Physiol. Res. 45: 169 – 176, 1996

MINIREVIEW

Cell Membrane-Bound Proteases: Not "Only" Proteolysis

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Received August 18, 1995
Accepted February 5, 1996

Summary

Ectopeptidases are widely distributed among various cell systems. Their expression on an appropriate cell type is finely regulated, reflecting the specific functional cell implications and engagement in defined physiological pathways. Protein turnover, ontogeny, inflammation, tissue remodelling, cell migration and tumor invasion are among the many physiological and pathological events in which cell-surface proteases play a crucial role, both as effector as well as regulatory molecules. It has recently become clear that also non-catalytic effects of membrane-bound proteases are of great importance in some biological regulations. They may generate specific signal transduction intracellularly, after reacting with certain target molecules. They may also play a pivotal role in cell-cell and cell-virus contact and recognition, as well as in binding to the extracellular matrix. This short review provides some insight into the multifunctional mechanisms attributed to cell membrane-bound proteases.

Key words

Protease – Cell Membrane – Integral Membrane Protein

I. Introduction

The importance of proteolytic enzymes in multiple functions of mammalian cells is widely recognized. The structure-function relationship of ectopeptidases has been attracting the interest of biochemists, biophysicists, physiologists and cell biologists for years. Cell surface membrane-bound proteases play a key role in protein turnover, in renal and intestinal processing of peptides, ontogenesis, cell migration, inflammation, hormonal regulation, immunity, oncogenesis, tumor metastasis and virus infection. Three main extracellularly functioning proteolytic systems are coordinated in various aspects of proteases and their biological functions (Chen 1992): a) secreted proteases and their inhibitors; b) cell-surface protease receptors that bind secreted proteases; c) integral membrane proteases.

Like other membrane integral proteins, membrane-bound proteases are anchored to the plasma membrane either through a glycosylphosphatidylinositol moiety or by a hydrophobic transmembrane peptide domain (Kenny and Hooper 1991, Moss 1994). In the latter case, the cell membrane-bound proteases belong either to transmembrane type I or type II glycoproteins, characterized by a large globular extracellular domain, a unique transmembrane domain, and a short cytoplasmic region (Table 1).

In this review, we will be focusing on general features of cell membrane-bound (integral) protease functions.

Membrane-bound proteases are widely distributed in various organs and cells (Křepela *et al.* 1985, Bauvois and Laouar 1993, McDermott and Gibson 1995, Šedo and Revoltella 1995). They frequently exhibit specific expression patterns and characteristics that are unique for a particular tissue, cell type and even cell compartment or domain, depending on the functional cell status (Bond 1991, Mari and Auberger 1995). Soluble forms or analogous counterparts of some ectopeptidases have been found in extracellular fluids, including blood plasma. For instance, membrane-bound and soluble forms of dipeptidylpeptidase IV (DPP-IV) and aminopeptidase N (AP-N) have revealed some differences in their amino acid primary sequences and in the composition of their carbohydrate chains (Watanabe *et al.* 1995, Duke-Cohan *et al.* 1995). The origin of these structural differences is still unclear. In the case of AP-N, the likely explanation is that the two enzyme forms, encoded by a single gene, may arise as a result of differential post-translational processing and transport. On the contrary, a functional soluble DPP-IV found in the blood plasma is believed to represent either a product of a single gene, perhaps allowing alternative splicing, or a product of a second DPP-IV gene. Angiotensin-I-converting enzyme (ACE) is probably secreted by regulated proteolytic conversion of a plasma membrane-bound enzyme form (Ramachandran *et al.* 1994). The significance of this dualism is not known. Evidence suggests that both cell membrane-bound and soluble blood plasma forms cooperate in the regulation of one particular physiological function (Tanaka *et al.* 1993).

Table 1
Typical cell membrane-bound proteases

Enzyme	EC number	Class	Membrane anchor[a]	N-C topology[b]	References
Exopeptidases					
aminopeptidase A	3.4.11.7	metallo	HP	type II	1
aminopeptidase N	3.4.11.2	metallo	HP	type II	1,2
aminopeptidase P	3.4.11.9	metallo	GPI	–	1,3
dipeptidyl-peptidase IV	3.4.15.5	serine	HP, GPI	type II	1-4
carboxypeptidase M	3.4.17.12	metallo	GPI	–	1,5
peptidyl-dipeptidase A[c]	3.4.15.1	metallo	HP	type I	1,2,6
pyroglutamyl-peptidase II	3.4.19.6	metallo	?	?	7
γ-glutamyltransferase[d]	2.3.2.2	?	HP	type I	1,2,8
X-Pro-dipeptidase	3.4.13.9	?	GPI	–	1,3,9
Endopeptidases					
neutral endopeptidase	3.4.24.11	metallo	HP, GPI[e]	type II	1,2,10,11
meprin	3.4.24.18	metallo	HP	type I	12

[a] *HP = transmembranous hydrophobic polypeptide domain, GPI = glycosylphosphatidylinositol moiety.*
[b] *It applies only for the enzymes anchored by their transmembranous hydrophobic polypeptide domain. Type I membrane-bound enzymes: the N- and C-terminal amino acids of the membrane-bound enzyme (subunit) polypeptide sequence located extra- and intracellularly, respectively. Type II membrane-bound enzymes: the N- and C-terminal amino acids of the membrane-bound enzyme (subunit) polypeptide sequence located intra- and extracellularly, respectively.*
[c] *Generally known as angiotensin-I converting enzyme.*
[d] *Besides the transferase activity, the enzyme has also a γ-glutamyl-peptide bond hydrolytic activity.*
[e] *Reported for a chimeric enzyme construct (Howell et al. 1994).*
References: 1) Turner et al. 1991; 2) Kenny and Hooper 1991; 3) Orawski and Simmons 1995; 4) Hartel et al. 1988; 5) Rehli et al. 1995; 6) Ramachandran et al. 1994; 7) O'Leary and O'Connor 1995; 8) Heisterkamp et al. 1991; 9) Previs et al. 1995; 10) Mari et al. 1992; 11) Howell et al. 1994; 12) Johnson and Hersh 1994

II. Modes of action

Three general modes of membrane-bound ectopeptidase action have been postulated (Bauvois *et al.* 1991).

Proteases functioning as molecules for signal transduction

Dipeptidylpeptidase IV, aminopeptidase N, neutral endopeptidase and aminopeptidase A have been shown to be identical with differentiation antigens CD26, CD13, CD10 and BP-1/gp60, respectively (Ship and Look 1993). In T-lymphocytes, CD26/DPP-IV participates in the regulation of IL-2 secretion, cell proliferation, activation of several cell functions, including cytotoxic activity, and stimulation of monocyte macrophage colony formation, probably by its involvement in specific cytokine-mediated events induced after appropriate signal transduction (Shipp and Look 1993, Šedo and Kraml 1994). Similarly, thymocyte Ala-aminopeptidase is involved in antigen-dependent T-cell activation (Naquet *et al.* 1989). Gamma-glutamyltranspeptidase (γGT) seems to participate in monocyte to macrophage differentiation (Bauvois *et al.* 1995) and also in cell apoptosis, at least in certain cell lines (Graber and Losa 1995).

The complex cascade of molecular and biochemical events activated by stimulation of cell-surface membrane-bound proteases and the induction of appropriate signal transduction events, mainly in the haematopoietic and immune cell systems, are attracting increasing attention (Ansorge *et al.* 1991, Bauvois and Laouar 1993, Fleischer 1994).

The functional and morphological association of DPP-IV with either CD45 protein tyrosine phosphatase or with the adenosine deamidase binding protein (Torimoto *et al.* 1991, Kameoka *et al.* 1993) is believed to be critical for activating CD26-mediated T-cell signalling. Recently, Mittrücker *et al.* (1995) described in detail a DPP-IV/CD26 activation pathway for T lymphocytes, requiring co-expression of the TcR/CD3 complex.

Taken together, at least some membrane-bound proteases can be considered to date as receptors, transmitting specific "signals" through the plasma membrane intracellularly.

Proteases functioning as molecules for cell-cell and cell-virus contacts, specific cell recognition and cell binding to the extracellular matrix

As integrins are involved in extracellular matrix-dependent cell activation and signal transduction (Juliano, 1994), a possible functional analogy has been proposed to occur when cell-substratum adhesion mediated by ectoproteases triggers modulation of intracellular signal transduction (Hanski *et al.* 1985, Piazza *et al.* 1989, Bauvois and Laouar 1993). In this context, the ability of certain ectoproteases to interact with components of the extracellular matrix (where many cytokines and growth factors are likely to be immobilized) and to potentially process the latter could cause a variety of endocrine, paracrine and autocrine effects.

This model has been proposed as a good illustration of several protease-mediated cross-talk mechanisms in a variety of complex regulations.

Johnson and coworkers (1993) proposed a role for the endothelial DPP-IV in the initial occurrence of site-specific tumour metastases. The endothelial surface DPP-IV is apparently selectively recognized by certain circulating cancer cells in the course of metastasis formation. Inhibition of AP-N and DPP-IV activities has been suggested as a new potential approach for suppression of human renal cell carcinoma and renal tubular epithelial cell spreading (Riemann *et al.* 1995).

CD13/AP-N acts as a major receptor for coronaviruses (Yeager *et al.* 1992, Delmas *et al.* 1994).

Recently, the participation of DPP-IV in HIV infection has been widely discussed. It was originally hypothesized that DPP-IV is an essential cofactor for HIV entry into target cells (Callebaut *et al.* 1993). This hypothesis was not confirmed by others (Lazaro *et al.* 1994) and subsequent results suggest that the catalytic activity of DPP-IV may decrease the efficiency of HIV-1 infection (Morimoto *et al.* 1994). In contrast, more recent observations suggest that the involvement of DPP-IV in AIDS pathogenesis (Dianzani *et al.* 1995) and in HIV-related immunosuppression (Gutheil *et al.* 1994) is highly probable (Oravecz *et al.* 1995).

Proteolytic functions

Lifetime regulation of the biologically active peptides and proteins, the turnover of the extracellular matrix and structural modifications of cell membrane components, all together affect the survival and functional activity of resident cells. The proteolytic effects of cell membrane-bound ectopeptidases can be "endocrine"; i.e. these peptidases may exert their activity distant from the action of their processed peptides (see for example angiotensin I-converting enzyme of the endothelial surface) (Ryan 1989). On the other hand, membrane-bound proteases may exert "autocrine" and "paracrine" effects, in their immediate pericellular microenvironment. Thus, soluble peptides (neuropetides, cytokines, growth factors, vasoactive peptides) exert their potent action locally because they are rapidly inactivated by specific inhibitors in the vicinity of cells or because they are directly cleaved by specific enzyme(s) expressed on the surface of their target cells (Nadel 1992). A large number of bioactive peptides and proteins grouped by their functional properties, has been described as the substrate of

specific membrane-bound proteases. They include pleotropic cytokines (IL-1β, IL-6 and TNF-α), vasoactive peptides (VIP, substance P), neuroendocrine hormones (endorphins, enkephalins, somatostatin, angiotensin). Furthermore, proteolytic mechanisms can lead to release of growth factors and cytokine receptors (TNF-α, nerve growth factor, CSF-1, IL-1, IL-2 and IFN-γ) as well as leukocyte antigens (FcRIII/CD16, FcRII/CD23, CD8, Me114) and, interestingly, also ectoenzymes themselves (cholineesterase, sialyltransferase/CD75, NEP, DPP-IV, AP-N and ACE) (Aoyama and Chen 1990, Chen 1992, Bauvois and Laouar 1993, Hoffmann *et al.* 1993, Yavelow *et al.* 1993, Laouar *et al.* 1994, Lucius *et al.* 1995).

It is also possible that in order to regulate larger hormones, several different peptidases are involved in a more complex cooperation by sequential cleavage of the peptides (Saint-Vis *et al.* 1995). Similarly, the proteolytic activation/inactivation sequence of events affecting biologically active peptides may turn out to play an important regulative role in cell differentiation and/or proliferation. Furthermore, T cell derived cytokines, such as IL-1β, IL-4, IL-13, IFN-γ, TGF-1β and TNF-α are known to regulate cell surface protease expression, probably on the transcriptional level (Riemann *et al.* 1995).

Various membrane-bound protease activities are known to be altered in tumour tissues when compared with the adjacent matched tissue (Šedo *et al.* 1991b, Procházka *et al.* 1991, Schlagenhauff *et al.* 1992, Asada *et al.* 1993). Direct involvement of ectoproteases in dynamic interactions between tumour epithelium and the adjacent connective tissue and their role in tumour progression has been investigated (Vassalli and Pepper 1994). In some cases, specific enzyme distribution at the subcellular level and distinct molecular form patterns of a particular protease have been observed in tumours as well as in their adjacent normal counterparts (Šedo *et al.* 1991a).

III. Non-enzymatic functions

Although peptide-bond cleavage still remains the best-known action of proteases, the particular proteolytic activity may (Tanaka *et al.* 1993, Kurachi *et al.* 1994, Reinhold *et al.* 1994, Kahne *et al.* 1995, Bristol *et al.* 1995), or may not (Delmas *et al.* 1994, Kurachi *et al.* 1994, Steeg *et al.* 1995, Yeager *et al.* 1992), be essential for the particular physiological function of an enzyme.

Surprisingly, a family of dipeptidyl peptidase IV-like proteins with high amino acid sequence homology to DPP-IV/CD26, but completely devoid of any catalytic activity, was recently identified in the central nervous system (Wada *et al.* 1992, de Lecea *et al.* 1994). Another DPP-IV structurally related but functionally distinct protein is represented by the human fibroblast activation protein-α which is inducible by several soluble cytokines and expressed in various malignant cells as well as in reactive stromal fibroblasts of some epithelial cancers. The physiological significance of this "variant enzyme" molecule is still not clear. However, its receptor, non-enzymatic function has been proposed (Scanlan *et al.* 1994).

Taken together, the above-mentioned findings suggest that an enzyme molecule might have been originally selected for its specific catalytic function, then elaborated, and subsequently reselected for a new function.

IV. Conclusions

Recent evidence suggests that at least some cell-surface membrane-bound ectoproteases are multifunctional proteins. A single protease could exert one or more of the above reviewed modes of action to perform its particular physiological function(s). The latter form may, however, differ in distinct organs or cell types, and be controlled by different, site-specific mechanisms (Bauvois and Laouar 1993, Shipp and Look 1993).

Expression patterns, mechanisms of regulation, as well as the physiological role(s) of various cell membrane-bound ectoproteases are only partially understood up to now. Perhaps, the recent findings that at least certain cell membrane-bound proteases function differently in normal and transformed cells may raise the possibility that such enzyme will become part of future diagnostic, prognostic as well as therapeutic applications (Kasafírek *et al.* 1992, Shipp and Look 1993, Chen *et al.* 1994, Vassalli and Pepper 1994).

Acknowledgment

Authors would like to thank Dr. Jan Pláteník for helpful discussion. This work was supported in part by grants 303/93/0541 (A.S.) and 2289-3 (E.K.) from the Grant Agency of the Czech Republic and from the Grant Agency of Ministry of Health of the Czech Republic, respectively.

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