

The Receptor for Urokinase-Plasminogen Activator

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Many human cells and cell lines possess a specific receptor that binds urokinase plasminogen activator (uPA) with an affinity of about 10^{-10} M. Bound enzyme is not internalized, is slowly dissociated, and retains its enzymatic activity. The amino acid sequence of uPA responsible for receptor binding is located within the first 35 aminoterminal residues, ie, in the growth factor domain. Binding, however, is not competed for by other proteins that contain the growth factor domain (including epidermal growth factor). Cells that produce uPA secrete the pro-uPA form, which subsequently binds to the receptor. A431 cells, in fact, have their receptors completely saturated with pro-uPA. It is proposed that uPA:uPA-receptor interaction plays a direct role in physiological and pathological processes that require cell migration.

Key words: cell migration metastasis, urokinase, receptor

A variety of events that require extracellular proteolysis can be regulated by the controlled production of one key proteolytic enzyme, plasmin. The presence of plasmin inhibitors in extracellular fluids, in blood, etc, ensures that plasmin becomes inactivated very quickly. Hence, the major control lies at the level of enzyme production from the inactive zymogen plasminogen. The enzymes that activate plasminogen thus exert a key-controlling role in extracellular proteolysis. These enzymes are the plasminogen activators (PA), namely tissue activator (tPA) and urokinase (uPA). Events that require extracellular proteolysis are those that lead to tissue-destruction, cell migrations, organ involution, and remodelling. Such events occur both in physiological (differentiation, mammary gland involution, trophoblast implantation, etc.) and pathological (cancer, metastasis, pemphigus) conditions. A very thorough review of this field has recently been published [1].

Of the two types of PA, tPA and uPA, the former has been mostly associated with the fibrinolytic function, while the latter has been associated with the regulation of extracellular proteolysis.

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Whether this view corresponds to physiological reality and does not represent a bias of the investigators is not really established. In any case, in this paper we shall only deal with the uPA form of plasminogen activator.

It would make sense that an enzyme required for cell migration and tissue destruction, ie that can control the dissolution of the basement membrane and of the extracellular matrix, would be sitting on the outer surface of the migrating or invading cells. The recent discovery [2,3] of a surface receptor specific for uPA indicates a possible cofactor in cell migration and will also give a tool to test directly the role of uPA in cell-associated extracellular proteolysis.

uPA is synthesized as a single-chain pre-pro-uPA [4], secreted as an inactive single-chain pro-uPA zymogen (411 residues) [5-8] and activated by a proteolytic cleavage at lysine 158 that generates a two-chain uPA molecule (residues 1-157 and 159-411) [9,10]. Two-chain uPA can also be degraded into a 135-residue amino-terminal fragment (ATF), and a carboxy-terminal active low molecular weight (Mr 33,000) uPA (LMW-uPA) (residues 136-157 and 159-411), which is also made up of two chains held together by a disulfide bond. The different forms of uPA can be isolated in pure form [3].

PROPERTIES OF THE uPA RECEPTOR

A specific binding site for uPA has been identified on the surface of human monocytes and monocyte-like U937 cells [2,3]. We have determined that the receptor binding sequence is located within the ATF by isolating the 135-residue-long peptide, labeling it with ^{125}I , and testing its binding to U937 cells. As shown in Figure 1, ATF binds to U937 cells and 50% competition is obtained with cold ATF at about 2×10^{-10} M. Table I lists the effects of uPA forms and fragments on ^{125}I -ATF or ^{125}I -uPA binding, and the concentration of the unlabeled forms required to obtain 50% competition.

These data show that all types of uPA bind the receptor, provided they contain the 135-amino-acid-long ATF. They also show that the catalytic activity is not

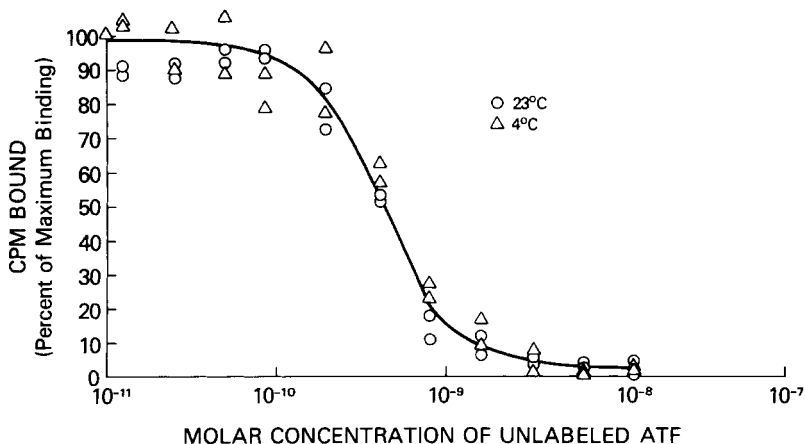


Fig. 1. Binding of ^{125}I -labeled ATF to U937 cells in the presence of different concentrations of unlabeled ATF, at two different temperatures. Incubation time: 2 hr. 100% binding was about 38,000 cpm at both temperatures. Nonspecific binding (less than 2,500 cpm) was subtracted at each time point.

TABLE I. Binding of uPA and Fragments, and Concentrations Required to Achieve 50% Competition

uPA Form	Competition		Concentration required for 50% competition of ^{125}I -ATF binding
	^{125}I -ATF	^{125}I -uPA	
Two-chain uPA	+	+	2×10^{-10}
ATF	+	+	2×10^{-10}
Single-chain pro-uPA (human)	+	+	5×10^{-10}
LMW-uPA (33,000 daltons)	-	-	-
Single-chain pro-uPA (mouse)	-	-	-

TABLE II. Specificity of ATF Binding to the uPA Receptor of U937 Cells

Competitor	Concentration	Residual binding (%)
None	—	100
Human insulin	5 nM	100
Mouse EGF	5 nM	100
Mouse EGF	100 μM	87
Bovine thrombin	2 μM	100
Human factor IX	5 nM	95
Human factor X	5 nM	121
Human tissue activator	2 nM	100
Human HMW uPA	5 nM	0
Human ATF	5 nM	0
Human single-chain pro-uPA	5 nM	5
Human LMW uPA	50 μM	100
Bovine plasminogen	10 nM	100
Bovine serum albumin	100 μM	100
Mouse single-chain pro-uPA	5 nM	100
Human IgG	100 μM	100
Aprotinin	2 μM	100

required, since pro-uPA binds the receptor, although with a slightly lower affinity, while LMW-uPA, which is fully active, does not. Finally, the data also show that mouse pro-uPA does not compete for human ^{125}I -ATF binding, thus showing a species-specificity in uPA-uPA receptor interaction, confirming the original observation of Vassalli and Belin (personal communication).

The specificity of the binding was tested by competition of ^{125}I -ATF binding from unlabeled proteins that share sequence homology with ATF, or that are known to have receptors in U937 cells (insulin, IgG) (Fig. 1, Table II).

None of these proteins (epidermal growth factor [EGF], coagulation factors IX and X, tissue activator tPA, plasminogen, thrombin) competes with ATF or HMW uPA in a binding assay, nor do insulin and human IgG for which U937 cells have specific receptors.

Several cells have been tested for the presence of uPA receptors. Table III lists the cell lines tested in our laboratory. The data seem to indicate that all cells, with the exception of A431, contain uPA receptors. As shown later, also A431 do in fact have uPA receptors. Thus, these appear to be ubiquitous at least among tumoral or transformed cell lines.

TABLE III. Presence of uPA Receptors on Different Human Cell Lines Tested by ¹²⁵I-ATF Binding

Exp. no.	Cell line	¹²⁵ I-ATF specific binding (cpm) ^a
1	U937; monocyte-like	650
1	HL60; myeloblastic	760
1	Mol T4; T-cell	234
1	Hut-78; T-cell	815
1	A431; epidermoid carcinoma	0
2	G937; human fibroblasts, transformed by SV40	5,000
2	HFS-10; human fibroblasts, transformed by SV40	2,000

^aDifferent ¹²⁵I-ATF preparations were used in experiments 1 and 2.

TABLE IV. Properties of uPA Receptors

1. Binds HMW uPA, ATF, pro-uPA but not LMW uPA—thus, all binding determinants are located in the aminoterminal domain. K_D 10^{-10} M; about 5×10^4 sites/cell [in U937 monocyte-like cells]
2. No internalization of the bound ligand can be detected for several hours at 37°C
3. Specificity is absolute, as related molecules such as EGF, factors IX and X, tPA, plasminogen, thrombin, do not compete; Binding is also species specific

Table IV summarizes the properties of uPA receptors. Special attention must be paid to the apparent absence of internalization, which distinguishes this receptor from others, like the EGF receptor [11].

SURFACE uPA IS IN FACT RECEPTOR-BOUND PRO-uPA

Since A431 cells synthesize and secrete relatively high levels of uPA [12], the apparent absence of uPA receptors (Table III) might be due to saturation of receptor with the biosynthetic ligand. This hypothesis proved to be correct on the basis of the following data: (1) Single-chain pro-uPA is present both in the medium and lysate of the A431 epidermoid carcinoma cell line [12]. (2) Most of the cell-associated pro-uPA copurifies with the membrane-rich fraction; part of it is present on the cell surface as shown by indirect immunofluorescence and surface iodination techniques. (3) Pro-uPA is not an integral membrane protein but is bound to a specific surface receptor; a mild acid treatment of the cells uncovers the surface receptors by dissociating pro-uPA. The properties of the receptor are the same as those of U937 cells (Table IV). Thus, A431 cells possess a surface uPA receptor, which is completely saturated with biosynthetic pro-uPA. (4) Resaturation of uncovered receptors may be followed by reincubating cells in normal culture medium; within 60 min, most of the sites have been reoccupied. Excess uPA-specific antibodies totally prevents the resaturation of the receptors. Thus, A431 cells first secrete pro-uPA and then bind it to the surface receptor [13]. The ability of A431 cells to secrete pro-uPA and to bind it to their own surface receptor represents an example of autocrine mechanism discovered in a tumor cell (Fig. 2). We have proposed that it may provide an unregulated expression of the invasive phenotype in malignant cells [13].

Binding of pro-uPA to receptors may shed light on previous data on membrane association of uPA: (1) A membrane form of plasminogen activator is synthesized upon transformation of chicken embryo fibroblasts with RSV [14]; (2) logarithmi-

Mouse	gly ser val leu gly ala pro asp glu ser asn cys gly cys gln asn gly
Human	ser <u>asn glu leu his gln val pro</u> ser asn cys <u>asp cys leu</u> asn gly
	1 10
	gly val cys val ser tyr lys tyr phe ser arg ile arg arg cys ser cys
	gly <u>thr</u> cys val ser <u>asn</u> lys tyr phe ser <u>asn</u> ile <u>his trp</u> cys <u>asn</u> cys
	20 30
	pro arg lys phe glu gly glu his cys glu ile
	pro <u>lys</u> lys phe <u>gly</u> gly <u>gln</u> his cys glu ile
	40

Fig. 2. Comparison of the aminoterminal region of human [8,10] and mouse [18] pro-uPA. Numbering refers to the sequence of the human enzyme. Mouse pro-uPA has one extra N-terminal residue.

cally-growing cells specifically express a membrane-associated uPA, as opposed to quiescent cells, which secrete tPA [15]; (3) correlation between levels of uPA and tumor prognosis only holds when the cell-associated activity is considered, both in experimental [16] and in human tumors [17].

RECEPTOR-BINDING SEQUENCE

In order to identify the aminoacid sequence in uPA responsible for the binding to the receptor, we have taken advantage of the species specificity of binding. The amino acid sequence of mouse and human uPA [18] differs at several single residues in the amino-terminal domain (Fig. 2). Two areas of gross difference are located at the amino- and carboxy-terminus of the ATF fragment and thus represent sequences possibly involved in receptor binding. The binding sequence has been narrowed down to residue 18–30 of human uPA on the basis of the following data (Appella, et al, manuscript in preparation): (1) Proteolytic cleavage of ATF at positions 23–24 or 25–26 totally abolish the binding; (2) a synthetic peptide covering residues 17–32 efficiently competes with ATF for uPA receptor binding; (3) a synthetic peptide covering residues 1–14 does not compete for ATF-binding nor does it influence the binding of the 17–32 peptide. The identification of the binding sequence will now allow experiments directed toward the understanding of the function of the uPA receptor in normal and malignant cells, under physiological or artificial conditions.

BINDING OF PRO-uPA TO THE uPA RECEPTOR

Proteolytic cleavage of pro-uPA at residue 157 induces a conformational change in the molecule that leads to a very large increase in specific activity. We have shown that pro-uPA binds the uPA receptor with an affinity very close to that of uPA or ATF (see Table I). One can speculate on why pro-uPA, which is much less active than uPA, should be bound to the receptor. It is possible that the surface receptor is the cellular site for activation of pro-uPA to uPA, through the action of a specific protease.

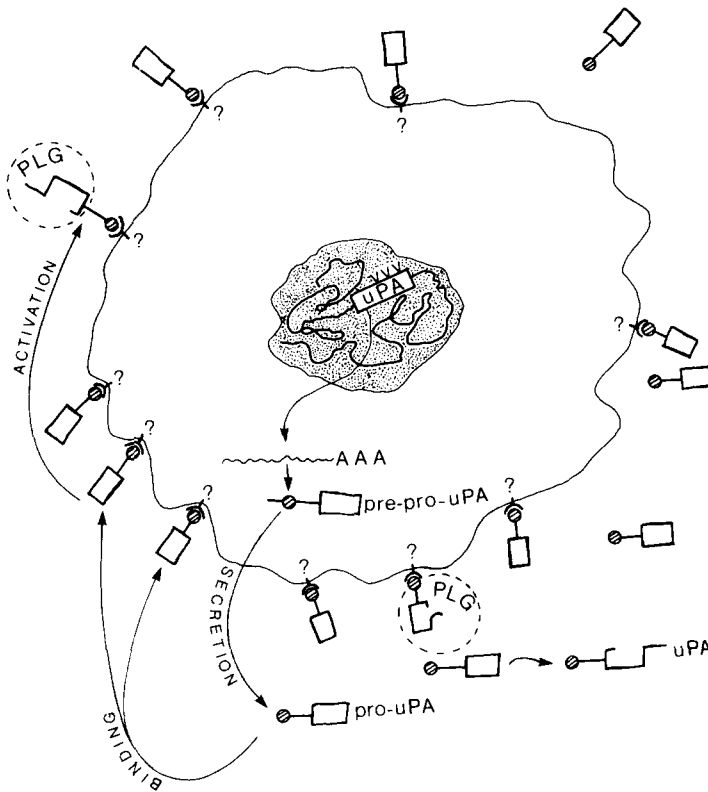


Fig. 3. Schematic representation of synthesis, secretion, and receptor binding of pro-uPA. The possibility of activation of pro-uPA to uPA is shown as a breakpoint in the rectangular region representing the catalytic portion of uPA. Dashed circles represent the receptor binding site. PLG, Plasminogen. The question marks indicate our lack of knowledge of receptor structure and function.

Alternatively, the binding of pro-uPA to the receptor might induce a conformational change that will activate pro-uPA with no need for a proteolytic cleavage. Whatever the mechanism, the advantage of an active receptor-bound uPA would be obvious. First, specific plasminogen activator protein inhibitors do not affect membrane-associated uPA activity, while totally blocking that of the secreted enzyme [19]. Second, the endothelial plasminogen activator inhibitor complexes the two-chain form of uPA, not the single-chain pro-uPA [20] and does not dissociate bound uPA (our unpublished data).

The identification of the binding sequence will now allow experiments directed toward the understanding of the function of the uPA receptor in normal and malignant cells, under physiological or artificial conditions.

REGULATION OF THE NUMBER OF uPA RECEPTORS

We have observed that substances that act as either mitogenic or differentiating agents can increase the number of uPA binding sites in U937 [3] and A431 cells. U937 monocytes can be differentiated to macrophage-like cells by the addition of

phorbol-myristate-acetate (PMA): the cells acquire the ability to adhere to the plastic culture dish, to enter in contact and phagocyte foreign particles such as Latex or Zymosan, to express Fc receptors, and to synthesize nonspecific esterases, etc. We have observed that cells treated with PMA for 48 hr also express the uPA receptor at about a ten-fold higher level [3]. The increase in binding sites results from the presence of a higher number of receptors exposed on the U937 cell surface: this is shown by cross-linking experiments using succinimidyl-suberate. This compound specifically cross-links ^{125}I -ATF to an approximately 55,000-dalton protein. The increase in uPA receptors in PMA-differentiated cells results in a drastic increase of the cross-linked protein band. Whether this is due to increased receptor synthesis or to a relocalization of this protein onto the cell surface remains to be established.

Increase of uPA receptor by treatment of cells with PMA is not limited to U937 cells. A431 epidermoid carcinoma cells also respond to PMA treatment, increasing the number of uPA receptors. The same effect is observed with epidermal growth factor. It must be noticed, however, that neither PMA nor EGF has any known differentiating effect on A431 cells. The kinetics of the effect of PMA and EGF on A431 uPA receptors closely resembles that of PMA on U937 cells.

CONCLUSIONS

The identification of the uPA receptor, the way through which it interacts with uPA (ie, occurring via a specific domain not involved in enzyme activity), and the absence of internalization all point to this molecule as a specific anchorage site for uPA on the cell surface. This suggests a role for the uPA-uPA receptor complex in the extracellular proteolysis required for the invasive behaviour of both normal and malignant cells. The identification of the amino acid sequence of uPA responsible for binding the receptor will allow a molecular approach to demonstrate a physiological and/or pathological function for this interaction.

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