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Biochimica et Biophysica Acta 1465 (2000) 275–280

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Review

Amino acid transporters in plants

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Received 1 November 1999; accepted 1 December 1999

Abstract

Amino acid transporters are essential participants in the resource allocation processes that support plant growth and development. Recent results have identified several new transporters that contribute to a wide array of physiological activities, and detailed molecular analysis has provided fundamental insights into the structure, function and regulation of these integral membrane proteins.

Keywords: Amino acid transporter; Plasma membrane; Symporter; Assimilate partitioning; Membrane protein; Cotransport

1. Introduction

Amino acids are the currency of nitrogen exchange in plants [1]. Although inorganic salts of nitrogen are initially acquired from the soil solution, these compounds are rapidly incorporated into amino acids in root or mature leaf tissue. While some of the newly synthesized amino nitrogen is used in protein biosynthesis or as the precursor of other essential nitrogen containing molecules in these tissues, most is transported in the plants vascular system from the sites of primary assimilation to satisfy the nutritional needs of other organs that do not play a major role in nitrogen assimilation. Those tissues, which include developing leaves, meristems and reproductive organs, must import amino acids to support growth and development (Fig. 1). Amino acid transport also plays a key role in leaf senescence and seed germination. In rice, for example, as much as 60% of the amino acids delivered to the developing seeds are derived from amino acids recovered from senescing leaves. Clearly, amino acid transport is a fundamental activity in plant growth. The aim of this review is to summarize recent advances in understanding the plant amino acid transporters that are central components of this essential resource allocation process.

The majority of amino acid transporters described in plants are proton–amino acid symporters [2]. These secondary active transporters couple amino acid uptake to the proton electrochemcal potential difference that is maintained across the plasma membrane of plant cells by a p-type proton-pumping ATPase [3]. These are electrogenic carriers that can be driven by either the ΔpH or $\Delta\Psi$ component of the proton-motive force [4,5]. Transport activity is inhibited by protonophores and by covalent modification of histidine residues by diethylpyrocarbonate [1,4,6].

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0005-2736/00/$ – see front matter
PII: S0005-2736(00)00144-9

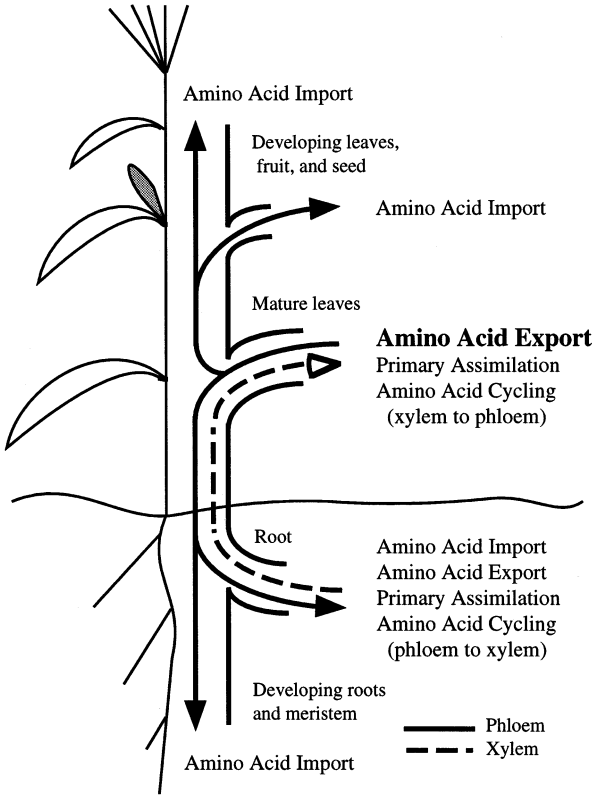

Fig. 1. Schematic representation of amino acid transport between the sites of primary nitrogen assimilation and the import-dependent tissues. Amino acids synthesized in the root from inorganic forms of nitrogen or symbiotic relationships are transported to mature leaf tissue in the xylem. Amino acids synthesized in the leaf after primary assimilation, or arriving from the root in the xylem, are transported out of the leaf in the phloem to satisfy the needs of heterotrophic sinks. Import-dependent tissues include developing leaves, roots, cortical cells in the stem, seed and fruits, and apical meristems (from [1] with permission).

Investigations of substrate binding have shown that the binding site is stereospecific, and that the α-amino and carboxyl groups are key determinants in defining substrate specificity [7,8].

2. There are multiple families of amino acid transporters in plants

Recent molecular cloning of several amino acid transporters by functional complementation in yeast has revealed that there are multiple gene families that encode different classes of amino acid transporters in plants ([9–14] for recent review). At least two superfamilies of amino acid transporters have been defined in plants; the amino acid, polyamine and choline transporters superfamily (APC) and amino acid transporter family (ATF) superfamily [14]. CAT1 is a high affinity transporter for basic amino acids in *Arabidopsis* that is the only well described member of the APC superfamily in plants. In contrast, the ATF superfamily has at least five sub-classes of transporters that have been described. These include the amino acid permeases (AAPs) with six members studied directly [14], the lysine, histidine transporters (LHTs) with one member described to date [15], the proline transporters (ProTs) with two members examined so far [16], the putative auxin transporters (AUXs) with AUX1 genetically defined as an auxin carrier [17] and a new member of the family (ANT1, aromatic and neutral amino acid transporter) demonstrated to transport aromatic amino acids, neutral amino acids, arginine and auxin (L. Chen, A. Ortiz-Lopez and D.R. Bush, unpublished data). There appear to be more than two dozen amino acid transporters in plants based on direct analysis of transport activity and on sequence similarities identified in expressed sequence tags and genome sequences [14]. Given that many of these transporters have overlapping substrate specificity, it seems clear that they must be distinguished from one another by tissue expression patterns (Table 1) and by their responses to environmental signals. For example, AAP2, AAP4 and AAP5 transcripts were found in *Arabidopsis* floral stems, where they might be involved in amino acid transport to the developing embryo. On the other hand, the expression of AAP3 is restricted to the roots, indicating a possible function in uptake from the phloem or in retrieving amino acids from the soil [18]. Similar differences in expression patterns have been observed for amino acid transporters in castor bean [19].

To understand better the significance of tissue-specific expression patterns, the Frommer lab has used promoter–GUS fusion analysis to investigate the function of AAP1 and AAP2 in seed development [20]. Prior experiments showed that AAP1 and AAP2 are highly expressed in flowers and siliques, and their expression is associated with the vascular system in cotyledons [9,21]. Hirner et al. [20] showed that the expression of both AAP1 and AAP2 are developmentally regulated and greatly induced at

Table 1
Summary of expression patterns for amino acid transporters described to date

AA carrier	Root	Sink leaf	Source leaf	Stem	Flower	Fruit	References
AtAAP1	−	−	+	−	+	+	[9,10,18,20,27]
AtAAP2	V	−	V	V	−	−	[20]
AtAAP3	+	−	+	−	−	−	[14]
AtAAP4	−	−	+	+	−	−	[14]
AtAAP5	+	−	+	+	+	+	[14]
AtAAP6	+	+	−	−	−	−	[14]
AtAUX1	+	?	?	?	?	?	[17,39]
AtProT1	+	+	+	+	+	+	[16]
AtProT2	+	+	+	+	+	−	[19]
AtCAT1	+	−	+	+	+	+	[40]
AtLHT1	+	+	+	+	+	+	[15]
AtANT1	−	?	+	+	+	?	[a]
RcAAP1	+	−	−	?	?	−	[19]
RcAAP2	+	−	−	?	?	−	[19]
RcAAP3	+	+	+	?	?	+	[41]
LeProT1	−	−	−	−	+	−	[22]

+ Indicates expression (any level); − indicates no detectable expression; ? unknown; V expression in vascular bundle.
[a] L. Chen, A. Ortiz-Lopez and D.R. Bush, unpublished data.

the heart stage of embryogenesis. The promoter–GUS analyses showed that AAP1 was expressed in the developing endosperm and cotyledon, whereas AAP2 was restricted to the vascular strand of siliques. Based on the timing and localization of its expression pattern, AAP1 was proposed to function in transporting amino acids into the developing endosperm and embryo, whereas AAP2 is particularly abundant in the vascular tissue of the stem and silique, suggesting a role in amino acid retrieval [20].

Environmental regulation of amino acid transport activity and gene expression also plays an important role in differentiating the function of these essential transport proteins. For example, although ProT1 and ProT2 are widely expressed proline-specific amino acid transporters in *Arabidopsis*, they respond differentially to changes in water and salt stress [16]. ProT2 expression was strongly induced under stress conditions whereas ProT1 expression was relatively unchanged and several AAPs were repressed. Increased proline transport capacity is consistent with the role proline plays as a compatible solute under water stress conditions. In addition to this environmental response, the expression of the tomato LeProT1 orthologue is restricted to the pollen and it appears to play a role in pollen maturation and germination. Significantly, 70% of the amino nitrogen in tomato pollen is proline, suggesting it plays an initial role as a compatible solute and later as energy source for tube elongation [22]. A pollen-specific amino acid transporter has also been identified in *Nicotiana sylvestris* [23].

Recent studies have shown that many important aspects of carbon and nitrogen metabolism are regulated by dynamic changes in C/N ratios where decreases in C or N resources up-regulate genes involved in their acquisition while abundance of these resources induces genes associated with use and storage [24–26]. Nitrogen assimilation in *Arabidopsis*, for example, is regulated by changes in metabolic status. Light and sugars, which both increase C/N balance, up-regulate the expression of genes involved in ammonia assimilation into glutamine and glutamate by chloroplastic GS (GLU1) and Fd-GOGAT (GLN2), while they repress AS (ASN1) and GDH expression [25]. In dark-adapted plants, however, carbon skeletons are less abundant (low C/N balance) and ASN1 gene expression is induced and there is a concomitant increase in asparagine levels observed in the phloem exudate [24]. Amino acid transporter gene expression also appears to be linked to the metabolic status of the plant C/N balance.

AAP1 (also known as NAT2) gene expression is regulated by light and carbon status. AAP1 transcript abundance in leaf tissue increases within 6 h in dark-adapted plants exposed to light. Likewise,

dark-adapted plants fed sucrose as an exogenous carbon source also increase message abundance [38]. Since both treatments increase sugar content, it is not possible to differentiate between a direct light signal versus indirect changes mediated by positive changes in the C/N ratio.

In addition to sugar and light-mediated changes in transcript abundance, AAP1 expression is also regulated by nitrate status. The AAP1 promoter has a nitrate response element, an A/T rich stretch A(G/C)TCA that is present at −367 of the AAP1 promoter region. This element is necessary for nitrate-dependent transcription and is commonly found in the promoter regions of nitrogen assimilatory enzymes from various species [28]. AAP1 transcripts increased several fold in *Arabidopsis* plants that were starved for nitrogen for 7 days and then fed 50 mM KNO_3 [38]. This is a significant observation because it suggests there is a global regulatory system that controls expression levels of multiple genes that are associated with distinct but complementary biochemical pathways. Thus, the coordinated expression of nitrate uptake, nitrogen assimilation and long distance transport genes allows plants to respond efficiently to dynamic changes in nitrogen availability.

In addition to the nitrate response element, there are other recognition sites that may regulate AAP1 expression. Several binding motifs of NIT-2, a global regulatory factor of nitrogen metabolism in fungi [29], are also present within the 5′ flanking sequence of AAP1, although the significance of this sequence in plant gene expression remains to be determined. ACGT core motifs are also found in the AAP1 promoter [20]. ACGT elements are found to be involved in abscisic acid response and in seed-specific expression [30–32]. The nitrate inducibility and seed-specific expression of AAP1 seem to correlate with the presence of these DNA motifs. The relatively complex regulation of AAP1 suggests that plant amino acid transporters respond to a variety of environmental and developmental signals.

Given the complex regulation of AAP1 expression in response to developmental and environmental signals, are there other regulatory pathways that control the expression of plant amino acid transporters? Recent insights into amino acid transporter regulation in yeast may also apply to plant amino acid transporters. SSY1 was initially identified in a mutant screen for yeast genes involved in branched-chain amino acid uptake [33]. SSY1 is a member of a major family of amino acid transporters in yeast and, significantly, it contains unique structural features that are similar to glucose-sensing members, SNF3 and RGT2, of the sugar transporter gene family in yeast. The primary function of these hexose transporter-like peptides appears to be monitoring sugar levels outside the yeast cell and transducing that information into different patterns of gene expression associated with carbon sufficient or deficient conditions [34]. Significantly, SSY1 has been shown to be involved in regulating the expression of at least six amino acid transporter genes [35,36], and these authors concluded that it is a sensor of external amino acids. In light of the recent observation that the amino acid content of phloem exudate exhibits diurnal variation [25], we may find that plants contain similar (or even homologous) sensing systems that monitor amino acid content in the apoplastic fluid and adjust, accordingly, the suite of amino acid transporters expressed by a given cell as a function of the amino acids present outside the cell.

3. Protein structure and function relationships in plant amino acid transporters

A complete analysis of amino acid transporter contributions to plant growth requires a detailed understanding of the structure and function relationships of these integral membrane proteins because specific amino acids and domains will be responsible for reversible regulation of transport activity and/or protein turnover. Moreover, amino acid residues involved in the transport reaction are potential targets for site-directed mutagenesis that alters transport activity. For example, substituting a basic amino acid for a single histidine residue in a proton–sucrose symporter has increased V_{max} transport activity by 10–15-fold [37].

A key question in any structural analysis of an integral membrane protein is the disposition of the polypeptide chain across the membrane. AAP1 (NAT2) was chosen for structural study as a prototypical example of the AAP gene family [27]. In order to determine the topology of AAP1, a human c-*myc* epitope was engineered onto the N- or C-termi-

nus of AAP1 to monitor their cellular localization using in vitro co-expression with microsomal membranes. The N-terminally tagged epitope was sensitive to proteolysis by proteinase K digestion, whereas the *myc* epitope on the C-terminus was resistant. Those results showed that the N-terminus of AAP1 is on the exposed surface of the microsomes and the C-terminus was inside the vesicle and thus protected from proteolysis. In addition, partial proteolysis of the in vitro translated C-terminally tagged protein generated six immunoprecipitable peptide fragments suggesting that AAP1 has six protein domains that are accessible to proteinase K. These results suggested AAP1 has 11 membrane spanning domains (Fig. 2). That conclusion was supported by immunofluorescent localization of epitope-tagged AAP1 in COS-1 cells. In those experiments [27], the C-terminally tagged protein was stippled with an fluorescently labeled antibody applied to intact cells whereas the N-terminally tagged protein was labeled only in permeabilized cells that allowed access to the intracellular face of the plasma membrane. An 11-transmembrane domain model for AAP1 was supported by several lines of evidence including the number and size of the proteolytic fragments, predictions derived from hydropathy analysis, the absence of protein glycosylation, and localization of the N- and C-termini on opposite sides of the plasma membrane.

Site-directed and random mutagenesis have also been used in a structural analysis of the AAP1 symporter. Previous results showed that plant amino acid symporters are inhibited by diethylpyrocarbonate-dependent modification of histidine residues [4] and kinetic analysis of the inactivation reaction suggests diethylpyrocarbonate binds at, or conformationally

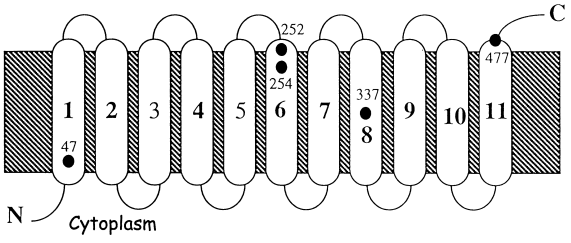

Fig. 2. Schematic diagram of AAP1 topology [38] with approximate location of functionally important amino acid residues identified using site-directed and random mutagenesis (L. Chen and D.R. Bush, unpublished data).

linked to, the substrate binding site of the transporter [1]. Two histidine residues in AAP1, H47 and H337, that are conserved in the AAP family were shown to be critical for amino acid transport function using site-directed mutagenesis. All amino acid substitutions of these residues dramatically altered function by impacting substrate binding or destabilizing the protein (L. Chen and D.R. Bush, unpublished data). In complementary experiments using random mutagenesis and selection for altered transport activity in AAP1, single amino acid substitutions were identified that dramatically affected the transport properties of AAP1. Significantly, two residues, D252 and A254, that are associated with membrane spanning domains differentially changed apparent K_ms for alanine and histidine, suggesting these residues may be involved in defining the substrate binding site (L. Chen and D.R. Bush, unpublished data).

In addition to a molecular analysis of AAP1 protein structure, it has been the focus of an electrophysiological dissection of its transport kinetics and reaction mechanism [5]. In these experiments, AAP1 was expressed in *Xenopus* oocytes and transport was measured using a two-electrode voltage-clamp method. The maximum current for both protons and amino acids was dependent on the activity of the cosubstrate, suggesting both ligands bind randomly to the symporter. Likewise, apparent affinities for the two substrates decreased in the presence of increasing concentration of the cosubstrate. This observation is consistent with a simultaneous transport mechanism. In a more detailed analysis of AAP5 transport kinetics and direct measurements of substrate flux, it was concluded that the stoichiometry of the AAPs is one proton per amino acid [8]. Significantly, lysine was transported in its cationic form while glutamic acid and histidine were neutral. Their analysis of AAP5 also identified the α-amino and carboxyl groups, as well as the β-carbon, as important determinants in defining substrate specificity. Those results are consistent with earlier experiments examining substrate binding using purified membrane vesicles and kinetic analysis of transport competition by a variety of amino acid analogues [7].

Amino acids are the key metabolite in nitrogen metabolism in plants because they represent the initial product of primary assimilation, and because

they are transported throughout the plant to satisfy the nutritional needs of heterotrophic tissues. Recent research has identified multiple gene families that encode at least a dozen amino acid transporters. Additional results dissecting the regulation and expression patterns of these carriers have shown how they contribute to every aspect of assimilate partitioning, ranging from phloem loading, to amino acid retrieval from senescing leaves and active accumulation in developing seeds. These advances are providing new insight into the biology of resource allocation, and they are laying the foundation for developing novel strategies to modify crop nutritional value by directly manipulating amino acid content in harvested organs.

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