentage of cell-associated radioactivity sensitive to trypsin is somewhat greater for HMW urokinase than it is for ATF. This difference may be related to the larger size and increased number of tryptic cleavage sites in HMW urokinase relative to ATF.

**PMA-Induced Differentiation of U937 Monocytes Increases ATF Binding.** PMA differentiates U937 monocytes into macrophage-like cells (20). We have examined the effect of differentiation on the binding of $^{125}$I-labeled ATF to the urokinase receptor. These experiments were performed at 4°C, conditions in which cell-associated radioactivity is restricted to the plasma membrane (refs. 26 and 27 and Table 2). To estimate ATF binding capacity in control and PMA-treated cells, increasing amounts of unlabeled ATF were allowed to compete for $^{125}$I-labeled ATF binding; the concentration of unlabeled ligand required to induce the onset of competition provides a measure of binding capacity. This method of analysis (Fig. 4*A*) shows that PMA-treated cells bind about 20-fold more ATF than their untreated counterparts. Saturation plots (28) of the binding data (Fig. 4*A Inset*) show that PMA treatment does not dramatically alter the relative affinity of the urokinase receptor (half-maximal binding is attained with 2 nM ATF in the differentiated cells), indicating that differentiation of U937 cells with PMA likely increases the number of ATF binding sites. Importantly, differentiation of the monocytes with PMA did not alter their binding of insulin (Fig. 4*B*).

We have considered the possibility that increased ATF binding to the macrophage-like cells might be a direct effect of PMA rather than a consequence of differentiation. By

Table 2. Trypsin treatment of receptor-bound ATF and urokinase

| Ligand | Cell-associated radioactivity, cpm: Incubation at 4°C | Incubation at 37°C |
|---|---|---|
| $^{125}$I-labeled ATF | | |
| Without trypsin | 897 | 1768 |
| With trypsin | 229 | 663 |
| $^{125}$I-labeled HMW urokinase | | |
| Without trypsin | 2800 | 2130 |
| With trypsin | 241 | 291 |

U937 cells were pre-loaded with $^{125}$I-labeled ATF or $^{125}$I-labeled HMW urokinase as described in the legend to Fig. 3 except that $10^5$ cpm of each ligand was used. Washed cells were suspended in 0.5 ml of fresh binding buffer and either treated with trypsin (0.2 mg/ml for 15 min at 37°C; see ref. 26) immediately or incubated at 37°C for 2 hr prior to trypsin treatment. Controls ("without trypsin") were always treated in parallel except that the incubation for 15 min at 37°C was performed in the absence of trypsin. Trypsin-sensitive radioactivity ranged from 60 to 75% and 85 to 92% of control binding for ATF and HMW-urokinase, respectively.

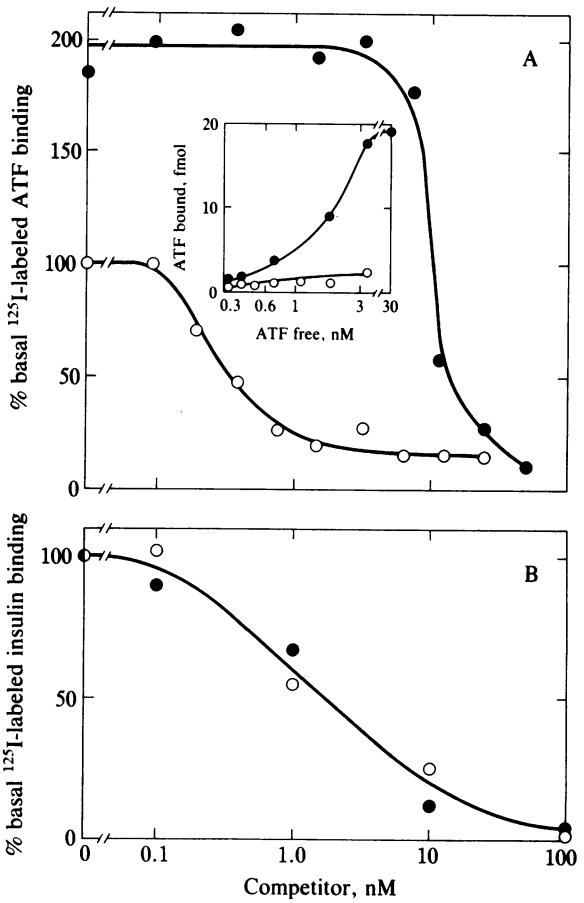


FIG. 4. PMA-induced differentiation alters urokinase binding. (*A*) ATF binding capacity in control (○) and differentiated (●) U937 cells was estimated by competition assay using $^{125}$I-labeled ATF (1.5 × $10^5$ cpm per tube) and unlabeled ATF as competitor. Results are presented as percent of basal binding ($^{125}$I-labeled ATF binding to control cells in the absence of competitor; 2500 cpm). Data are corrected for nonspecific binding (about 20% of total binding). (*Inset*) ATF binding data plotted as saturation curves. (*B*) Comparison of binding capacity of untreated (○) and PMA-treated (●) cells for insulin (determined as described for *A* using 8 × $10^4$ cpm of $^{125}$I-labeled insulin; basal $^{125}$I-labeled insulin binding was about 800 cpm).

treating U937 cells with PMA for 24 or 48 hr, we could obtain a population of adherent macrophage-like cells as well as a nonadherent cell population that had yet to differentiate. (Note, however, that both cell populations were exposed to PMA for the same period of time.) Control studies, performed as described in Fig. 4*A*, showed that the adherent cells bound 10-fold more ATF than the nonadherent population (data not shown). Thus, the increased ATF binding described in Fig. 4 is likely a consequence of differentiation.

## DISCUSSION

We have prepared specific fragments of urokinase to examine the domains responsible for receptor binding. The results show that urokinase binds to specific cell surface receptors on U937 cells solely through the amino-terminal portion of the enzyme. Interestingly, the majority of cell-associated ATF or HMW urokinase remains on the cell surface during incubation at 37°C. These results suggest a model for urokinase binding to U937 monocytes in which the amino-terminal portion of the enzyme binds to a plasma membrane receptor while the catalytic, carboxyl-terminal domain of the molecule remains exposed on the plasma membrane to stimulate extracellular proteolysis.

*In vivo*, the binding of urokinase to monocytes might serve to localize proteolysis and facilitate migration of these cells through the walls of blood vessels. In addition to supporting previous studies demonstrating that cell migration is plasminogen-dependent and impaired by protease inhibitors (6, 29), our model of urokinase binding lends itself to direct testing; ATF should be an inhibitor of receptor-mediated urokinase action. It is also likely that cell-surface urokinase can serve additional purposes; studies by others have indicated a role for plasminogen activator in lymphocyte activation and tumor antigenicity (30–32).

Differentiation of U937 monocytes into macrophage-like cells specifically increases ATF binding to the urokinase receptor. Other studies have shown that stimulation of peritoneal macrophages induces secretion of urokinase (33). This coordinate regulation of ligand and receptor expression emphasizes the likely role of cell-surface urokinase in the inflammatory response and agrees with the well-documented stimulation of proteolysis that accompanies inflammation (1). A detailed comparison of the monocyte and macrophage urokinase receptors is an important matter for further study.

The presence of a urokinase receptor on monocytes has implications in tumor cell biology. Although many malignant tumors and tumor cells secrete high amounts of urokinase, not all of them do (4). Some tumor cells might circumvent the need for increased urokinase synthesis by having urokinase receptors that could concentrate the enzyme on their cell surface and localize extracellular proteolysis. Should this be the case, the discrepancies in plasminogen activator levels in different tumor cells (4) might be reconciled.

Higher eukaryotes have evolved different mechanisms to partition proteins between the cell surface and the extracellular fluid. For example, differential RNA splicing can give rise to both membrane and secreted forms of IgM (34). Cells can also have membrane receptors that bind proteins present in plasma. In these receptor systems, ligand binding initiates a cellular response by transmitting a signal and also limits the response by inducing rapid internalization and degradation of the effector (26). U937 cells secrete (35) and bind urokinase, but they internalize the ligand poorly. These data suggest a novel variation of the autocrine mechanism (36): a secreted effector binds to its plasma membrane receptor and acts extracellularly while on the cell surface.

M.P.S. is greatly indebted to Michael Sporn for hospitality in his laboratory. George Grunberger, Phillip Gorden, and Andrea Fattorossi, provided cell lines and helpful discussions. A. Negri and S. Ronchi performed amino acid analysis. Joseph De Larco and Susannah Gal provided helpful suggestions concerning the manuscript. The Italian Association for Cancer Research provided a fellowship to M.P.S. Part of this work was supported by grants from the Consiglio Nazionale delle Ricerche (Italy), Progetti Finalizzati Ingegneria Genetica and Oncologia, to F.B. Part of this work was carried out while F. B. was a Fogarty Scholar-in-Residence at the National Institutes of Health.

1. Reich, E. (1978) in *Molecular Basis of Biological Degradative Processes*, eds. Berlin, R. D., Herrmann, H., Lepow, I. H. & Tanzer, J. M. (Academic, New York), pp. 155–169.
2. Danø, K., Andreasen, P. A., Grondhal-Hansen, J., Kristensen, P., Nielsen, L. S. & Skriver, L. (1985) *Adv. Cancer Res.*, in press.
3. Ossowski, L. & Reich, E. (1983) *Cell* **35**, 611–619.
4. Mullins, D. E. & Rohrlich, S. T. (1983) *Biochim. Biophys. Acta* **695**, 177–214.
5. Rosenstreich, D. L. (1981) in *Cellular Functions in Immunity and Inflammation*, eds. Oppenheim, J. J., Rosenstreich, D. L. & Potter, M. (Elsevier/North-Holland, New York), pp. 127–159.
6. Ossowski, L., Quigley, J. P., Kellerman, G. M. & Reich, E. (1973) *J. Exp. Med.* **138**, 1056–1064.
7. Vassalli, J. D., Baccino, D. & Belin, D. (1985) *J. Cell Biol.* **100**, 86–92.
8. Sobel, G. W., Mohler, S. R., Jones, N. W., Dowdy, A. B. C. & Guest, M. M. (1952) *Am. J. Physiol.* **171**, 768–769.
9. Salerno, G., Verde, P., Nolli, M. L., Corti, A., Szots, H., Meo, T., Johnson, J., Bullock, S., Cassani, G. & Blasi, F. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 110–114.
10. Verde, P., Stoppelli, M. P., Galeffi, P., DiNocera, P. P. & Blasi, F. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4727–4731.
11. Wun, T., Ossowski, L. & Reich, E. (1982) *J. Biol. Chem.* **257**, 7262–7268.
12. Nielsen, L. S., Hansen, J. G., Skriver, L., Wilson, E. L., Kaltoft, K., Zeuthen, J. & Danø, K. (1982) *Biochemistry* **21**, 6410–6415.
13. Günzler, W. A., Steffens, G. J., Otting, F., Buse, G. & Flohe, L. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 133–141.
14. Günzler, W. A., Steffens, G. J., Otting, F., Kim, S. M., Frankus, E. & Flohe, L. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 1155–1165.
15. Savage, C. R. & Cohen, S. (1972) *J. Biol. Chem.* **247**, 7609–7611.
16. Assoian, R., Thomas, N., Kaiser, E. & Tager, H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5147–5151.
17. Assoian, R. K., Frolik, C. A., Roberts, A. B., Miller, D. M. & Sporn, M. B. (1984) *Cell* **36**, 35–41.
18. Sundström, C. & Nilsson, K. (1976) *Int. J. Cancer* **17**, 565–577.
19. Contrell, D. A. & Smith, K. A. (1983) *J. Exp. Med.* **158**, 1895–1911.
20. Ralph, P., Williams, N., Moore, M. A. S. & Litkovsky, P. B. (1982) *Cell. Immunol.* **71**, 215–223.
21. Cline, M. J. & Lehrer, R. I. (1968) *Blood* **32**, 423–435.
22. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
23. Plough, J. & Kjeldgaard, N. O. (1957) *Biochim. Biophys. Acta* **24**, 278–282.
24. Wun, T. E., Schleuning, W. D. & Reich, E. (1982) *J. Biol. Chem.* **257**, 3276–3283.
25. Robert, A., Grunberger, G., Carpentier, J. L., Dayer, J. M., Orci, L. & Gorden, P. (1984) *Endocrinology* **114**, 247–253.
26. King, A. C. & Cuatrecasas, P. (1982) *J. Biol. Chem.* **257**, 3053–3060.
27. Pastan, I. & Willingham, M. C. (1983) *Trends Biochem. Sci.* **8**, 250–254.
28. Rodbard, D. (1973) in *Receptors for Reproductive Hormones*, eds. O'Malley, B. W. & Means, A. R. (Plenum, New York), pp. 289–326.
29. Ossowski, L., Quigley, J. P. & Reich, E. (1975) in *Proteases and Biological Control*, eds. Reich, E., Rifkin, D. B. & Shaw, E. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 901–913.
30. Cohen, S. D., Israel, E., Spiess-Meier, B. & Wainberg, M. A. (1981) *J. Immunol.* **126**, 1415–1420.
31. Miller, C. L., Graziano, C. J. & Lim, R. C. (1982) *J. Immunol.* **128**, 2194–2200.
32. Reich, E. (1974) in *Control of Proliferation in Animal Cells*, eds. Clarkson, B. & Baserga, R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 351–358.
33. Unkeless, J. C., Gordon, S. & Reich, E. (1974) *J. Exp. Med.* **139**, 834–850.
34. Early, P., Huang, H., Davis, M., Calame, K., Bond, M., Wall, R. & Hood, L. (1980) *Cell* **20**, 313–319.
35. Vassalli, J. D., Dayer, J. M., Wohlwend, A. & Belin, D. (1984) *J. Exp. Med.* **159**, 1653–1668.
36. Sporn, M. B. & Todaro, G. J. (1980) *N. Engl. J. Med.* **303**, 878–880.