Regulation of Macrophtage Receptor-bound Plasmin by Autoproteolysis

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(Received for publication, October 4, 1994)

The activation of plasminogen by macrophage is regulated by their expression of receptors for urokinase and plasminogen. In these studies we have examined plasminogen binding to adherent human THP-1 macrophage. Plasmin bound to the THP-1 cells in a time- and dose-dependent manner (Kd 15.8 ± 6.3 nM; Ebmax, 1.4 ± 0.3 x 10^6/cell). The lysine analog e-aminocaproic acid competitively inhibited plasmin binding. The fraction of membrane-bound plasmin, however, became increasingly resistant to displacement with e-aminocaproic acid. Over a 24-h period, membrane-bound plasmin activity fell 80% despite the presence of catalytically active plasmin in the incubation media. The loss of receptor-bound plasmin activity was not due to proteolytic alterations of its receptor since [125I]-Lys-plasminogen bound to THP-1 cells pretreated with plasmin with similar affinity as to untreated cells. Following a 24-h incubation of [125I]-Lys-plasminogen or [125I]-plasmin with THP-1 cells, several degradative fragments were apparent in their conditioned media. The smaller degradative fragments (28 and 36 kDa) lacked cell binding activity and were demonstrated to be active by casein-zymography. A 48-kDa fragment bound to cells in a lysine-dependent manner but was not active. In contrast, phenethylsulfonyl fluoride-inactivated [125I]-plasmin retained its binding activity over 24 h, and degradative fragments were not present in the conditioned media. The binding of [125I]-Lys-plasminogen to THP-1 cells was also examined in the presence of excess α2 plasmin inhibitor. Despite the absence of fluidphase plasmin activity, membrane-bound [125I]-Lys-plasminogen (ogen) decreased over 24 h. At 24 h a radiolabeled 48-kDa fragment was observed in the conditioned media and together with [125I]-Lys-plasminogen (ogen) was bound to cells. Unlike [125I]-Lys-plasmin, the 48-kDa fragment did not form a complex with α2 plasmin inhibitor. Thus, autoproteolysis of receptor-bound plasmin results in fragments with truncated physiologic properties that possess either cell binding or catalytic activities. We propose that autoproteolysis is a mechanism for regulating membrane-bound plasmin activity.

The abbreviations used are: TGF-β, transforming growth factor beta; uPA, urokinase-plasminogen activator; α2-antiplasmin, α2-A, α-aminocaproic acid; FBS, fetal bovine serum; DPBS, Dulbecco's phosphate-buffered saline; MSFM, macrophage serum-free media; PMSF, phenylmethylsulfonyl fluoride; PAGE, polyacrylamide gel electrophoresis.

1 The abbreviations used are: TGF-β, transforming growth factor beta; uPA, urokinase-dependent plasminogen activation; α2-ACA, α-aminocaproic acid; FBS, fetal bovine serum; DPBS, Dulbecco's phosphate-buffered saline; MSFM, macrophage serum-free media; PMSF, phenylmethylsulfonyl fluoride; PAGE, polyacrylamide gel electrophoresis.
Regulation of Macrophage Receptor-bound Plasmin

Dose- and time-dependent binding of plasmin to adherent THP-1 cells. Cells were plated into 96-well plates (1 x 10^5/well) in RPMI 1640 supplemented with 10% FBS. The next day media were removed and cells washed three times with DPBS. Left panel, cells were incubated with plasmin (0.1-40 nM) at 37°C for 2 h ( ), or plasmin and 25 nM e-ACA to block lysine-dependent binding ( ). Right panel, cells were incubated with 1.0 pg/ml plasmin for 0-2 h ( ) followed by a 10-min exposure to e-ACA to displace membrane-bound plasmin ( ). Membrane-bound plasmin was determined as described under "Materials and Methods." Data represent the mean ± S.E. of six separate wells.

RESULTS

Binding of Plasmin and Plasminogen to THP-1 Cells—A variety of cell types, including monocytes, express large numbers of relatively low affinity binding sites for plasminogen (16-24, 26). The binding of plasminogen appears to be lysine dependent since the lysine analog e-ACA competitively blocks plasminogen binding to cells. In these experiments, we have measured the binding of catalytically active plasmin to adherent THP-1 macrophage. As seen in Fig. 1, plasmin binds to adherent THP-1 cells in a dose- and time-dependent manner. The dose-dependent plasmin binding is completely blocked if e-ACA is present during the incubation period. Based on Scatchard analysis of four separate experiments, the estimated Kd of binding was determined to be 15.8 ± 6.2 nM (mean ± S.E.) with 1.4 ± 0.26 x 10^8 binding sites/cell. The observed binding affinity of plasmin to the THP-1 macrophage was significantly greater than reported for plasminogen binding to either endothelial cells (Kd = 210 ± 235 nM) (19) or suspension cultures of U937 monocytes (Kd = 800 ± 500 nM) (16). However, it was of similar magnitude to that reported for plasmin binding to endothelial cells (Kd = 77 ± 18 nM) (19). The plasminogen in the FBS used to grow the THP-1 cells did not affect plasmin(ogen) binding. Results of at least 20 experiments have demonstrated that THP-1 cells incubated with 10% FBS alone had trace or no detectable plasmin(ogen) on their surfaces by Western blot and/or activity measurements (data not shown).

Although e-ACA competitively inhibits plasmin binding to...
adherent THP-1 cells (Fig. 1, left panel), the fraction of membrane-bound plasmin that is resistant to subsequent displacement with e-ACA increases with time (Fig. 1, right panel). In addition, adherent THP-1 cells were incubated with plasmin for 1–24 h and membrane-bound plasmin determined. There was a time-dependent loss of THP-1 membrane-bound plasmin activity (Fig. 2). Following 24 h of incubation, ~80% of the membrane-bound plasmin activity observed at 1 h was lost. Furthermore, the remaining membrane-bound plasmin (20%) was not susceptible to displacement by e-ACA.

In an effort to identify the mechanism(s) responsible for the observed loss of enzymatically active plasmin from the THP-1 surface, we determined whether the binding properties of the THP-1 plasminogen receptor were sensitive to plasmin cleavage. For this purpose, the binding of 125I-Lys-plasminogen to cells preincubated with plasmin was determined (Fig. 3). Lys-plasminogen is the plasmin-modified form of Glu-plasminogen (26, 31). Lys-plasminogen is formed at the cell surface and is the form of plasminogen that is preferentially bound (26). The binding of 125I-Lys-plasminogen to plasmin-treated THP-1 cells was similar to the binding of plasmin to untreated cells (Fig. 1). Based on Scatchard analysis of four separate experiments, the estimated KD of binding is 14.7 ± 1.41 nM, and the numbers of binding sites is 1.96 ± 0.30 x 10^6/cell. Therefore, plasmin-mediated proteolysis of the plasminogen receptor could not be responsible for the observed loss of plasmin from the cell surface.

Autoproteolysis Leads to Catalytically Active Plasmin without Cell Binding Activity—We determined whether the region of plasmin responsible for cell binding is lost or modified during an extended incubation with adherent THP-1 macrophage. Cells were incubated with plasmin (1 μg/ml) for 24 h after which their conditioned media were recovered and assayed for remaining plasmin activity. The binding of 24 h conditioned plasmin to freshly plated THP-1 cells was compared to control plasmin (Table I). THP-1 cells incubated with control plasmin for 1 h bound >100-fold more plasmin than cells incubated with conditioned plasmin. These data suggest that either cells are releasing an inhibitor of plasmin binding, or plasmin itself is modified during an extended incubation with the THP-1 cells. In order to determine whether THP-1 cells released an inhibitor of plasmin binding to its receptor, fresh plasmin was added to the 24-h conditioned media and membrane-bound plasmin determined. As seen in Table I, the binding of plasmin to THP-1 cells in the presence of conditioned media was restored by the addition of fresh plasmin. Furthermore, the ability of plasmin to bind to cells was lost when plasmin was incubated in the absence of cells for 24 h. Therefore, we conclude that plasmin can form an enzymatically active species that is not recognized by the plasminogen receptors.

A possible mechanism for the observed loss in plasmin’s cell binding properties is autoproteolysis. Therefore, we examined SDS-PAGE autoradiography alterations in 125I-Lys-plasminogen incubated with adherent THP-1 cells for 1 and 24 h. Under nonreducing conditions, the major fraction of labeled plasminogen in the 1-h conditioned media co-migrated with intact 125I-Lys-plasminogen (Fig. 4). Radiolabeled degradative fragments between 48 and 40 kDa, which were absent in the starting material, became visible following 1 h of incubation with THP-1 cells. In contrast, at 24 h the majority of the radiolabeled plasminogen had shifted to the 48- and 40-kDa peptide fragments. Smaller degradative fragments, as well as intact 125I-Lys-plasminogen were faintly visible at 24 h.

Identification of Active Plasmin Fragments by Zymography—In order to determine which of the plasmin fragments in THP-1-conditioned media were enzymatically active, cells were incubated with unlabeled plasminogen for 24 h and analyzed by SDS-PAGE zymography. Plasmin fragments expressing proteolytic activity degrade casein included in the

**Table I**

<table>
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<th>Loss of plasmin's cell binding activity</th>
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<td><strong>Media activity μg/ml</strong></td>
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<td>Pls t0</td>
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<tr>
<td>1000</td>
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<tr>
<td>Pls t1</td>
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<tr>
<td>946 ± 75</td>
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<td><strong>Membrane-bound activity ng/ml</strong></td>
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<tr>
<td>THP-1</td>
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<td>0.03 ± 0.1</td>
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<tr>
<td>THP-1 + Pls t0</td>
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<td>16.69 ± 0.05</td>
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<td>THP-1 + Pls t1</td>
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<td>0.13 ± 0.01</td>
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<td>THP-1 + Pls t2</td>
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<td>13.37 ± 0.54</td>
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<td><strong>Active Plasmin Fragments by Zymography</strong></td>
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<td>—— In order to determine which of the plasmin fragments in THP-1-conditioned media were enzymatically active, cells were incubated with unlabeled plasminogen for 24 h and analyzed by SDS-PAGE zymography. Plasmin fragments expressing proteolytic activity degrade casein included in the</td>
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polycrylamide gel. Areas of casein digestion appear unstained following staining of the gel with Coomassie Blue. As positive controls, both plasminogen and plasminogen preincubated with uPA for 1 h were examined. Plasmin activity in the plasminogen preparation was clearly evident in the zymograph (Fig. 5). When plasminogen was activated by 1 h of preincubation with uPA, a wide range (80-20 kDa) of enzymatically active plasmin fragments were formed. In contrast, conditioned media recovered from THP-1 cells incubated with plasminogen for 24 h contained fewer degradative forms of enzymatically active plasmin. Several high and two lower molecular weight caseinolytic bands were observed. The lower molecular mass caseinolytic bands (28 and 36 kDa) correspond to the low molecular mass 125I-labeled plasminogen fragments observed in the media of THP-1 cells at 24 h (Fig. 4). However, caseinolytic activity did not co-migrate with a prominent radiolabeled fragment of plasmin (i.e. 48 kDa). When media derived from THP-1 cells cultured in the absence of plasminogen were analyzed by SDS-PAGE zymography, no caseinolytic bands were observed (data not shown). Taken together, (Table 1, Figs. 4 and 5), these data demonstrate that following activation, plasmin autoproteolysis results in the generation of proteolytically active fragments that do not express cell binding properties.

PMSF-inactivated 125I-Plasmin Does Not Lose Its Cell Binding Properties—If autoproteolysis is responsible for the loss in cell-bound plasmin, then the binding of inactivated 125I-plasmin to cells would not be expected to change over time. Therefore, we compared the binding of 125I-plasmin and PMSF-inactivated 125I-plasmin to adherent THP-1 cells at 1 and 24 h (Fig. 6). By autoradiography, the radiolabeled plasmin preparation contained a 48-kDa degradative fragment. A similar degradative fragment was observed in the conditioned media when THP-1 cells were incubated with 125I-Lys-plasminogen for 24 h (Fig. 4). Following a 1-h incubation, both intact plasmin and the 48-kDa radiolabeled fragment. As observed utilizing 125I-Lys-plasminogen as a tracer

**Fig. 4.** Degradative alterations of 125I-Lys-plasminogen incubated with THP-1 cells. Cells were plated into 12-well plates (5 x 10⁵/well) as described in Fig. 1. Following adherence the cells were washed and media replaced with MSFM containing 1 μg/ml 125I-Lys-plasminogen. Conditioned medium was recovered at 1 and 24 h. Alterations in molecular weight of 125I-Lys-Plasminogen was determined by SDS-PAGE followed by autoradiography.

**Fig. 5.** Zymographic identification of enzymatically active plasmin fragments. Cells were plated into T-25 flasks (5 x 10⁶) as described in Fig. 1. Following adherence the cells were washed and media replaced with MSFM containing 1 μg/ml plasminogen. The conditioned medium was collected 24 h later and concentrated 5:1 by ultrafiltration as described under "Materials and Methods." SDS sample buffer was added to aliquots of THP-1-conditioned medium, plasminogen and plasminogen preincubated (1 h) with high molecular weight uPA. Samples were electrophoresed in composite polyacrylamide-casein gels. Active plasmin fragments were identified by zymography.

**Fig. 6.** Effect of PMSF inactivation on the binding of 125I-plasmin to THP-1 cells. Cells were plated into 6-well plates (2 x 10⁶/well) as described in Fig. 1. Following adherence the cells were washed and media replaced with MSFM containing 1 μg/ml 125I-plasmin or PMSF-inactivated 125I-plasmin. Conditioned media and cell lysates were recovered at 1 and 24 h. Alterations in molecular weight of 125I-plasmin was determined by SDS-PAGE followed by autoradiography. (Fig. 4), at 24 h the majority of the 125I-plasmin was recovered in the 48-, 46-kDa, and several other smaller degradative fragments (Fig. 6). Associated with the formation of degradative fragments of 125I-plasmin, at 24 h 125I-plasmin recovered from THP-1 cells was dramatically reduced as compared to 1 h. In
marked contrast, no degradative change in the active site inhibited \(^{125}\text{I}\)-plasmin was observed in the media over 24 h, and similar amounts of PMSF-inactivated \(^{125}\text{I}\)-plasmin were bound to cells following 24 h of incubation (Fig. 6). The \(\varepsilon\)-ACA-resistant fraction of PMSF-plasmin was comparable to that of untreated plasmin. These data suggest that autoproteolysis can regulate cell binding.

Loss of Anti-kringle IgG Binding to Autoproteolysed Plasmin—Figs. 4 and 6 demonstrate the generation of plasmin degradation fragments over a 24-h incubation with THP-1 cells. However, in both experiments small quantities of intact plasminogen were present in the conditioned media at 24 h. The lack of substantial membrane-bound activity at 24 h (Fig. 6), despite the presence of material that co-migrates with intact plasminogen, suggests that other alterations in plasminogen may have taken place. Since the kringle domains of plasminogen’s A-chain mediate its binding to both fibrin and endothelial cells (20, 21), we determined whether plasmin autoproteolysis was associated with a loss of reactivity to a monoclonal anti-kringle IgG. When conditioned media from THP-1 cells incubated with plasmin for 1–24 h were examined by Western blot, a clear time-dependent reduction in kringle immunoreactivity of recovered plasmin was observed (Fig. 7). Little or no intact immunoreactive plasmin could be demonstrated at 24 h. These data suggest that the failure of the remaining intact plasminogen in the 24-h conditioned media to bind THP-1 cells was due to alterations in its cell-binding domain(s).

Plasmin Autoproteolysis Occurs on the Cell Surface in the Presence of Excess \(\alpha_2\) Anti-plasmin—Under physiologic conditions fluid-phase plasmin will be rapidly inactivated by plasmin inhibitors (32). Therefore, it is not clear from experiments described above whether autoproteolysis could contribute to the physiologic regulation of membrane-bound plasmin activity. Consequently, we determined whether autoproteolysis would occur on the cell surface in the presence of 20-fold molar excess of \(\alpha_2\) plasmin inhibitor. THP-1 cells were preincubated with TGF-\(\beta\) overnight to up-regulate their expression of both uPA and the uPA receptor.\(^2\) However, TGF-\(\beta\) does not affect plasminogen binding.\(^2\) When TGF-\(\beta\)-primed macrophages were incubated with media containing \(^{125}\text{I}\)-Lys-plasminogen and \(\alpha_2\) plasmin inhibitor for 1 h, the conditioned media contained two major radiolabeled bands (~80 and 140 kDa). The lower molecular weight band comigrated with intact \(^{125}\text{I}\)-Lys-plasminogen. We have concluded that the higher molecular weight band is an SDS-resistant complex of \(^{125}\text{I}\)-Lys-plasmin and \(\alpha_2\) plasmin inhibitor (65–70 kDa) for three reasons: \(\alpha_2\) plasmin inhibitor rapidly forms a denaturation-resistant complex with plasmin (32). This band was not observed in the absence of \(\alpha_2\) plasmin inhibitor. Plasmin activity was undetectable in the media samples containing \(\alpha_2\) plasmin inhibitor (data not shown). At 1 h intact \(^{125}\text{I}\)-Lys-plasminogen was found associated with the THP-1 cells. Exposure of the cells with \(\varepsilon\)-ACA released a majority of the bound \(^{125}\text{I}\)-Lys-plasminogen, however, these differences are not readily apparent because films were overexposed to visualize other radioactive fragments.

The 24-h conditioned media also contained intact \(^{125}\text{I}\)-Lys-plasminogen and complexes of \(^{125}\text{I}\)-Lys-plasmin and \(\alpha_2\) plasmin inhibitor; however, the proportion of \(^{125}\text{I}\)-Lys-plasmin associated with \(\alpha_2\) plasmin inhibitor was increased. In addition, despite the absence of detectable plasmin activity in the fluid-phase, degradative fragments of \(^{125}\text{I}\)-Lys-plasminogen were

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**FIG. 7. The loss of anti-kringle IgG binding to plasmin.** Cells were plated into 12-well plates (0.5 x 10\(^5\)well) as described in Fig. 1. Following adherence the cells were washed and media replaced with MSFM containing 1 µg/ml plasmin. Conditioned media were recovered at 1–24 h and the immunoreactivity of recovered plasmin was determined by Western blot utilizing monoclonal anti-kringle IgG.

**FIG. 8. Effect of \(\alpha_2\) plasmin inhibitor on the binding of \(^{125}\text{I}\)-Lys-plasminogen to THP-1 cells.** Cells were incubated with TGF-\(\beta\) (5 ng/ml) overnight, harvested, and plated into 12-well plates (5 x 10\(^5\)well) as described in Fig. 1. Following adherence the cells were washed and media replaced with MSFM containing TGF-\(\beta\), 1 µg/ml \(^{125}\text{I}\)-Lys-plasminogen, and 20 µg/ml \(\alpha_2\) plasmin inhibitor. Conditioned media were recovered at 1 and 24 h. Alterations in molecular weight of \(^{125}\text{I}\)-Lys-plasminogen were determined by SDS-PAGE followed by autoradiography.

**FIG. 9. A model depicting autolytic regulation of receptor-bound plasmin activity.** Plasminogen and uPA bind to their respective receptors. Plasminogen initially binds to the cell surface in a lysine-dependent manner via its kringle domains. Plasminogen is preferentially cleaved by uPA on the cell surface generating catalytically active plasmin. Plasmin which has dissociated from its receptor is inhibited by \(\alpha_2\) plasmin inhibitor. Membrane-bound plasmin is autoproteolysed generating inactive fragments that remain bound to the receptor. Catalytically active fragments released as a consequence of autoproteolysis are inhibited by \(\alpha_2\) plasmin inhibitor. A fraction of membrane-bound plasmin becomes resistant to displacement by the lysine analog \(\varepsilon\)-ACA and is protected from plasmin degradation.
visible. Similar degradation fragments of greater intensity were observed in the absence of α2 plasmin inhibitor. The amount of 125I-Lys-plasminogen bound to cells at 24 h was markedly reduced. In addition to intact 125I-Lys-plasminogen, a radiolabeled fragment also observed in the media was bound to the cells. Both the intact 125I-Lys-plasminogen and the fragment were displaced by exposure to ε-ACA indicating lysine-dependent binding. Since fluid-phase plasmin activity was completely inhibited by the excess of α2 plasmin inhibitor, these data demonstrate that plasmin autoproteolysis occurs on the cell surface despite the presence of excess plasmin inhibitor.

**DISCUSSION**

The broad substrate specificities of plasmin require that cells localize its activation in order to prevent collateral tissue damage. The directed activation of plasminogen by macrophage is affected by their expression of receptors for uPA and plasminogen (6, 18–19). The regulation of these receptors and the fate of ligands bound to them have great implications for a diverse group of macrophage functions including matrix remodeling, migration, and mobilization of matrix-bound growth factors.

In contrast to the uPA receptor (34–37), little has been reported concerning regulation of plasminogen receptors (38). In these studies we demonstrate that plasmin is subject to autoproteolysis which leads to a loss in receptor recognition but not catalytic activity. When THP-1 macrophages were incubated with either plasmin or plasminogen in serum-free media, membrane-bound plasmin activity declined dramatically over 24 h despite the presence of catalytically active plasmin in the media (Fig. 2, Table I). Plasmin-mediated proteolysis of the plasminogen (ogen) receptor was not responsible for the loss in membrane-bound plasmin activity since preincubation of THP-1 cells with plasmin did not affect binding of 125I-Lys-plasminogen (Fig. 3). When conditioned media from cells incubated with either 125I-Lys-plasminogen or 125I-plasmin were examined by SDS-PAGE and autoradiography, autoproteolysis of the radiolabeled material had clearly taken place (Figs. 4 and 5). Following a 24-h incubation with THP-1 cells, two major degradative fragments of 125I-Lys-plasminogen were visible at 48 and 40 kDa. In addition, faintly visible intact 125I-Lys-plasminogen and small degradative fragments were observed. Similar fragments were observed when cells were incubated with 125I-plasmin. Both intact 125I-plasminogen and the 48-kDa fragment were found associated with the THP-1 cells and could be displaced by ε-ACA (Fig. 6). Although the 48-kDa plasmin fragment bound THP-1 cells in a lysine-dependent manner, it did not appear to possess plasmin activity as judged by casein zymography (Fig. 5). In contrast to either 125I-Lys-plasminogen and 125I-plasmin, degradative fragments did not form when cells were incubated with PMSF-inactivated 125I-plasmin. Furthermore, similar amounts of PMSF-inactivated 125I-plasmin were bound to cells at 1 and 24 h (Fig. 6). Thus, it appears that under serum-free conditions, autoproteolysis dissociates plasmin into fragments with truncated physiologic properties that possess either cell binding or catalytic activities.

The modification of structure and biological properties of native (Glu)-plasminogen (92 kDa) by plasmin and other proteases has been described previously. A "preactivated" form of plasminogen termed Lys7-plasminogen (84 kDa) is obtained by plasmin-mediated cleavage of the NH2-terminal 76 amino acid peptide (39). Lys7'-plasminogen is formed on the cell surface and binds to cells with higher affinity than Glu'-plasminogen (26, 31). Likewise, the kinetics of Lys-plasminogen conversion to Lys7'-plasmin by tissue plasminogen activator is markedly enhanced (26). When plasmin was incubated in a protein-free aqueous buffer (pH 6.5), its catalytic activity decreases rapidly over time (40). The observed decrease in catalytic activity was due to autodegradation of the B-chain (26.5 kDa) which contains the catalytic domain of plasminogen (40). In contrast, most of the A-chain (63 kDa), which contains plasmin's five kringle domains, remained unaltered (40). Micro-plasmin (intact B-chain; 26.5 kDa) was obtained by incubating plasmin in a protein-free aqueous buffer at pH 11 (39, 40). At an alkaline pH, the B-chain was protected from degradation, whereas the A-chain was partially degraded to a protein of 58 kDa (40, 41). This partially degraded A-chain bound lysine-Sepharose demonstrating the preservation of functional kringle(s) that mediate lysine-dependent binding (40). Mini-plasminogen (38 kDa) containing kringle 5 and the B-chain was obtained by digestion of plasminogen with elastase (42).

Lysine-dependent plasminogen binding to both fibrin and endothelial cells is inhibited by kringle 1–5 (55 kDa) and mini-plasminogen, but not micro-plasminogen or kringle 1–3 (20, 21, 43). Kringle 4 does not exhibit significant binding to either fibrin or endothelial cells (20, 43). These data demonstrate that kringle 5 plays a pivotal role in the lysine-dependent binding of plasminogen to the endothelial cell surface and fibrin. In experiments reported here, autoproteolysis results in an enzymatically inactive plasmin fragment (46 kDa) that binds cells in a lysine-dependent manner and catalytically active plasmin fragments (~28 and 36 kDa) which are devoid of cell binding properties. These active fragments may represent micro-plasmin or mini-plasmin with an altered kringle conformation.

Plasminogen receptors provide a mechanism for localization of zymogen, its activation, and protection from inactivation (16, 19, 25). Since fluid-phase plasmin is rapidly inhibited by α2 plasmin inhibitor (kcat, 2.8 × 107 M−1 s−1) (32), it is not clear if autoproteolysis could play a role in regulating membrane-bound plasmin activity under physiologic conditions. Therefore, we examined whether autoproteolysis could take place in the presence of excess α2 plasmin inhibitor. When THP-1 cells were incubated with 125I-Lys-plasminogen and the plasmin inhibitor, intact membrane-bound 125I-plasminogen decreased dramatically over 24 h despite the absence of detectable fluid-phase plasmin activity (Fig. 8). As observed in the absence of the plasmin inhibitor, intact 125I-Lys-plasminogen and a radiolabeled 48-kDa fragment observed in the conditioned media were bound to the cells. The binding of both 125I-Lys-plasminogen and the 48-kDa fragment were lysine dependent since they were displaced by ε-ACA. The failure of the fragment to form a complex with α2 plasmin inhibitor corroborates our conclusion that it lacks an active site. Smaller radiolabeled fragments which were observed in the absence of α2 plasmin inhibitor and demonstrated to be active by zymography were not present. These data demonstrate that in the presence of plasmin inhibitors, plasmin autoproteolysis is restricted to the cell surface and generates fragments that possess either cell binding or catalytic activities.

In these studies, we have also demonstrated that plasmin binding to THP-1 cells is initially ε-ACA sensitive but becomes resistant to displacement with ε-ACA over time. Following a 24-h incubation with plasmin or 125I-Lys-plasminogen, all membrane-bound plasmin activity or 125I-Lys-plasminogen was resistant to ε-ACA displacement and further autoproteolysis. It has been recently reported that endothelial cells in suspension cross-linked plasmin to their surfaces via expression of transglutaminase activity (28). However, in these studies the inability of ε-ACA to displace plasmin bound to adherent THP-1 was not due to transglutaminase-mediated cross-linking, since cell-associated large molecular weight complexes containing 125I-Lys-plasminogen were not observed in SDS-PAGE. Thus, ε-ACA-resistant and transglutaminase-independent binding of
plasmin represents an undefined mechanism for localizing plasmin to the cell surface.

The current model of plasminogen binding and activation provides mechanisms for localization of zymogen, its regulated activation, and protection from inactivation. However, the model does not provide a mechanism for cell clearance of receptor-bound protease. Based on results of experiments reported here, we propose that autoproteolysis is a mechanism for regulating receptor-bound plasmin activity under conditions of high pericellular plasmin concentration (Fig. 9).

REFERENCES