Note
The Occurrence of Matriptase C-Terminal Fragments on the Apical and Basolateral Sides of Madin–Darby Canine Kidney Epithelial Cells

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Matriptase is a type II transmembrane serine protease. In the present study, matriptase C-terminal fragments containing the catalytic serine protease domain were found to occur on the apical and basolateral sides of Madin–Darby canine kidney epithelial cells transfected with a cDNA encoding the protease. This suggests that matriptase interacts with various potential substrates when expressed in simple epithelia.

Key words: apical and basolateral domains; C-terminal fragment; distribution; matriptase; Madin–Darby canine kidney epithelial cells

Matriptase (also known as membrane-type serine protease 1, etc.) is a type II transmembrane serine protease that is strongly expressed in simple epithelial cells such as enterocytes and kidney tubular cells.¹⁻³ It was first synthesized as a zymogen comprising 855 amino-acid residues, which requires processing by cleavage between Arg614 and Val615 (activation cleavage) to generate the disulfide-linked-two-chain active enzyme. (Fig. 1) Two-chain matriptase is known to cleave and activate a number of macromolecules, including pro-hepatocyte growth factor (pro-HGF) and prostatin zymogen.⁴⁻⁵ These characteristics suggest that matriptase plays an important role in maintaining epithelial integrity.

The plasma membranes of simple epithelial cells are characterized by two structurally and functionally different domains: the apical and basolateral domains. We have found that matriptase is processed post-translationally by cleavage between Gly149 and Ser150, and that the N-terminal fragment (NTF, which contains the cytoplasmic and transmembrane regions) (Met1–Gly149) (Fig. 1) is present on the basolateral membranes but not on the apical membranes of enterocytes in the jejenum of adult rats.⁴ In that study, however, we could not identify the epithelial-cell domain on which the matriptase C-terminal fragment (CTF, which constitutes most of the extracellular part, including the catalytic serine protease domain) (Ser150–Val855) (Fig. 1) is targeted.⁴ We and others found recently by immunofluorescence microscopy that the matriptase CTF occurs on basolateral membranes but not on apical membranes when a cDNA encoding full-length matriptase was transfected into Madin–Darby canine kidney (MDCK) epithelial cells (this cell line has been widely used as a model of simple epithelial cells).⁶⁻⁷ These findings suggest that when matriptase is expressed in simple epithelium, it can interact with potential substrates occurring on the basolateral side (e.g., pro-HGF) but not with those occurring on the apical side (e.g., prostatin zymogen), but there remains the possibility that matriptase CTF is also delivered to the apical side of the epithelium. In fact, a soluble matriptase (matriptase CTF) has been found in biological fluids, including milk and urine.⁷ In the present study, we found that matriptase CTF molecules occurred on the apical and basolateral sides of MDCK cells transfected with a cDNA encoding full-length rat matriptase.

We have established an MDCK line stably expressing full-length rat matriptase (Met1–Val855), in which a Myc-epitope/hexahistidine tag is fused to the C-terminus (designated matriptase-Myc/(His)₆ (Fig. 1)).⁸ The stable transformant was cultured in minimum essential medium supplemented with 10% fetal bovine serum, as described previously.⁹ The cells were seeded on Transwell™ with polycarbonate filter inserts (24-mm diameter, 0.4 µm pore size; Costar Corning, Acton, MA) at a density of 1 x 10⁵ cells per well. When the trans-epithelial resistance of the monolayer exceeded 700 W/cm², the cells were washed 3 times with phosphate-buffered saline (8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 136 mM NaCl, 2.7 mM KCl, pH 7.4), exposed to serum-free minimum essential medium (1 ml for the upper, or apical chambers and 2 ml for the bottom, or basolateral chambers), and incubated for 24 h. After incubation, the apical and basolateral media were harvested. After the addition of a protease inhibitor cocktail (Complete™, Roche, Mannheim, Germany), each medium was concentrated to 50 µl by ultrafiltration using Microcon®-50 (50,000 MWCO, Millipore, Bedford, MA), and stored at −20°C until use. After medium harvest, domain-selective biotinylation was conducted using a cell membrane-impermeable biotin derivative (sulfo-NHS-SS-biotin, Pierce, Rockford, IL).

¹ Abbreviations: CTF, C-terminal fragment; HGF, hepatocyte growth factor; MDCK cells, Madin–Darby canine kidney cells; NTF, N-terminal fragment
as described previously.\textsuperscript{6} Samples were analyzed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting using a mouse anti-Myc antibody (Invitrogen, Carlsbad, CA) (for analysis of CTF) and a rabbit anti-matriptase NTF antibody (designated Tmc172) as primary antibodies.\textsuperscript{4,6}

Fig. 1. Schematic Representation of Rat Matriptase and Its Expression Constructs.

Matriptase is synthesized as a zymogen form consisting of 855 amino acids (refer to Matriptase, proteolytically-inactive, at the top). The N- and C-termini are indicated by NH\textsubscript{2} and COOH. Amino acid numbering starts from the putative N-terminus of the protein. The NTF and CTF parts are indicated by lines, and the association is illustrated with three broken lines. The predicted disulfide linkages between the two cysteine residues corresponding to Cys604 and Cys731 in matriptase are shown as S-S. The amino acid sequence around the matriptase activation cleavage site is indicated in a single-letter code with amino acid numbering at the C-terminal residue (G149). S-CTF-Myc\textsubscript{(His)}\textsubscript{6} consists of all domains of rat matriptase with the C-terminal Myc\textsubscript{(His)}\textsubscript{6}-tag (MHT). The recognition site for Tmc172 is indicated by the amino acid sequence in the single-letter code with amino acid numbering at the C-terminal residue (G149). S-CTF-Myc\textsubscript{(His)}\textsubscript{6} is a secreted variant of recombinant matriptase in which the NTF is replaced by the human immunoglobulin kappa chain signal peptide and S-tag (ST). The Myc\textsubscript{(His)}\textsubscript{6}-tag is also fused to its C-terminus. TM, transmembrane domain; SEA, sea-urchin sperm protein–enterokinase–agrin domain; CUB, complement factor 1R–urchin embryonic growth factor–bone morphogenetic protein domain; L, low-density lipoprotein receptor A module domain; CD, catalytic serine protease domain.

It is unclear why considerable numbers of matriptase CTF molecules occurred in the apical medium in spite of serum starvation does not significantly affect the distribution of matriptase CTF. (Fig. 1),\textsuperscript{6} was found to occur in both the apical and basolateral media (Fig. 2, middle panel, indicated by open arrowhead). In addition, a 33-kDa band was found to occur in both media (Fig. 2, middle panel, indicated by open arrowhead). We have confirmed that the 33-kDa band is produced from the catalytic domain of proteolytically-active, disulfide-linked-two-chain CTF (Miyake, Tsuzuki, Mochida, Fusiki, and Inouye, unpublished results) (also refer to Fig. 1). The ratio of CTF signal (93- and 33-kDa bands) between the apical and basolateral media was about 1:3 (N = 8). After 8 h, incubation of cells with serum-free media, CTF signals were detected from both media with apical to basolateral ratios of about 1:3 (data not shown). These results indicate that matriptase CTF occurs predominantly on the basolateral side of MDCK cells. To our knowledge, however, this is the first evidence that matriptase CTF can also be targeted to the apical side of cultured simple epithelial cells. In addition, these results indicate that matriptase CTF is shed soon after the molecule reaches the basolateral-surface membranes.
Representative Western Blots Showing the Distribution of Matriptase-Myc/(His)6 and S-CTF-Myc/(His)6 in the Transfected MDCK Cells.

Stable transformants were grown on filter inserts. The distribution of NTF on the cell-surface membranes (Surface) of MDCK cells expressing matriptase-Myc/(His)6 was evaluated by domain-selective biotinylation assay (left panel). After medium harvest, the cells were labeled with a biotin derivative on the apical (AP) or the basolateral (BL) side. After labeling, the cells were extracted with Triton X-100. Biotinylated proteins in the Triton extract were precipitated with an immobilized avidin (Neutravidin, Pierce) and subjected to SDS–PAGE under reducing conditions (16% polyacrylamide) and Western transfer. The signal for NTF (indicated by the arrowhead on the right of the blot) was produced by incubation of the blot with an anti-matriptase NTF antibody (middle panel). A 94-kDa signal (indicated by the arrowhead on the right of the blot) was also produced by anti-Myc antibody (right panel). Note that no signals for activated two-chain molecules were produced from the S-CTF-Myc/(His)6 variant. In the analysis of NTF of matriptase-Myc/(His)6 and S-CTF-Myc/(His)6 were subjected to SDS–PAGE under reducing conditions (12% polyacrylamide) and Western transfer. The 93- and 33-kDa signals (indicated by closed and open arrowheads on the right of the blot, respectively) were produced by incubation of the blot with an anti-Myc antibody (middle panel). A 94-kDa signal (indicated by the arrowhead on the right of the blot) was also produced by anti-Myc antibody (right panel).

Mature CTF/My/(His)6 is that some CTF molecules fail to bind to NTF, thereby secreted into the apical medium depending on the putative apical-sorting signal, but there is no direct evidence of the occurrence of free CTF molecules (CTF molecules that do not bind to NTF molecules) in the intracellular environment. An alternative model can be proposed that considerable numbers of matriptase molecules (both CTF and NTF molecules) are delivered to the apical-surface membrane through the signal for apical sorting carried by the CTF even in the presence of the signal for basolateral sorting carried by the NTF (juxtanemembrane cytoplasmic amino acid residues: Lys45, Val47, and Arg50).6) In other words, the putative apical-sorting signal and the basolateral-sorting signals6) might work in a competitive manner to allow matriptase to be delivered to both the apical and basolateral ends in simple epithelia. This model is to be favored if the lack of a signal for NTF at the apical-surface membrane (Fig. 2, left panel) is due to rapid degradation and/or internalization at the site.

In summary, we found that matriptase CTF molecules are delivered to both the apical and the basolateral side of MDCK cells expressing the full-length enzyme. However, matriptase CTF appeared mainly as the proteolytically-inactive form when expressed in the cells (Fig. 2, middle panel). Co-expression of HGF activator inhibitor type I might be important for the occurrence of proteolytically-active CTF in MDCK cells.1) Regardless, the present findings suggest that matriptase, when expressed in intact simple epithelia, can interact with various potential substrates, and they confirm that this protease plays a key role in the maintenance of epithelial structures.

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References


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<th>Matriptase-Myc/(His)6</th>
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<tbody>
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<td>NTF (Surface) (kDa)</td>
<td>(Medium) (kDa)</td>
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<tr>
<td>AP BL</td>
<td>AP BL</td>
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<tr>
<td>27 -</td>
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