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69. Membrane alanyl aminopeptidase

**Databanks**

**MEROPS name:** Aminopeptidase N  
**MEROPS classification:** clan MA(E), family M1, peptidase M01.001  
**IUBMB:** EC 3.4.11.2  
**CAS registry:** 9054-63-1  
**Species distribution:** Chordata  
**Sequence known from:** Bos taurus, Canis familiaris, Felis catus, Homo sapiens, Mus musculus, Ornithogalum curvispica, Pleuronectes americanus, Rattus norvegicus, Sus scrofa

**Name and History**

The early history of membrane alanyl aminopeptidase (mAAP) relates to its role as Cys-Gly dipeptidase or cysteiny1-glycinase when Binkley and colleagues proposed that this peptidase activity present in apparently purified RNA preparations contributed to polypeptide biosynthesis by acting in reverse in a sequential fashion (Binkley, 1952; Binkley et al., 1957). In 1957, Giorgio Semenza used newly developed chromatographic procedures to purify, and demonstrate unequivocally, that cysteinyl-glycinase was a protein distinct from Binkley's RNA preparation (Semenza, 1957a) and subsequently developed a convenient assay procedure (Semenza, 1957b). The enzyme has also been referred to in its earlier days as aminopeptidase M (for microsomal or membrane aminopeptidase), reflecting its tight association with a microsomal membrane fraction in pig kidney from which it was first purified. The use of the name aminopeptidase M is still occasionally seen today in the literature and the enzyme has also been confused with the cytosolic 'leucine aminopeptidase' because of their overlapping substrate specificities and similar tissue distributions. In 1980 it was suggested that
the enzyme should be renamed aminopeptidase N, reflecting its preference for action on neutral amino acids (Feracci & Maroux, 1980) and that terminology is still in common use today. The name membrane alanyl aminopeptidase was introduced to clarify the nature and localization of the enzyme and to distinguish it from its cytosolic counterpart.

The enzyme is widespread but is particularly abundant in the brush border membranes of kidney, small intestine and placenta and is also rich in liver. Much of the original characterization of mAAP was performed on the renal or intestinal enzymes. However, the presence of the enzyme in brain has attracted substantial interest since the discovery that it can participate in the hydrolysis and inactivation of the enkephalins by hydrolysis of the Tyr1-Gly2 bond (Gros et al., 1985; Matsas et al., 1985). mAAP also turns out to be identical with the human cluster differentiation antigen CD13 expressed on the surface of myeloid progenitors, monocytes, granulocytes and myeloid leukemia cells (Look et al., 1989).

Activity and Specificity

mAAP has a broad substrate specificity removing N-terminal amino acids (Xaa-Xbb-) from almost all unsubstituted oligopeptides and from an amide or arylamide. It has usually been assayed with derivatives of alanine, e.g. Ala+=NHMeC or the NHPHNO2 or NNNap derivatives, because Ala is the most favored residue. Leu+=NHMeC and other bulky hydrophobic amino acid derivatives are also good substrates but leucinamide is poorly hydrolyzed. For aminoacyl derivatives, the favored order is reported to be Ala+, Phe+, Tyr+, Leu+, Arg+, Thr+, Trp+, Lys+, Ser+, Asp+, His+ and Val+. Pro- and α-or γ-Glu-derivatives are very slowly attacked. When a prolyl residue is preceded by a bulky hydrophobic residue, e.g. Leu, Tyr or Trp, unusual secondary reactions can occasionally arise such that the X-Pro+ combination is released as an intact dipeptide (see, for example, McDonald & Barrett, 1986). Dipeptides are readily hydrolyzed, e.g. Cys+Gly, as in the original studies on this activity (Semenza, 1957a,b). Sub-site interactions are important and hence chain length greatly affects the rates, although precise rules governing specificity have not been defined. The specific recognition by mAAP of the N-terminal amino acid of its substrate appears to involve a critical glutamate residue in the active site (Glu350) (Luciani et al., 1998).

The pH optimum is around 7.0 although the optimum can rise to 9.0 as the substrate concentration is increased. However, the $K_m$ is lowest in the pH range 7.0–7.5. Metal chelating agents are effective inhibitors, consistent with the metallopeptidase nature of the enzyme, and sulfhydryl reagents are without effect. A comparison of the effects of a range of metallopeptidase inhibitors on membrane aminopeptidases has been carried out by Tieku & Hooper (1992). Amastatin (originally described as an inhibitor of glutamyl aminopeptidase (aminopeptidase A) (Chapter 72)) is also a very effective inhibitor of mAAP, with an increase in potency when preincubated with the enzyme, the $K_i$ value decreasing from 20 μM to 20 nM, i.e. it is a slow, tight-binding inhibitor which involves a conformational change in the enzyme–inhibitor complex (Rich et al., 1984). The kinetics of this reaction have been examined in detail by Rich et al. (1984). Probestin is also a potent inhibitor with a reported $IC_50$ of 50 nM (Tieku & Hooper, 1992). Bestatin is also a well-recognized inhibitor of mAAP although considerably less potent than amastatin or probestin (Stephenson & Kenny, 1987; Tieku & Hooper, 1992). Actinonin ($IC_50 = 2 \mu M$) can be considered a relatively specific inhibitor of mAAP compared with other membrane aminopeptidases (Tieku & Hooper, 1992).

The enzyme is only very weakly inhibited by puromycin (see Distinguishing Features). Based on such inhibitory data, a selective enzyme assay for mAAP has been devised (Gillespie et al., 1992). A new range of potent and selective inhibitors of mAAP have been described based on derivatives of 3-amino-2-tetralone (Schalk et al., 1994), some of which exhibit $K_i$ values in the nanomolar range. The proposed mode of binding of these compounds is as bidentate ligands with the amino and carbonyl functions coordinating to the active-site zinc. Highly potent and selective mAAP inhibitors have been designed, which utilize phosphinic compounds that mimic the transition state of substrates of the enzyme (Chen et al., 1999). Prodrugs of dual inhibitors of the two enkephalin-degrading enzymes, mAAP and neprilysin, also based on a phosphinic acid design, are highly effective as anti-nociceptive compounds (Chen et al., 2001).

Structural Chemistry

mAAP is a type II integral membrane protein located on the plasma membrane as an ectoenzyme. The pl is approximately 5. The native enzyme exists as a homodimer of subunit $M_r 140000–150000$ in most species (Riemann et al., 1999), although it is reported to be monomeric in the rabbit (Feracci & Maroux, 1980). It is heavily glycosylated, with carbohydrate accounting for at least 20% of the mass of the protein. The polypeptide chain is susceptible to proteolysis, generating two fragments of $M_r$ approximately 90000 and 45000 that have been referred to in the earlier literature as β and γ subunits respectively (the intact chain being the α subunit). This artifact of preparation led to the suggestion that the native enzyme may be a trimer (Maroux et al., 1973).

The enzyme was originally cloned from a human intestinal cDNA library (Olsen et al., 1988) and subsequently from rat (Watt & Yip, 1989; Malfroy et al., 1989) and rabbit kidney (Yang et al., 1993). The rat enzyme comprises a 966 amino acid polypeptide with a small cytoplasmic domain, a 24 amino acid hydrophobic segment close to the N-terminus which serves as the membrane anchor region and the bulk of the polypeptide chain including the active site present as an ectodomain. The sequence includes nine potential N-linked glycosylation sites and a typical zinc-binding motif (His-Glu-Xaa-Xaa-His) in which the two closely spaced histidines represent two of the zinc ligands. The third zinc ligand is a glutamate and the protein contains one Zn$^{2+}$ per subunit. Chemical modification experiments have been used to identify arginyl, histidyl, tyrosyl and aspartyl/glutamyl residues at the active site (Helene et al., 1991). The Lactococcus lactis pepN gene encodes an aminopeptidase homologous to mAAP with almost 30% identity between the bacterial and mammalian proteins and with particularly high conservation around the active-site region (Tan et al., 1992).
**Preparation**

In the kidney, mAAP represents as much as 8% of the brush border membrane protein, thereby providing a convenient and abundant source to initiate purification. It was first isolated from pig kidney as 'cysteinyl-glycinase' (Semenza, 1957a) and subsequently as an aminopeptidase (Wachsmuth et al., 1966). The protein can be purified in either hydrophilic or amphipathic form by proteinase (trypsin, papain) treatment or detergent solubilization respectively. Conventional chromatographic procedures can then be used to isolate the enzyme (e.g. Feracci & Maroux, 1980). The pig small intestinal mAAP has also been purified by immunoadsorbent chromatography (Sjöström et al., 1978). A 130 kDa glycoprotein purified from pig kidney brush border membranes by affinity chromatography on immobilized 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonate (SITS) followed by concanavalin A-Sepharose, turned out serendipitously to be mAAP (See & Reithmeier, 1990), suggesting that the protein possesses an anion-binding site. This procedure provides a convenient purification method for the enzyme, which represents the major concanavalin A-binding protein in brush border membranes. mAAP in the larval midgut cell membranes of the silkworm, *Bombyx mori*, is partially sensitive to release by phosphatidylinositol-specific phospholipase C, suggesting that in this species the enzyme may be anchored through a glycopid anchor rather than a transmembrane domain (Takesue et al., 1992).

**Biological Aspects**

mAAP is widely distributed among species and tissues although it is of greatest abundance in brush border membranes of the kidney, mucosal cells of the small intestine and in the liver. It is also present in the lung where it is identical to the p146 type II alveolar epithelial cell antigen (Funkhouser et al., 1991) and is located on endothelial cells in blood vessels. On polarized epithelial cells, mAAP is localized to the apical domain and is targeted there through an apical sorting signal thought to be located in the catalytic head group region of the protein (Vogel et al., 1992). In the kidney, mAAP contributes to the extracellular catabolism of glutathione (Curthoys, 1987). The cysteinyl-glycine generated during the catabolism of glutathione by -glutamyltranspeptidase is hydrolyzed by the two ectoenzymes mAAP and membrane dipeptidase (Chapter 302) contributing approximately equally (McIntyre & Curthoys, 1982). In the intestine, the enzyme functions in the final stages of protein and peptide digestion.

A detailed localization of the enzyme has been carried out in the brain because of its potential involvement in terminating the actions of certain neuropeptides, especially the enkephalins (Solhonne et al., 1987; Barnes et al., 1988, 1994) and angiotensin III, which is a key brain regulator of vasopressin release (Reaux et al., 1999). In addition to being present on endothelial cells and synaptic membranes, mAAP is found on astrocytes and pericytes (Barnes et al., 1994; Kunz et al., 1994). It is abundant in the choroid plexus and can therefore also serve to prevent access to the brain of potentially damaging circulating peptides. On vascular cells, mAAP may serve to metabolize certain vasoactive peptides (Ward et al., 1990). An important location of mAAP is in hematopoietic cells, where it is referred to as CD13 (Look et al., 1989). Here, its expression is restricted primarily to myeloid cells, but it is also found on antigen-presenting cells, melanoma cells and lymphocytes. On granulocytes it may cooperate with neprilysin (Chapter 108) to downregulate responses to chemotactic factors such as formyl-Met-Leu-Phe (Shipp & Look, 1993). More generally in the immune system it may serve to inactivate certain cytokines (Hoffmann et al., 1993; Kanayama et al., 1995; Riemann et al., 1999). The immunopotentiating and reported antitumor activities of betasin may relate to inhibition of mAAP (Leyhausen et al., 1983). Reduction in expression or activity of mAAP results in inhibition of growth of T cells, probably through a mechanism involving glycogen synthase kinase-3 (Lendeckel et al., 2000). Its roles in the immune system and correlation with neoplastic transformation are summarized in Riemann et al. (1999). mAAP itself has been implicated in angiogenesis (Bhagwat et al., 2001) and cell motility, and is a poor prognostic factor in colon cancer (Hashida et al., 2002). The expression of mAAP has also been explored in human prostate cancer, for which the enzyme may be a valuable histological marker (Bogenrieder et al., 1997; Ishii et al., 2001). mAAP appears to regulate the cycle-dependent bioavailability of interleukin 8 in the endometrium and its activity is, in turn, regulated by estrogen (Seli et al., 2001).

The human mAAP gene, which occupies 35 kb, is localized on chromosome 15 (Kruse et al., 1988; Watt & Willard, 1990). Separate promoters control transcription of the human gene in myeloid and intestinal epithelial cells (Shapiro et al., 1991). The pig mAAP gene has been cloned and localized to porcine chromosome 7 (Olsen et al., 1989; Poulsen et al., 1991).

A novel feature of mAAP is its ability to serve as a receptor for certain viruses, especially coronavirus 229E, an RNA virus that causes upper respiratory tract infections in humans (Yeager et al., 1992). Mutagenesis studies suggest that the virus-binding site lies close to the active site region, although enzyme activity is not essential for virus binding. Human mAAP also appears to mediate human cytomegalovirus infection although, again, enzyme activity is not essential for infection (Soderberg et al., 1993). Another coronavirus, transmissible gastroenteritis virus, which causes a fatal diarrhea in newborn pigs, uses intestinal mAAP as its receptor (Delmas et al., 1992). mAAP appears to be the major receptor for the CryLAc toxin of *Bacillus thuringiensis* in *Lymantria dispar* (gypsy moth) (Lee et al., 1996; de Maagd et al. 2001).

mAAP is synthesized in a fully active form. Substance P and bradykinin, which are not substrates for mAAP, have been reported as natural inhibitors of the enzyme with *K* values in the low micromolar range (Xu et al., 1995). However, it is unlikely that they play any physiological role in regulating enzyme activity and the enzyme is therefore probably essentially unregulated at the surface of cells. A recent study has, however, shown that oxidoreductase-mediated modulation of the thiol status of the cell surface markedly affects the activity of mAAP (Firla et al., 2002) and the enzyme is upregulated in response to hypoxia (Bhagwat et al., 2001). Expression of CD13 may vary during cell growth and differentiation and certain cytokines, e.g. interleukin 4 and interferon , can upregulate levels of mAAP mRNA and protein (Riemann et al. 1999).
Distinguishing Features

mAAP can be distinguished from the cytosolic leucine aminopeptidase by its membrane association and its poor hydrolysis of leucinamide (see above). It can be distinguished from another aminopeptidase in brain (aminopeptidase BS; Chapter 76) capable of hydrolyzing the enkephalins by its relative insensitivity to puromycin ($K_i = 78 \text{ mM}$ compared with 1 mM for the puromycin-sensitive activity). Actinomycin is a relatively selective inhibitor. The dipeptidase activity of mAAP can be distinguished from that of the mammalian membrane dipeptidase (Chapter 302) by the sensitivity of the latter to cilastatin (Littlewood et al., 1989).

Related Peptidases

Several mammalian aminopeptidases with homology to mAAP have been cloned, including the puromycin-sensitive aminopeptidase PS (Chapter 76), which has been implicated in cell growth and viability (Constam et al., 1995), and human placental leucine aminopeptidase/oxytocinase (Chapter 74), which is also a type II integral membrane protein and may play a role in the degradation of oxytocin and vasopressin (Rogi et al., 1996). The major protein present in GLUT4 vesicles in fat and muscle tissues is a glycoprotein of $M_r 160,000$ that has structural homology to mAAP and exhibits aminopeptidase activity in vitro (Kandror et al., 1994). The cytosolic leukotriene $A_4$ hydrolase (Chapter 80) also has aminopeptidase activity and belongs to the mAAP family (Toh et al., 1990).

Further Reading

For reviews, see Wang & Cooper (1996), Lendeckel et al. (1999) and Riemann et al. (1999).

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Databanks

**MEROPS name:** Aminopeptidase Ey

**MEROPS classification:** clan MA(E), family M1, peptidase M01.016

**IUBMB:** EC 3.4.11.20

**Species distribution:** Gallus gallus

**Name and History**

The introduction of a sensitive fluorogenic substrate, Leu-NHMec, and a chromogenic substrate, Leu-NHPhNO₂, at pH 7.5 led to the discovery of aminopeptidase Ey in hen's egg yolk (Ichishima et al., 1989). The enzyme has a broad specificity for N-terminal amino acid residues at the P1 position (Tanaka & Ichishima, 1993b).

**Activity and Specificity**

Aminopeptidase Ey has a broad specificity for amino acid residues at the P1 position of substrates (Tanaka & Ichishima, 1993b). The enzyme degrades a variety of peptides having various N-terminal amino acids: hydrophobic, basic and acidic amino acids including proline (Tanaka & Ichishima, 1993b). It rapidly degrades Leu-enkephalin [Tyr-Gly-Gly-Phe-Leu] to the C-terminus (Tanaka & Ichishima, 1993b). Tyrosine sulfate, a post-translationally modified amino acid, is easily liberated from cholecystokinin octapeptide: Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂ (Tanaka & Ichishima, 1993b).

Aminopeptidase Ey hydrolyzes N-terminal Xaa-Pro bonds in a chicken brain pentapeptide (Leu-Pro-Leu-Arg-Phe-NH₂), substance P fragment 1–4 (Arg-Pro-Lys-Pro-Pro-Lys), and bradykinin fragment 1–5 (Arg-Pro-Gly-Phe-Pro-Asp-Arg-Leu-NH₂) (Tanaka & Ichishima, 1993b).

The enzyme releases proline from Pro-Leu-Pro-Leu-Arg-Phe-NH₂ (Tanaka & Ichishima, 1993b), while it is unable to release proline from melanocyte-stimulating hormone release-inhibiting factor (Pro-Leu-Gly-NH₂) (MSH-release inhibiting factor), or from schistosomula-amine (Pro-Asp-Val-Asp-His-Val-Phe-Leu-NH₂) (Tanaka & Ichishima, 1993b).

The pH optimum of the enzyme for Leu-Leu-Tyr-Leu-NHMec and Leu-NHPhNO₂ hydrolysis is about pH 7.5.