

Urokinase-Type Plasminogen Activator: Proenzyme, Receptor, and Inhibitors

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NEW information has recently become available that we believe may be of value for a more precise focusing of research on the role of plasminogen activators (PAs) in cellular physiology and pathology. PAs convert the abundant extracellular zymogen plasminogen into plasmin, an active protease that, directly or indirectly, can promote degradation of all components of the extracellular matrix. The urokinase-type PA (u-PA) is one of two activators with similar catalytic specificities that have been described in each mammalian species so far studied. Many cell types synthesize and secrete u-PA at different stages in their life cycle; it has been a consistent finding that the expression of u-PA-catalyzed proteolysis is under the control of hormonal-type mediators, the nature of which depends on the cell population considered. u-PA is thought to play a central role in regulating extracellular proteolysis in a variety of normal and pathological processes involving tissue destruction and cell migration, e.g., organ involution, inflammatory reactions, and invasive growth of trophoblasts and cancer cells (for reviews, see 8, 18, 44, 47, 49, 50). Recent findings show that u-PA is synthesized and released from mammalian cells as a proenzyme with little or no activity (pro-u-PA), that cells possess a specific binding site for u-PA and pro-u-PA, and that they can also synthesize different rapidly acting, high affinity inhibitors of PAs. Thus, activation of pro-u-PA, u-PA receptor occupancy, and PA-inhibitors may finely regulate the location and extent of plasminogen activation.

The Proenzyme

The human u-PA gene, located on chromosome 10, is 6.4-kb long. It is organized in 11 exons and gives rise to a 2.5-kb-long mRNA, which in turn yields a single-chain glycosylated polypeptide of ~50,000 D (25, 45, 48, 52, 63, 67). Pig and mouse u-PA show strong homologies with the human enzyme; mouse u-PA, however, contains no N-glycosylation site (7, 37). It is the single-chain form of u-PA that is released from a variety of cultured normal and neoplastic cell types (20, 29, 39, 55, 59, 64, 70), and that appears to be the predominant extracellular form of u-PA in the intact organism (30, 54). Single-chain pro-u-PA is converted by limited proteolysis into an active M_r 50,000 enzyme consisting of two polypeptide chains held together by one disulfide bond; this conversion can be catalyzed by plasmin. Results from several laboratories have demonstrated that single-chain u-PA is a zymogen that in contrast to the two-chain active enzyme has little or no proteolytic activity against the natural substrate

plasminogen, does not react with macromolecular antiproteases, lacks amidolytic activity against synthetic plasminogen activator substrates, and is unable to react with the active site titrant diisopropylfluorophosphate (2, 18, 26, 29, 39, 55, 64, 70). It has recently been reported (33, 60), however, that certain purified preparations of single-chain u-PA may also have catalytic activity; recombinant single-chain u-PA produced in *Escherichia coli* had a catalytic efficiency superior to that of the two-chain enzyme, and single-chain u-PA purified from different mammalian sources had variable and low, but detectable, catalytic activity. The basis for these differences in the activity of single-chain u-PA preparations is not known. It may be related to differences in the extent of glycosylation, in the three-dimensional folding, or in a partial degradation of the protein, depending on the cell of origin or the procedure of purification.

The u-PA Receptor

Several types of cells possess specific high affinity (K_d ~ 10^{-10} M) plasma membrane binding sites for u-PA (9, 57, 66). Human peripheral blood monocytes, the monocyte-like U937 cell, fibroblasts, and HT1080 fibrosarcoma cells all have ~ 10^5 such specific receptors per cell. Receptor binding does not require u-PA catalytic activity, and single-chain u-PA binds with about the same affinity as does the M_r ~50,000 two-chain enzyme. The region of u-PA that binds to the receptor is located on the 135-residue-long amino terminal fragment of the A-chain; this amino terminal fragment peptide, which comprises two structural domains, a "growth factor" region with homology to EGF and a kringle (43), binds to the receptor with the same affinity as does intact u-PA. Recent data show that the binding region is located within the second loop of the growth factor domain (4). In contrast, the M_r ~33,000 form of u-PA, a partial degradation product of the M_r ~50,000 enzyme that lacks the amino-terminal fragment but maintains catalytic activity, does not interact with the receptor. Bound u-PA does not dissociate rapidly from the cell surface ($t_{1/2}$ >5 h), nor is it appreciably endocytosed and/or degraded. The bound enzyme maintains its plasminogen activating ability, and binding of both one-chain and two-chain u-PA to the u-PA receptor on cultured human monocytes-macrophages results in the generation of plasmin-mediated pericellular proteolysis. Thus the presence of a u-PA receptor allows cells to acquire surface-bound plasminogen-activating ability. It will be of interest to determine whether the receptor-bound one-chain protein itself catalyzes plasmin formation, or whether it is first cleaved to the two-chain enzyme. The receptor-bound single

1. *Abbreviations used in this paper:* PA, plasminogen activator; u-PA, urokinase-type plasminogen activator.

chain pro-u-PA can be converted to two-chain u-PA by plasmin with high efficiency (16).

Cultured human monocytes and monocyte-like U937 cells do produce u-PA, although at a rather low rate. In contrast, many malignant cells produce high levels of u-PA. Do they express a u-PA receptor? Human epidermoid carcinoma-derived A431 and fibrosarcoma HT1080 cells bind little or no ¹²⁵I-labeled u-PA. However, uncovering of u-PA receptors can be achieved by dissociation of single-chain pro-u-PA from the cell surface by short exposure to low pH conditions (6, 58). These cells, therefore, possess u-PA receptors that are very similar to, and possibly identical with, those of U937 cells, except that they are fully occupied by cellular pro-u-PA. Immunochemical and kinetic studies have shown that pro-u-PA is first secreted from A431 cells and then binds to the plasma membrane (58). Thus, cells that produce little or no u-PA may have free u-PA receptors, while those that produce larger amounts of the proenzyme have receptors constantly saturated through an autocrine process, and may now release excess u-PA into the extracellular milieu.

PA Inhibitors (PAI)

Two distinct protease inhibitors specific for PAs have recently been identified and purified. One of these, PAI type-1, is released by endothelial cells and some neoplastic cell lines (2, 21, 23, 32, 34, 62, 65); it is also present in thrombocytes and blood plasma (22, 68). The other, PAI type-2, first purified from placenta extracts (5), is also released by cultured monocytes-macrophages (12, 31, 51, 56, 64, 69). These two inhibitors are proteins of $M_r \sim 50,000$; they differ in immunological reactivity and in some physiological characteristics, such as activation by denaturing agents (27). Recent protein and c-DNA sequence data (3, 24, 31, 41, 42) have shown that both inhibitors belong to the serine protease inhibitor family (serpins, see reference 11), PAI type-1 being an Argserpin with arginine at its reactive center (3). A third PA inhibitor is the fibroblast-produced protease-nexin that, in contrast to the PA-specific inhibitors, also inhibits plasmin, thrombin, and other trypsin-like serine proteases (53); complexes of protease-nexin with proteases bind to fibroblasts in a manner that is independent of the u-PA receptor, and are endocytosed and degraded (35). These three PA inhibitors react with u-PA, but not with pro-u-PA (2, 20, 64). Several cultured cell types produce both pro-u-PA and one of the PA inhibitors; the proenzyme and the inhibitors have been found to coexist in the extracellular milieu. The reaction rate of the three inhibitors for u-PA is several orders of magnitude higher than that of the major plasma protease inhibitors; it is thus likely that they play an important part in controlling u-PA-initiated extracellular proteolysis *in vivo*. It is noteworthy that in endotoxin-treated macrophage cultures a PA inhibitor is induced, which inhibits the secreted enzyme but does not affect the cell-associated (presumably receptor-bound) activity (13). Furthermore, the addition of protease nexin or of anti-u-PA antibodies blocks the degradation of fibroblast-produced extracellular matrix by human HT 1080 cells (10). The role of the u-PA receptor in this reaction is certainly one of the first questions that should be posed.

The endothelial cell inhibitor is proteolytically converted to an inactive form by u-PA (38) and it may be speculated that, apart from inhibiting PA, this inhibitor, its conversion

product, or a putative released peptide may have other functions. Another exciting subject for further study is the regulation of inhibitor production; hormones and growth factors have been found to affect inhibitor levels in different cell types (1, 14, 15, 17, 19, 36, 40).

Modulation of u-PA Activity on Cell Surfaces and in Body Fluids

To summarize our current understanding of the cell biology of u-PA, it is now established that: (a) the product of the u-PA gene is a single-chain proenzyme with little or no activity, which is released as such from at least some cell types; (b) released pro-u-PA can be bound to cell surfaces via a high affinity binding site; (c) pro-u-PA does not react with PA inhibitors, unlike active two-chain u-PA.

These data suggest a model, probably oversimplified, in which receptor-associated u-PA is the active form in cell migration and tissue destruction. Extracellular pro-u-PA interacts with its plasma membrane receptor, and, after activation, triggers localized proteolysis. It is possible that the binding to the receptor may either render the proenzyme more susceptible to activation by other factors (plasmin, u-PA itself, or other still unidentified components of the system), or directly induce activity in single-chain u-PA, e.g., by an allosteric effect. Furthermore, receptor-bound two-chain u-PA may be protected from certain inhibitors, in contrast to free two-chain u-PA that is rapidly inactivated by PA inhibitors.

In any event, it is clear that u-PA binding to its plasma membrane receptor results in focalization of plasminogen activation to the close environment of the cell surface; this appears as an optimal configuration if u-PA is to participate in the proteolytic events that are required for cell migration. In this respect, and although a discussion of the biology of the other mammalian plasminogen activator tissue-type PA is beyond the scope of the present review, it should be pointed out that tissue-type PA binds to fibrin (28, 46, 61); this interaction, which increases the enzyme's catalytic activity, suggests a preferred role for tissue-type PA in the maintenance of fluidity of the extracellular milieu.

Implications and Perspectives

Conflicting data have been obtained relating production of u-PA to malignancy. In light of the most recent information, this question should be reinvestigated; studies that include determination of two-chain versus single-chain forms of u-PA, of receptor distribution and occupancy, and of PA inhibitors are likely to give a clearer picture of the role of u-PA in tumor biology. Similarly, the multiple steps at which u-PA-catalyzed proteolysis can be modulated in response to growth factors and hormones can now be dissected at the molecular level. Finally, the discovery of a u-PA receptor and of PA-specific inhibitors suggests possible approaches for interfering with the cascade reactions leading to plasminogen activation, and may thus help in designing a therapeutic regimen for the control of extracellular proteolysis.

The evolving picture of the increasing number of factors involved in plasminogen activation is reminiscent of the unraveling of other proteolytic cascades, e.g., the coagulation and complement systems. It is likely that additional factors will be discovered that will complete our, at present, frag-

mentary understanding of the biochemistry of this system. For example, the activation of pro-u-PA under physiological conditions is still poorly understood. The possible role of u-PA receptors in this process appears an attractive target for further studies.

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