# Plasmin cleaves the juxtamembrane domain and releases truncated species of the urokinase receptor (CD87) from human bronchial epithelial cells

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**Abstract The three-domain (D1D2D3) urokinase receptor (CD87) is highly susceptible to cleavage within the D1–D2 linker sequence, but also within the juxtamembrane region by yet poorly characterized proteinases, allowing the release of D1 and D2D3 species in various (patho)physiological body fluids. Using immunoblot analysis and ELISA applied to a recombinant soluble CD87 and to CD87-expressing epithelial cells, we establish that exogenous or in situ generated plasmin proteolyzes CD87 in the D1–D2 linker and D3 carboxyterminal sequences, producing a major soluble D2D3 species. Mass spectrometry analysis of the fragmentation of CD87-related synthetic peptides, and aminoterminal sequencing of D2D3 reveal $Arg^{83}$, $Arg^{89}$, and $Arg^{281}$ as residues targeted by plasmin within human CD87.**


*Keywords:* Urokinase-type plasminogen activator receptor; Plasmin; Proteolysis; Human bronchial epithelial cell

## 1. Introduction

In the lungs, as in many organs, the plasminogen (PLG) activation system is involved in various (patho)physiological processes (reviewed in [1,2]) such as the control of fibrinolysis, tissue remodeling during wound healing and fibrosis [3,4], as well as cell migration during cancer metastasis [5] and leukocyte recruitment during inflammation [6]. It operates through dynamic, highly regulated interactions between various molecular components including serine(Ser)-proteinases (reviewed in [2,7]). Indeed, the receptor-dependent colocalization of PLG and the urokinase-type PLG activator (uPA) on cell surfaces supports a reciprocal zymogen activation and induces a pericellular proteolytic cascade through conversion of PLG into active plasmin. Plasmin has a broad enzymatic activity and, either directly or through activation of matrix metalloproteinases (MMP), participates to the degradation of the extracellular matrices and basement membranes, and modulates the activity of extracellular effectors and cell membrane receptors [2,8–11].

CD87/uPAR, a highly glycosylated GPI-anchored membrane protein, is yet the only known high-affinity receptor for uPA. It is present on the surface of many cell types (e.g., leukocytes, vascular endothelial cells, smooth muscle cells and epithelial cells [12–16]) and displays three homologous domains (D1, D2 and D3 from the amino- to the carboxyterminus), each containing a conserved arrangement of disulfide bonds, and separated by short interdomain linker sequences (reviewed in [1,17]). Besides binding uPA, which requires the presence of domain D1 [17], CD87 also interacts with other extracellular ligands (e.g., vitronectin and various integrins) [18] and can express an intrinsic chemotactic activity localized within the D1–D2 linker sequence [1], thus contributing to the regulation of pericellular proteolysis as well as cellular adherence and migration (reviewed in [1,18]).

A major feature of CD87 is its susceptibility to endoproteolysis within the D1–D2 linker sequence, which contains cleavage sites for proteinases such as uPA itself, plasmin, and various MMP or leukocyte proinflammatory proteinases [19–22]. Accordingly, a soluble D1 domain can be detected in body fluids such as urine, and is markedly increased in pathological states associated with various types of carcinomas or infectious and inflammatory disorders [15,23,24]. However, soluble forms of full-length (D1D2D3) or truncated (D2D3) CD87 species are observed in urine and in plasma, and are similarly increased in diseases [5,23], for which they are now proposed as prognostic markers [23–26], although mechanistic explanations for their release remain scarce [22,27,28].

The present study provides new insights on the mechanism of release of the soluble CD87 species detected in (patho)physiological human fluids, by showing that not only the D1–D2 linker sequence, but also the carboxyterminus of CD87 is sensitive to plasmin.

## 2. Materials and methods

### 2.1. Materials

Polyhistidine-tagged recombinant human CD87 corresponding to the sequence $Leu^1$–$Arg^{281}$ [17] (rhCD87[$His]_6$) was from R&D Systems (Minneapolis, MN, USA). Purified pancreatic α-chymotrypsin (αCT,

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57 U/mg), bacterial phosphatidylinositol-specific phospholipase (PI-PL) C (5100 U/mg), phenylmethylsulfonyl fluoride (PMSF) and aprotinin were from Sigma–Aldrich (Saint-Louis, MO, USA). Purified human uPA (80 000 IU/mg), predominantly of the high molecular weight, two-chain form, and human Glu-PLG were from American Diagnostica (Greenwich, CT, USA). Purified human plasmin (10 caseinolytic units/mg) and its chromogenic substrate S-2251 (H-D-valyl-L-leucyl-lysine-*p*-nitroanilide dihydrochloride) were from Chromogenix (Milan, Italy).

Two synthetic peptides mapping the human CD87 sequences $Ser^{81}$–$Cys^{95}$ within the D1–D2 linker domain and $Asn^{272}$–$Gly^{283}$ at the carboxyterminus of D3 were prepared with >95% purity by Eurogentec (Seraing, Belgium): the 15-mer SGRAVTYSRSRYLEC and the 12-mer NHPDLDVQYRSG, thereafter designated as the D1–D2 and the D3 peptide, respectively.

The anti-CD87 mouse mAbs 3931 (anti-D1) and 3932 (anti-D2) were from American Diagnostica; His-probe (H-15), a rabbit polyclonal antibody against the $[His]_6$ peptide tag, was from Santa Cruz Biotechnologies (Santa Cruz, CA, USA); HRP-conjugated Abs against mouse or rabbit IgG (ImmunoPure® were from Pierce; Rockford, IL, USA).

### 2.2. Cells

16HBE14o$^-$ cells, hereafter designated as 16HBE, are human SV40-transfected bronchial epithelial cells [29] and are a gift from Dr. C. Gruenert (University of Vermont, Colchester, VT, USA). Cells were grown as previously described [30]. 16HBE cells were cultured to confluence in 24-wells plate and when needed, biotinylation of cell surface molecules was performed as previously reported [31]. Briefly, cell monolayers were washed with HBSS containing $CaCl_2$ and $MgCl_2$, 1 mM each (HBSS-CaMg), then were covered with 250 μl of PBS-CaMg, pH 8.0, containing sulfosuccinimidyl-6-(biotinamido)hexanoate (EZ-Link™ Sulfo-NHS-LC-biotin, Pierce), at 1 mg/ml. After 30 min of incubation, the medium was replaced by PBS containing 50 mM glycine in order to quench unreacted biotin, then cell monolayers were washed twice with HBSS, before further treatment. All steps were at 25 °C.

### 2.3. Exposure of recombinant CD87 or CD87-expressing cells to proteinases

$RhCD87[His]_6$ adjusted to 1 μg/ml (≈20 nM) in HBSS either was mixed with varying concentrations of purified proteinases or left untreated as control. Following incubation for 30 min at 37 °C, the enzymatic activity was neutralized by addition of 10 μM aprotinin (uPA, plasmin) or 1 mM PMSF (αCT).

16HBE cell monolayers, covered with 200 μl HBSS-CaMg, received varying amounts of proteinases and incubation continued at 37 °C for 15 min before blocking enzymatic activities as above. In some experiments, cells were incubated with 1 U/ml PI-PLC for 30 min under similar conditions. Extracellular fluids were collected and centrifuged at 18 000 × *g* for 30 min at 4 °C to eliminate cell debris and biotinylated 16HBE cell monolayers were solubilized at 4 °C for 30 min in a RIPA medium containing a cocktail of proteinase inhibitors [22]. Insoluble material was removed by centrifugation at 18 000 × *g* for 30 min at 4 °C and protein concentrations were measured using the BCA Protein Assay (Pierce).

### 2.4. uPA-mediated PLG activation on 16HBE cell monolayers

The procedure for plasmin generation on 16HBE cell monolayers was adapted from that reported for the bronchial epithelial cell line BEAS-2B [16]. 16HBE monolayers were acid washed in order to dissociate endogenous uPA from its receptor, then saturated for 2 h at 4 °C with 50 nM exogenous uPA in HBSS containing 0.5% BSA (w/v). Glu-PLG (30 μg/ml, ≈0.35 μM), or control PLG-free medium was then added in each well, and incubation continued for 15–60 min at 37 °C. Extracellular fluids were collected and centrifuged at 18 000 × *g* for 30 min at 4 °C. For plasmin enzymatic activity determination, 25 μl of fluids was added to 75 μl of the chromogenic substrate S-2251 (3 mM stock solution) in a 96-well microplate, then incubated at 37 °C. Plasmin activity was evaluated through kinetic analysis of paranitroanilide release measured as the variation of optic density at 405 nm and calculated relatively to a standard curve established with purified plasmin. In control experiments performed in the absence of cells, we checked that only the combination of uPA and PLG resulted in active plasmin generation, and that none of the reagents taken separately had either PLG-converting or S-2251-hydrolyzing activity.

### 2.5. Immunoblot analysis of recombinant, cellular or soluble CD87

When required, biotinylated membrane proteins were separated from non-biotinylated proteins on NeutrAvidin™-agarose beads (Pierce) as previously reported [22]. Proteins were solubilized in the presence of 2% (w/v) SDS and 5 mM *N*-ethylmaleimide for 5 min at 100 °C, and disulfide bonds were reduced by adding 5% (v/v) 2-mercaptoethanol before separation by SDS–PAGE and transfer onto a PVDF membrane [22]. Membranes were blocked with dried skimmed milk and probed with the primary antibody at the concentration indicated in the figure legends. Bound antibodies were detected by a HRP-coupled secondary antibody and chemiluminescent reaction (ECL+, Amersham Biosciences, Little Chalfont, UK).

### 2.6. Determination of cleavage sites for proteinases within CD87

*MALDI-MS.* CD87-related synthetic peptides were adjusted to 65 μM in HBSS and incubated at 37 °C in a 10 μl volume with purified proteinases in a peptide/enzyme molar ratio varying from 25 to 100, and for 5–15 min. Controls consisted of proteinase-free peptides or of proteinase dilutions alone incubated at 37 °C for the longest period of time. Enzymatic activity was stopped by acidifying the reaction mixture. Lyophilized samples were prepared for MALDI-MS, and measurements were taken on a Voyager-DE STR spectrometer (Applied Biosystems Inc., Framingham, MA, USA), exactly as previously reported [22]. Identification of the truncated peptides was carried out with the GPMAW software, version 6.0 (Lighthouse Data, Odense, Denmark).

*Aminoterminal amino acid sequencing.* $RhCD87[His]_6$ (0.1 mg/ml, ≈2 μM in HBSS) was incubated overnight at 37 °C with 10 nM of purified proteinases or left untreated as control [12,19]. Proteins were solubilized and reduced as described above before SDS–PAGE separation and transfer onto PVDF membranes. Protein bands visualized by Amido black staining were excised and subjected to aminoterminal microsequencing on an Applied Biosystem ABI 494 protein sequencer.

### 2.7. Detection of soluble CD87 by quantitative ELISA

The concentration of soluble CD87 (sCD87) in cell-free 16HBE extracellular fluids was determined using a specific quantitative ELISA kit, according to the manufacturer's instructions (Quantikine® ELISA for human uPAR, R&D Systems).

## 3. Results and discussion

### 3.1. CD87 is cleaved by plasmin in the D1–D2 linker sequence as well as at its carboxyterminus

The susceptibility of CD87 to purified proteinases was first assessed using a recombinant human molecule without the GPI moiety, but fused to a polyhistidine carboxyterminal tag. Immunoblotting analysis of the proteinase-treated $rhCD87[His]_6$ first confirmed the previously described susceptibility of the D1–D2 linker domain to uPA, plasmin and αCT, the latter used as a reference proteinase known to regulate the structure and function of CD87 [12,19,32,33]. Indeed, exposure to 250 nM of either purified enzyme for 30 min resulted in the disappearance of the full-length three-domain $rhCD87[His]_6$, which was accompanied by the production of a D2D3 truncated form (Fig. 1A) and the release of a free D1 domain, which, however, was further degraded by both αCT and plasmin into fragments undetectable in our assay (Fig. 1B). Cleavage of $rhCD87[His]_6$ was much less efficient when using uPA as compared to PL (Fig. 1A), unless extending the exposure of $rhCD87[His]_6$ to the proteinase up to several ( $\geqslant$ 12) h (data not shown), in agreement with previous observations [12].

We next examined whether αCT, uPA or plasmin has the capacity to cleave CD87 within the juxtamembrane region of the D3 domain, in addition to the D1–D2 linker sequence, as we recently reported for the neutrophil Ser-proteinase ca-

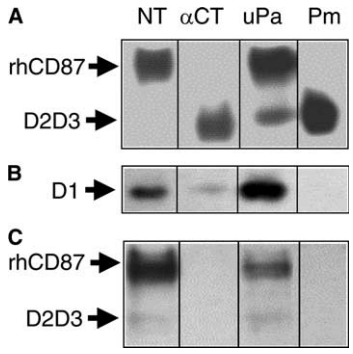


Fig. 1. rhCD87[His]$_6$ is proteolyzed within the D1–D2 linker sequence and at the carboxyterminus of domain D3. rhCD87[His]$_6$ was exposed at 37 °C to 250 nM αCT, uPA or plasmin (Pm), or to no proteinase (non-treated, NT) for 30 min, then electrophoresed (15 ng per well) by SDS–PAGE under reducing conditions, and immunoblotted using the anti-CD87 mAbs 3932 (anti-D2, 0.05 μg/ml, panel A) and 3931 (anti-D1, 1 μg/ml, panel B), or the His-Probe Ab (anti-[His]$_6$, 0.1 μg/ml, panel C) to probe the carboxyterminal polyhistidine tag. Portions of the films corresponding to the location of relevant antigens and representative of three experiments are depicted.

thepsin G [22], by testing whether the carboxyterminal polyhistidine tag was retained following exposure to proteinases. While intact, non-treated rhCD87[His]$_6$ produced signals of similar intensities whether reacted with the anti-D2 or the anti-[His]$_6$ antibodies (compare Figs. 1A and C), the D2D3 species generated by either αCT or plasmin were totally unreactive with the anti-[His]$_6$ probe. By contrast, exposure to uPA did not result in a similar drastic decrease of the tag-related signal, although converting some intact CD87 into D2D3 species. These results indicate that, while efficiently acting on the D1–D2 linker sequence, αCT and plasmin also cleave CD87 in the carboxyterminus of domain D3, while uPA has little activity on both regions.

Determination of the cleavage sites was first approached through MALDI-MS analysis of the proteolytic fragmentation of synthetic peptides mapping the proteinase-sensitive domains in human CD87. As reported in Table 1, exposure of the D1–D2 peptide to proteinases confirmed previous data, with αCT resulting in a single cleavage following a Tyr residue corresponding to Tyr$^{87}$ in human CD87 [17], while exposure to uPA produced cleavages after Arg residues corresponding to Arg$^{83}$ and Arg$^{89}$ [12]. However, contrasting with uPA, plasmin showed a major cleavage site after the Arg residue corresponding to Arg$^{89}$, a cleavage which has been reported among others [12]. On another hand, exposure of the D3 peptide to CT induced its complete cleavage following a Tyr residue corresponding to Tyr$^{280}$, while exposure to plasmin and, in a much lesser extent, to uPA both resulted in cleavage following the Arg residue corresponding to Arg$^{281}$, thus confirming the susceptibility of the CD87 carboxyterminus to proteolysis. A second approach used the aminoterminal sequencing of the truncated D2D3 species generated through exposure of rhCD87[His]$_6$ to uPA and plasmin. For both proteinases, two aminoterminal sequences, AVTYSR and SRYLE, were revealed, confirming cleavages in the D1–D2 linker region of CD87 after both Arg$^{83}$ and Arg$^{89}$. The former sequence was preponderant in the case of uPA, as previously observed [19], while plasmin produced predominantly the latter.

Table 1
MALDI-MS analysis of proteinase-induced fragmentation of CD87-related peptides

| | Enzymatic treatment[a] | $[M+H]^{+}$[b] | Peptides[c] |
|---|---|---|---|
| D1–D2 peptide | None | 1788.88 | SGRAVTYSRSRYLEC |
| | αCT | 1012.56 | SRSRYLEC |
| | | 795.43 | SGRAVTY |
| | uPA | 1447.51 | AVTYSRSRYLEC |
| | | 770.30 | SRYLEC |
| | Plasmin | 1039.51 | SGRAVTYSR |
| | | 770.28 | SRYLEC |
| D3 peptide | None | 1441.69 | NHPDLDVQYRSG |
| | αCT | 1142.50 | NHPDLDVQY |
| | uPA | 1298.69 | NHPDLDVQYR |
| | Plasmin | 1298.78 | NHPDLDVQYR |

[a] Peptides (65 μM) were exposed for 5–15 min at 37 °C to proteinases in peptide/enzyme molar ratios varying from 25 to 100, or to a control proteinase-free medium.
[b] Values for the $[M+H]^{+}$ monoisotopic ions observed in an experiment representative of 4 for the D1–D2 peptide and 2 for the D3 peptide.
[c] Truncated peptides were deduced from the masses fitted to the amino acid composition of the original D1–D2 and D3 peptides using the GPMAW software.

### 3.2. *Plasmin cleaves membrane CD87 on human bronchial epithelial cell monolayers and releases intact and truncated CD87 species*

Confluent monolayers of the 16HBE human bronchial epithelial cell line were used as a model of the CD87-expressing human airway epithelium [34] in order to evaluate the activity of the proteinases on cellular CD87. Immunoblotting analysis of membrane CD87 (mCD87) from biotinylated 16HBE cells indicated that, as in many cell types [12–14,22], epithelial mCD87 naturally occurs as two molecular species, a major full-length, three-domain (D1D2D3) form with $M_r \approx 62\,500$, and a minor truncated D2D3 form with $M_r \approx 48\,700$ (see Fig. 2A, NT sample). The former is reactive with both the anti-D1 and anti-D2 mAbs, while the latter is unreactive with the anti-D1 probe (data not illustrated). Both species are released into the extracellular fluid through exposure of cells to a bacterial PI-PLC, which hydrolyzes GPI structures [17,27]. Under optimal conditions of exposure to PI-PLC, large amounts of mCD87 are released [22,27], and the relative amounts of full-length and truncated species mirror those initially present on the cell surface (see Figs. 2A and B, PI-PLC samples).

Exposure of 16HBE monolayers to purified plasmin in the range 10–250 nM for 15 min resulted in a proteolytic transition from the full-length mD1D2D3 to the truncated mD2D3, which accumulated in the cell membrane (Fig. 2A), thus reflecting a specific, limited cleavage of mCD87 in the D1–D2 linker sequence by this proteinase as it has been reported on thyroid cells [21]. However, we did not observe evidence for a shift from intact to truncated mCD87 in the membrane of bronchial epithelial cells exposed to uPA as high as 250 nM (see Fig. 2A, uPA sample). These latter data contrasted with those previously reported using either monocytic or normal thyroid cells which, upon exposure to a lower concentration of uPA (10 nM), showed a membrane increase of the D2D3 species within 30 min [19,21]. These discrepancies may result from intrinsic structural variabilities of CD87 expressed in various cell types, since a resistance to cleavage by uPA has

been noted in tumoral versus normal thyroid cells, which may arise from an altered glycosylation of CD87 [21].

In parallel, the analysis of the extracellular fluids recovered from cell monolayers showed evidence for a plasmin-induced release of soluble CD87 species (sCD87). Indeed, while exposure to uPA did not result in the generation of any detectable sCD87, as judged by a quantitative ELISA (Fig. 2C), exposure to increasing concentrations of plasmin induced a progressive, concentration-dependent accumulation of antigenic sCD87 in fluids up to $3.0 \pm 0.4$ ng/ml ($n = 5$), which amounted ≈90% of the material released by PI-PLC ($3.5 \pm 0.8$ ng/ml, $n = 5$). Immunoblotting analysis showed the presence of both sD1D2D3 and sD2D3 species in these fluids (Fig. 2B), thus indicating a cleavage of the receptor in its juxtamembrane domain, with sD1D2D3 detectable at plasmin concentrations as low as 10 nM, while a major sD2D3 species accumulated up to 250 nM of enzyme. By contrast, the species released through the GPI hydrolysis by PI-PLC are mostly of the full-length form. It is of note that none of the anti-D1 Abs available for this study could detect any sD1 in the extracellular fluids, likely due to further degradation by plasmin.

### 3.3. uPA-dependent plasmin generation induces in situ proteolysis of membrane CD87 and generates a major soluble D2D3 species

We further wondered whether uPA, which appears to be rather inactive per se on epithelial CD87, was however able to participate in the structural alteration of its own receptor through generation of active plasmin. We therefore adapted to our bronchial epithelial cell monolayers a system of PLG activation which is dependent on surface-bound uPA [16]. Acid-washed 16HBE monolayers were incubated with a near-saturating concentration of uPA and then exposed to PLG for different periods of time. Measurement of enzymatically active plasmin in the extracellular fluids indicated a marked time-dependent increase of plasmin concentration, up to $195 \pm 30$ nM for a 60 min incubation (Fig. 3A, white bars), while no detectable active plasmin was generated when cell monolayers were exposed to a PLG-free control medium. In preliminary experiments performed on acid-washed cell monolayers, we observed that incubation with PLG without prior addition of exogenous uPA to cells led, after 60 min at 37 °C, to the generation of active plasmin which amounted about 10% of that generated when cells were previously saturated with 50 nM exogenous uPA. We assumed that this residual intrinsic PLG-converting cellular activity related to minute amounts of endogenous epithelial uPA which were either not completely removed by the acid treatment, and/or were released by cells subsequently to the acid treatment, knowing that airway epithelial cells do express uPA [4].

In situ generation of appreciable amounts of active plasmin was accompanied by the release of antigenic sCD87 in the extracellular fluids, which concentration increased, on a time-dependent basis, up to $6.5 \pm 1.5$ ng/ml 60 min after addition of PLG, as judged by quantitative ELISA (Fig. 3A, black bars). In parallel, immunoblotting analysis performed on the same fluids allowed to identify the sD2D3 species (Fig. 3B).

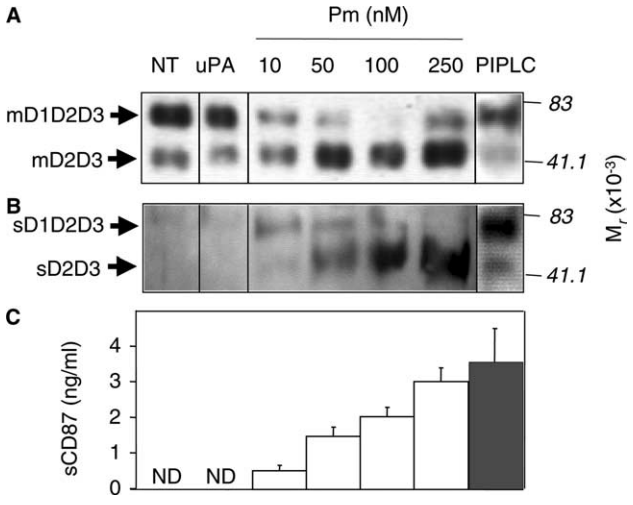


Fig. 2. Plasmin cleaves membrane CD87 on human bronchial epithelial cells and releases soluble receptor species. Confluent 16HBE cell monolayers were surface-biotinylated, then left untreated (NT) at 37 °C for 15 min, or exposed to 1 U/ml PI-PLC, or incubated with either 250 nM uPA or 10–250 nM plasmin (Pm), before removal of the extracellular fluids and solubilization of both fluids and cells. Membrane biotinylated proteins extracted from 10 μg of total proteins (A) or proteins from the extracellular fluids released by a number of cells equivalent to 20 μg of total proteins (B) were separated by SDS–PAGE and immunoblotted under reducing conditions with mAb 3932 (0.25 μg/ml). Films are representatives of three experiments and the positions and $M_r$ values of some calibration standard proteins are indicated. (C) Concentrations of sCD87 in the extracellular fluids were assayed by quantitative ELISA and histograms represent means ± S.E.M. of five independent experiments.

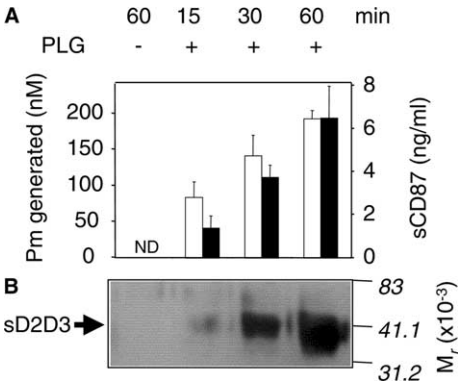


Fig. 3. Cell-bound uPA initiates proteolysis and release of CD87 through plasmin generation. 16HBE cell monolayers were acid washed and saturated with 50 nM uPA for 2 h at 4 °C, before exposure of cells to either 30 g/ml Glu-PLG or PLG-free medium for 15–60 min at 37 °C. The extracellular fluids were collected, then the concentration of generated plasmin (Pm) was measured through hydrolysis of the S-2251 chromogenic substrate, and the concentration of sCD87 was assayed by quantitative ELISA (A, white and black bars, respectively, depicting means ± S.E.M. of three independent experiments), while extracellular proteins were separated by SDS–PAGE under reducing conditions and immunoblotted with mAb 3932 (0.25 μg/ml) (B). Films are representatives of three experiments.

## 4. Conclusions

Altogether, these data are consistent with a model of proteolytic events which not only result in the cleavage of the D1–D2 linker sequence by plasmin and possibly by uPA [12,19,21], with the concomitant release of a free D1 domain which is likely further fragmented by plasmin (Fig. 4), but also in the

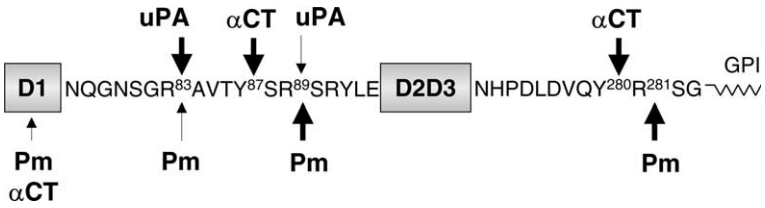


Fig. 4. Location of cleavage sites within human CD87 for the proteinases αCT, uPA and plasmin. Cleavage sites for αCT, uPA and plasmin (Pm) within the D1–D2 linker and in the carboxyterminus of domain D3 were determined by MALDI-MS analysis on synthetic peptides and/or aminoterminal sequencing on a proteolyzed recombinant human CD87, as described in the text. Thin arrows indicate minor cleavage sites. Cleavage sites for αCT and uPA within D1–D2 are similar to those previously reported [19,32]. It is of note that: (i) αCT and uPA cleave preferentially upstream the chemotactic SRSRY motif [1,32], while plasmin cleaves preferentially within this motif; (ii) plasmin as well as αCT cleave also within D1.

efficient cleavage of the carboxyterminus of CD87 by plasmin, thus releasing mostly a D2D3 truncated species (Fig. 4). This remarkable feature has not been reported yet and sheds new lights on the biology of the receptor. Indeed, the binding of uPA to its own receptor triggers a proteolytic downregulation of the full-size, functional uPAR expression via the pericellular generation of active plasmin. This negative feedback regulatory process is likely to modulate further plasmin generation and thus fibrinolysis, tissue remodeling and cell migration.

Noticeable quantities of soluble full-length and/or truncated CD87 species (essentially D2D3) are detected in plasma and urine of healthy individuals, and increase during various types of carcinomas [5,15,24]. In these malignancies, these fragments are yet strong and independent factors for the tumor cell metastatic propensity and correlate with poor survival prognosis [5,24]. Quite similarly, increased systemic or locoregional concentrations of soluble CD87 accompany infectious and inflammatory diseases [35], and can correlate with the outcome [25,26]. The source of such soluble CD87 species in human body fluids has therefore long been discussed. Whereas they can derive from tumor cells and non-malignant stromal cells, as well as from inflammatory cells [1,5,17], the mechanisms for release can include exposure to pathogens or their products, such as PI-PLC [17,36], and exposure to host enzymes such as PI-PLD, known to be overexpressed in some cancers [28], and proteinases. Indeed, the susceptibility of the D1–D2 linker sequence of CD87 to various Ser-proteinases, including uPA, plasmin, several MMP and leukocyte Ser-proteinases [17,20,22], elucidates the mechanism for the release of free D1. By contrast, mechanistic details on the proteolytic release of either intact or D2D3 truncated species from cell membranes are still scarce, except for our recent observation of a juxtamembrane cleavage of CD87 by the leukocyte Ser-proteinase cathepsin G [22]. We now extend this model and propose that during inflammatory and/or tumoral diseases, locally produced plasmin could be responsible, at least in part for the elevated levels of soluble full-length and/or truncated D2D3 receptor species.

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