

Aminopeptidases isolated from plants of great economic value – role and characteristics

Joanna KANIA*, Danuta GILLNER – Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland

Please cite as: CHEMIK 2015, 69, 8, 463–468

Introduction

Aminopeptidases (EC 3.4.11.XX) belong to the group of proteases, important enzymes which play key roles in many life processes. They catalyze the hydrolysis of amino acids located at the *N*-terminus of peptide and are involved in proteins degradation to free amino acids. Leucine aminopeptidase (LAP, EC 3.4.11.1) typically belong to the M1 or M17 family and preferably hydrolyse leucine (Leu) from *N*-terminus of proteins.

Several types of aminopeptidases are usually identified in one plant. They are responsible for different activities. Some studies focused on the role of aminopeptidases in the life processes, revealed that leucine aminopeptidases are involved in the process of plant aging (wilting), germination, protective processes, transport of auxins or meiosis.

Leucine aminopeptidase (LAP) from *Arabidopsis thaliana* (mouse-ear cress) is one of the most studied enzyme from this class. *A. thaliana* is a small flowering plant and because of its small genome it is a model species in biology, botany and genetics of plants. Leucine aminopeptidase from *Arabidopsis* (molecular mass of 54.5 kDa), takes an important part in protein metabolism, stress response and wilting. Loss of LAP activity in *Arabidopsis* results in early leaves senescence and phenotype sensitive to stress. *Arabidopsis* LAP shows maximum activity in the alkaline pH (8.5) and temperature of 70°C. Preferentially it hydrolyzes leucine and phenylalanine from *N*-terminus of the peptide chain [1–3]. Important role of aminopeptidases in *Arabidopsis* inspired other scientist to study the activity of these enzymes in cereals, vegetables, fruits and even weeds [4–7]. In 2012 it was found that plant leucine aminopeptidases may have additional functions in plants. Studies on the leucine aminopeptidase LAP-N and LAP-A from tomato and LAP1 and LAP2 from *Arabidopsis* have shown that these enzymes can function as molecular chaperones (proteins responsible for correct folding of other macromolecules and proteins) [8]. Aminopeptidases can be used as a molecular targets for new herbicides. These enzymes may also be used in protein hydrolysates production or enhancing the taste of food (eg. from soy, by removing *N*-terminal hydrophobic amino acids responsible for bitter taste) [9]. Studies concerning inhibition of the aminopeptidases activity are widely described in the literature. It is known that amastatin or bestatin (natural compounds) and 4-chloromercuric-benzoic acid are good inhibitors of certain aminopeptidases [9, 10]. In the case of metalloaminopeptidases chelating agents such as EDTA and 1,10-phenanthroline are very efficient inhibitors [5].

Aminopeptidases due to participation in many key physiological processes have found and may find in the future a lot of applications. They may, for example serve as a molecular target for new herbicides (selective inhibition of these enzymes will block many physiological processes in plants). Hydrolysis of amino acids from the *N*-terminus of the protein is useful in the production of protein hydrolysates or in enhancing the food flavor (eg. removal of hydrophobic amino acids responsible for the bitter taste in protein hydrolysates from soybeans) [11].

Cereals

Barley. Research on aminopeptidase isolated from barley (*Hordeum vulgare* L.) was first described in 1974. Barley seedlings aminopeptidase was tested for substrate specificity toward di- and tripeptides. The highest activity was observed for Leu-Gly-Gly and Met-Leu-Gly substrates. The immediate hydrolysis was observed for Leu-Tyr and Leu-Gly dipeptide substrates [12]. The other aminopeptidases from barley flour [13], barley seeds, barley sprouts growing in a light and sprouts developing in the dark were also characterized. In barley sprouts developing in dark three types of LAP were identified (LAP 1, LAP 2, LAP 3). They were monomeric enzymes with a molecular mass of 57 kDa. Their activities were similar in all vegetative plant organs. Optimum pH was 7.0 for LAP 1 and LAP 2, but 8.0 for LAP 3 [14]. Further studies have shown that up to 6 aminopeptidases with the pH optimum in the range of 7.4–8.2 can be identified in germinating barley. Two of them had broad substrate specificity, while the other four were specific towards Leu, Phe, Pro/His and Arg substrates. The activity changed during plant growth, adapting to the needs and occurring processes. The scientists concluded that aminopeptidases from barley are involved in germination, tissue development and growth [15]. Another enzyme discovered in barley is the methionine aminopeptidase (MAP). MAPs remove methionine from the *N*-terminus of the peptide chain. It is usually the first post-translational modification of the protein. Barley MAP showed increased expression in response to abscisic acid (ABA) and low temperatures [16]. Recent studies of aminopeptidases from barley seeds resulted in isolation of 58 kDa protein, with pH optimum of 7.5, showing a typical substrate specificity of the leucine aminopeptidase, favoring bulky amino acids such as Phe and Leu [17].

Oat. Aminopeptidase isolated from oat leaves with the pH optimum of 8.4, shows broad substrate specificity, but preferably hydrolyzes Lys, Arg, Phe and Leu. It is inhibited by heavy metals as well as 2-mercaptoethanol. Studies have shown that this aminopeptidase may be located in the plant photosynthetic organelles [18, 19].

Triticale. Proline aminopeptidase (PAP) has been identified in triticale sprouts. The isolated enzyme is a tetramer consisting of identical subunits with a molecular mass of about 54 kDa. Triticale PAP shows the highest activity at pH 7.5 and at the temperature of 37°C. The enzyme is highly specific toward Pro substrate, but is also active with other amino acids such as Ala, Phe and Leu. In three-week old triticale seedlings PAP activity increases in response to water deficit and strong salinity. Similar effect is observed after addition of heavy metals (cadmium and aluminum) to the medium [20, 21].

Maize. Research on leucine aminopeptidases from maize are limited mainly to genetic testing. Their main purpose is to determine the genetic diversity within and among plant populations. In maize endosperm four forms of LAP were discovered and described due to its location in gel electrophoresis as A, B, C and D [22, 23].

Nightshades family

The nightshades (*Solanaceae*) family includes, among others, tomato, potato and tobacco. In tomato four types of aminopeptidase

Corresponding author:

Joanna KANIA – M.Sc., Eng., e-mail: Joanna.Kania@polsl.pl

has been identified, two are LAP-like enzymes with molecular weight of 66 and 77 kDa, the other two are neutral LAP-N and acidic LAP-A, both of 55 kDa. Crystallographic studies have shown that LAP-A is a dimer of trimers. Each monomer contains two metal ions bridged by a water or a hydroxyl ion at the active site [24].

Most plants have a LAP-like and LAP-N protein. LAP-A however occurs almost exclusively in *Solanaceae* plants and is expressed under the influence of harmful factors [25, 26]. The LAP-N and LAP-A have highly conserved C-terminal domain containing amino acid residues necessary to perform catalysis and to coordinate Zn^{2+} . N-terminal sequences are more diverse. Both aminopeptidases are synthesized as preproteins and then processed to a mature form [27, 28]. LAP-A has the ability to impair development of herbivore insects, LAP-N has no influence in wound responses and insect defense [29]. Factors influencing the expression of LAP-A include plant hormones: systemin, jasmonates (e.g. methyl jasmonate), and abscisic acid [30, 31, 32]. The level of abscisic acid is increased in the plants under water deficit or in case of high salinity [33]. It has been proven that mechanical injury of tomato leaves results in accumulation of LAP-A which is detected mostly within the chloroplasts of spongy and palisade mesophyll cells [9, 28, 29]. Also, herbivores attack increases the expression and activity of LAP-A. It has been proven in the experiment in which larvae of *Spodoptera littoralis* was used [34]. Moreover LAP has an influence on herbivore. Larvae of *Manduca sexta* fed on *Solanum nigrum* leaves with silenced gene of LAP were heavier than larvae fed on control plants [29]. Increased LAP-A activity is also observed in the presence of cadmium ions. Cadmium is highly toxic to living organisms. The presence of cadmium causes damage to plant roots, inhibits cell growth, and impair photosynthesis which in turn leads to the plant death. The activity of LAP-A in tomato roots increases ten-fold in response to cadmium ions [35]. Also coronatine, phytotoxins produced by *Pseudomonas syringae* increases the expression of LAP-A in tomato [36]. All the factors affecting the activity and expression of LAP-A are illustrated in Figure 1.

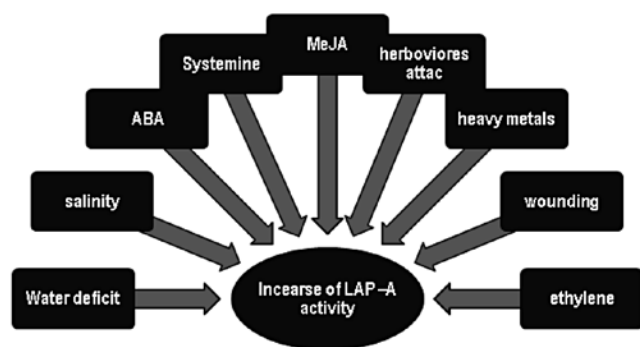


Fig. 1. Factors leading to increased LAP-A activity [9, 28÷36]

Vegetables

Aminopeptidase from the seeds of *Phaseolus vulgaris* (common bean) with molecular weight of 31 kDa and optimum pH of 7.0 showed a broad substrate specificity, but the leucine derivative was preferred. The inhibitory studies demonstrated that the aminopeptidase depends on metal ions and thiol groups present in the medium [37].

The aminopeptidases isolated from cotyledons, dry and germinating seeds of *Vicia faba* (broad bean, faba bean) has also been characterized. The molecular weight of faba bean LAP is approx. 60 kDa. The enzyme activity is stimulated by dithiothreitol (DTT) and mercaptoethanol and inhibited by Cu^{2+} and Zn^{2+} ions. The enzyme is most active against leucine derivatives, with the pH optimum of 7.5 [38].

Cucumber (*Cucumis sativus*) is another popular vegetable tested for aminopeptidase activity. It is metalloprotease, composed of two subunits with high molecular weight (200 kDa). It exhibits a highest substrate specificity toward Ala, and the optimum pH in the range of 8.0–9.0 [39]. In the case of carrot (*Daucus carota*) the aminopeptidase is specific to alanine and arginine [40]. Aminopeptidase isolated from cotyledons of soybean (*Glycine max*) has a molecular weight of approx. 56 kDa. The enzyme preferentially hydrolyzes derivatives of Glu and Asp, its optimum temperature is 45°C and pH optimum is in the range 7.9–9.0 [41].

A broad research of plant aminopeptidases was performed in *Cajanus cajan* (pigeon pea) which is a species of legume plants popular in Asia, Africa and Latin America. In the leaves of pigeon pea two LAP-like proteins (AP1, AP2) were detected, with a molecular mass of 60 kDa (AP1) and 41 kDa (AP2). In AP1 highest activity was observed with Ala and Leu derivatives. Enzyme activity significantly increased in the presence of methyl jasmonate (MeJa) and also after wounding. Aminopeptidase activity was also tested in *Cajanus cajan* during germination and in response to pathogens infection (fungi *F. oxysporum* and *A. niger*). Two aminopeptidases has been detected. The activity of one of them increased significantly in response to pathogens [42, 43].

Summary

Plant aminopeptidases are very interesting enzymes playing crucial role in plants physiology. The study described in literature shows that these enzymes are involved in key life processes such as: protein turnover, aging, germination and growth. Moreover it was proven that some of these enzymes are directly involved in plant defense response to harmful factors such as salinity, water deficit or presence of heavy metals. Such information is extremely important from the standpoint of plant protection products. Among the wide variety of aminopeptidases identified in plants, the majority shows broad substrate specificity with highest activity toward Leu, but also Ala, Phe, Pro or Met. Most of the studied enzymes have pH optimum in the range of 7.0 and 9.0. Recent studies showed that except LAP-A from tomato, harmful factors activate also triticale PAP, barley MAP and pigeon pea AP1. The exact role of aminopeptidases in physiological processes as well as in response to stress factors is still a subject of interest for many research groups worldwide.

Literature

1. Waditee-Sirisattha R., Hattori A., Shibato J., Rakwal R., Sirisattha S., Takabe T., Tsujimoto M.: *Role of the Arabidopsis leucine aminopeptidase* 2. Plant Signal. Behav. 2011, **6**, 1581–1583.
2. Waditee-Sirisattha R., Shibato J., Rakwal R., Sirisattha S., Hattori A., Nakano T., Takabe T., Tsujimoto M.: *The Arabidopsis aminopeptidase LAP2 regulates plant growth, leaf longevity and stress response*. New Phytol. 2011, **191**, 958–969.
3. Bartling D., Weiler E.: *Leucine aminopeptidase from Arabidopsis thaliana. Molecular evidence for a phylogenetically conserved enzyme of protein turnover in higher plants*. Eur. J. Biochem. 1992, **205**, 425–431.
4. Gettys K., Hancoc J., Cavaliere A.: *Salt tolerance of in vitro activity of leucine aminopeptidase, peroxidase nad malate dehydrogrnaze in the halophytes Spartina alterniflora and S. patens*. Bot. Gaz. 1980, **141**, 453–457.
5. Premarathne A., Leung D.: *Characterization of activity of a potential food-grade leucine aminopeptidase from kiwifruit*, Enzyme Research, 2010, No pp. given.
6. Lomate P., Hivrale V.: *Induction of leucine aminopeptidase (LAP) like activity with wounding and methyl jasmonate in pigeonpea (Cajanus cajan) suggests the role of these enzymes in plant defense in leguminosae*. Plant Psychol. Bioch. 2011, **49**, 609–616.
7. Wilson H.: *Genetic control and distribution of leucine aminopeptidase in the cultivated Chenopods (Chenopodium) and related weed taxa*. Biochem Genet. 1976, **14**, 913–919.

8. Scranton M., Yee A., Park S., Walling L.: *Plant leucine aminopeptidases moonlight as molecular chaperones to alleviate stress-induced damage*. J. Biol. Chem. 2012, **287**, 18408–18417.
9. Gu Y., Holzer F., Walling L.: *Overexpression, purification and biochemical characterization of the wound-induced leucine aminopeptidase of tomato*. Eur. J. Biochem. 1999, **263**, 726–735.
10. Herbers K., Prat S., Willmitzer L.: *Functional analysis of a leucine aminopeptidase from Solanum tuberosum L.* Planta 1994, **194**, 230–240.
11. Marinova M., Thi Kim Cun N., Tchorbanov B.: *Enzymatic hydrolysis of soy protein isolate by food grade proteinases and aminopeptidases of plant origin*. Biotechnol. & Biotechnol. 2008, **22**, 835–838.
12. Sopanen T., Mikola J.: *Purification and partial characterization of barley leucine aminopeptidase*. Plant Physiol. 1975, **55**, 809–814.
13. Doi H., Kawakami M.: *Purification of aminopeptidase from Australian classified barley flour*. Int. J. Biochem. Cell. Biol. 1997, **29**, 345–352.
14. Ogiwara N., Amano T., Satoh M., Shioi Y.: *Leucine aminopeptidase from etiolated barley seedlings: characterization and partial purification of isoforms*. Plant Sci. 2005, **168**, 575–581.
15. Strelec I., Vukeli B., Vitale L.: *Aminopeptidases of germinated and non-germinated barley*. Food Technol. Biotechnol. 2009, **47**, 296–303.
16. Jeong H., Shin J., Ok S.: *Barley DNA-binding methionine aminopeptidase, which changes the localization from the nucleus to the cytoplasm by low temperature, is involved in freezing tolerance*. Plant Sci. 2011, **180**, 53–60.
17. Oszywa B., Makowski M., Pawelczak M.: *Purification and partial characterization of aminopeptidase from barley (Hordeum vulgare L.) seeds*. Plant Physiol. Bioch. 2013, **65**, 75–80.
18. Casano L., Desimone M., Trippi V.: *Proteolytic Activity at Alkaline pH in Oat Leaves, Isolation of an Aminopeptidase*. Plant Physiol. 1989, **91**, 1414–1418.
19. Gajda A., Pawelczak M., Drag M.: *Substrate specificity screening of oat (Avena sativa) seeds aminopeptidase demonstrate unusually broad tolerance in SI pocket*. Plant Psychol. Bioch. 2012, **54**, 6–9.
20. Szawłowska U., Zdunek-Zastocka E., Bielawski W.: *Biochemical characterisation of prolyl aminopeptidase from shoots of triticale seedlings and its activity changes in response to suboptimal growth conditions*. Plant Psychol. Bioch. 2011, **49**, 1342–1349.
21. Szawłowska U., Grabowska A., Zdunek-Zastocka E., Bielawski W.: *TsPAP1 encodes a novel plant prolyl aminopeptidase whose expression is induced in response to suboptimal growth conditions*. Biochem. Biophys. Res. Commun. 2012, **419**, 104–109.
22. Beckman L., Scandalios J., Brewbacker J.: *Genetics of leucine aminopeptidase isozymes in maize*. Genetics 1964, **50**, 899–904.
23. Scandalios J.: *Leucine aminopeptidase isozymes in maize development*. J. Hered. 1975, **56**, 177–180.
24. Duprez K., Scranton M., Walling L., Fana L.: *Structure of tomato wound-induced leucine aminopeptidase sheds light on substrate specificity*. Acta Cryst. 2014, **70**, 1649–1658.
25. Gu Y., Pautot V., Holzer F., Walling L.: *A complex array of proteins related to the multimeric leucine aminopeptidase of Tomato*. Plant Physiol. 1996, **110**, 1257–1266.
26. Chao W., Pautot V., Holzer F., Walling L.: *Leucine aminopeptidases: the ubiquity of LAP-N and the specificity of LAP-A*. Planta 2000, **210**, 563–573.
27. Tu C., Park S., Walling L.: *Isolation and characterization of the natural leucine aminopeptidase (LapN) of tomato*. Plant Physiol. 2003, **132**, 243–255.
28. Gu Y., Walling L.: *Specificity of the wound-induced leucine aminopeptidase (LAP-A) of tomato, activity on dipeptide and tripeptide substrates*. Eur. J. Biochem. 2000, **267**, 1178–1187.
29. Hartl M., Merker H., Schmidt D., Baldwin I.: *Optimized virus-induced gene silencing in Solanum nigrum reveals the defensive function of leucine aminopeptidase against herbivores and the shortcomings of empty vector controls*. New Phytol. 2008, **179**, 356–365.
30. Narváez-Vásquez J., Tu C., Park S., Walling L.: *Targeting and localization of wound-inducible leucine aminopeptidase A in tomato leaves*. Planta, 2008, **227**, 341–351.
31. Fowler J., Narváez-Vásquez J., Aromdee D., Pautot V., Holzer F., Walling L.: *Leucine aminopeptidase regulates defense wound signaling in tomato downstream of jasmonic acid*. Plant Cell, 2009, **21**, 1239–1251.
32. Clarence A., Pearce R., Pearce G.: *Systemins: A functionally defined family of peptide signals that regulate defensive genes in Solanaceae species*. Proc. Natl. Acad. Sci. USA 2003, **100**, 14577–14580.
33. Manaa A., Ahmed H., Valot B., Bouchet J., Aschi-Smiti S., Causseand M., Faurobert M.: *Salt and genotype impact on plant physiology and root proteome variations in tomato*. J. Exp. Bot. 2011, **62**, 2797–2813.
34. Patout V., Holzer F., Reisch B., Walling L.: *Leucine aminopeptidase: An inducible component of the defense response in Lycopersicon esculentum (tomato)*. Proc. Natl. Acad. Sci. USA 1993, **90**, 9906–9910.
35. Bouilila-Zoghalmi L., Gallusci P., Holzer F., Basset G., Hjeblali W., Chaibi W., Walling L., Brouquisse R.: *Up-regulation of leucine aminopeptidase-A in cadmium-treated tomato roots*. Planta 2011, **234**, 857–863.
36. Pautot V., Holzer F., Chauvaux J., Walling L.: *The induction of tomato leucine aminopeptidase genes (LAPA) after Pseudomonas syringae pv. tomato infection is primarily a wound response triggered by coronatine*. Mol. Plant Microbe In. 2001, **14**, 214–224.
37. Abdala A., Takeda L., Freitas J., Alves K.: *Phaseolus vulgaris seed aminopeptidase Purification and partial characterization of Phaseolus vulgaris seed aminopeptidase*. Braz. J. Med. Biol. Res. 1999, **32**, 1489–1492.
38. Citharel J., Garreau C.: *Aminopeptidase from the cotyledons of vicia faba l. var. minor seeds: partial purification and some properties*. New Phytol. 1987, **107**, 499–506.
39. Yamauchi Y., Ejiri Y., Tanaka K.: *Purification of an aminopeptidase preferentially releasing N-terminal alanine from cucumber leaves and its identification as a plant aminopeptidase N*. Biosci. Biotechnol. Biochem. 2001, **65**, 2802–2805.
40. Hegedus I., Vojtassak J., Bilisics L., Borovkov N., Siekel P.: *Activity of some aminopeptidases in immobilized cells of Daucus carota*. Biologia Plantarum, 2000, **43**, 462–466.
41. Asano M., Nakamura N., Kawai M., Miwa T., Nio N.: *Purification and characterization of an N-terminal Acidic Amino Acid-Specific aminopeptidase from soybean cotyledons (Glycine max)*. Biosci. Biotechnol. Biochem. 2010, **74**, 113–118.
42. Lomate P., Hivrale V.: *Induction of leucine aminopeptidase (LAP) like activity with wounding and methyl jasmonate in pigeonpea (Cajanus cajan) suggests the role of these enzymes in plant defense in leguminosae*. Plant Psychol. Bioch. 2011, **49**, 609–616.
43. Lomate P., Hivrale V.: *Changes and induction of aminopeptidase activities in response to pathogen infection during germination of pigeonpea (Cajanus cajan) seeds*. J. Plant Physiol. 2011, **168**, 1735–1742.

*Joanna KANIA – M.Sc., Eng., graduated from the Faculty of Chemistry, Silesian University of Technology in Gliwice. Currently she is a Ph.D. student in the Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology. Research interests: plant aminopeptidases, especially aminopeptidases from oilseed rape their characteristics and influence of various factors on aminopeptidase activity both *in vitro* and *in vivo*.
e-mail: Joanna.Kania@polsl.pl

Danuta GILLNER – Ph.D., D.Sc., Eng., graduated from the Faculty of Chemistry, Silesian University of Technology in Gliwice. Currently works at the Department of Organic and Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology. Area of research interest: biotechnology, enzymology (isolation, purification, characteristics, structure, inhibition), metalloproteases, biotransformations, organic synthesis.