Transport of primary metabolites across the plant vacuolar membrane

H. Ekkehard Neuhaus*

Pflanzenphysiologie, Technische Universität Kaiserslautern, Postfach 3049, D-67653 Kaiserslautern, Germany

Received 16 January 2007; revised 2 February 2007; accepted 3 February 2007

Available online 12 February 2007

Edited by Ulf-Ingo Flügge and Julian Schroeder

Abstract Mesophyll cells and most types of storage cells harbor large central vacuoles representing the main cellular store for sugars and other primary metabolites like carboxylic- or and amino acids. The general biochemical characteristics of sugar transport across the vacuolar membrane are already known since a couple of years but only recently the first tonoplast sugar carriers have been identified on the molecular level. A candidate sucrose carrier has been identified in a proteomic approach. In Arabidopsis, the tonoplast monosaccharide transporters (TMT) represent a small protein family comprising only three members, which reside in the vacuolar membrane. Two of three tmt genes are induced upon cold, drought or salt stress and tmt knock out mutants exhibit altered monosaccharide levels upon cold induction. These observations indicate that TMT proteins represent the first examples of tonoplast sugar carriers involved in the cellular response upon osmotic stress stimuli.

© 2007 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Vacuole; Carboxylic-acid; Sugar transporters

1. General features of central vacuoles

Central vacuoles are surprisingly large, can occupy (e.g. in CAM plants or fruit tissues) more than 80% of the total cell volume [1] and are separated from the surrounding cytosol by a single, semi-permeable membrane, the so called tonoplast. Already these facts make the central vacuole uniquely suited to fulfill essential storage functions. Therefore, it is not surprising that the number of different inorganic and organic solutes found in the vacuole is extremely large. In fact, the substantial structural and functional diversity of compounds stored in the vacuole identifies this organelle as a core structure in plant cells important for processes like the cellular energy management, accumulation of reserves and nutrients, regulation of cellular pressure, detoxification and ecological interactions.

Several of the compounds given below accumulate via secondary active transporters in the vacuole against an existing concentration gradient. The driving forces for these carriers are either a proton- or an electrochemical gradient generated by the activity of two types of proton pumps; a vacuolar-type (V-type) H⁺-ATPase and a H⁺-PPhase [2–5]. As far as we know, these two proton pumps are present in all types of plant vacuoles [6,7].

Organic compounds which typically accumulate in central vacuoles are e.g. carbohydrates like mono- and disaccharides (for details see below), linear and cyclic polyols, three types of fructans with slightly different structures and various carboxylic acids [8–12]. Malate, citrate and fumarate enter the vacuole via specific transport proteins and represent major carboxylic acids in plants [13–15]. For malate two distinct transport mechanisms have been identified: this dicarboxylic acid enters the vacuole either by a anion channel specific for malate³⁻ [16], or by a recently identified solute carrier [17] (Fig. 1). The latter carrier joins structurally the family of the so called sodium-coupled carbohydrate carriers as present in the plasma membrane of animal cells [18]. Interestingly, the absence of the Arabidopsis homolog AtTDT (Arabidopsis thaliana tonoplast dicarboxylate transporter) alters both, the metabolism of organic acids and the regulation of the cytosolic pH substantially [19] without affecting the phenotype of the corresponding knock out mutant lines when compared with wild-type plants [17].

Central vacuoles also play a critical role in plant nutrition because they accumulate sulfate, phosphate and nitrate [20,21]. Not only nitrate, which serves as the precursor for amino acid synthesis, but also the intermediate and toxic ammonia molecule [22], and the final amino acids themselves are stored in the central vacuoles [23,24]. The observation that the vacuolar concentration of several amino acids is lower than in the surrounding cytosol [25] is, however, not consistent with the facilitated diffusion mode of transport catalyzed by reconstituted tonoplast amino-acid carrier activity [26]. The first candidate amino acid carriers have been identified in the proteome of Arabidopsis mesophyll tonoplasts or as transporter-GFP fusion proteins after transient transformation [27,28]. It will be interesting to analyze the biochemical properties of corresponding recombinant proteins in the near future.

In addition to the compounds above, ecological relevant solutes like anthocyanins, flavonoids and a wide array of conjugated endogenously synthesized toxic- or xenobiotic compounds typically accumulate in the vacuole and several of these substances cross the tonoplast via ABC-type carriers [29–32]. Thus in sum, there is no other cellular compartment harboring a comparable large number of different solutes (see [33] for a recent complete overview).

In this review I will focus only on the transport of one class of compounds, namely sugars in form of the disaccharide
2. Transport of mono- and disaccharides across the tonoplast

The ability for net sugar synthesis represents a main feature of plant physiology. Sugars fulfill a remarkable number of essential functions as they are a general source for metabolic energy, serve as a precursor for starch and cellulose synthesis, and are a metabolic starting point of carboxylate- and amino acid synthesis [34]. Therefore, it is not surprising that cells from all types of organisms control the endogenous sugar levels and perceive the actual concentrations via sophisticated sensing systems [35–37].

The vacuole stores large concentrations of the disaccharide sucrose, but also the monosaccharides glucose and fructose are typically present in high levels [38]. In vacuoles from leaves of C3- and CAM species sucrose import occurs by an ATP independent mechanism solely driven by the existing concentration gradient between the cytosol and the vacuolar lumen [39,40] (Fig. 1). In contrast, vacuoles from sugar beet taproots seem to exploit the existing proton-motive force to import sucrose via an H⁺ antiport mechanism against the concentration gradient [41] (Fig. 1).

Given that sucrose accumulation in the vacuoles is of high importance for the control of photosynthesis [42] and for primary metabolism in storage tissues [38] it appears surprising that no corresponding proteins have so far been characterized on both, the functional and molecular level. A genome analysis indicated that Arabidopsis harbours about ten disaccharide transporter isoforms [51,52]. Within these large monosaccharide family a small group comprising only three isoforms exhibits unique structural characteristics. These carriers, named *Arabidopsis thaliana* tonoplast monosaccharide transporter, *AtTMT* [53], exhibit about 12 predicted transmembrane domains, as known from other carriers belonging to the major facilitator superfamily [54], but harbour an extraordinarily long, hydrophilic loop connecting trans-membrane domains six and seven [53]. This loop comprises up to 320 amino acids in length and is therefore 4–5 times longer than the corresponding loop domain in other so far known monosaccharide transport proteins. A specific function of this loop domain is so far unclear, but it appears remarkable that in yeast, are located in the plant tonoplast [43] (Fig. 1). This location has been substantiated by two approaches: Firstly, corresponding *HvSUT4* and *AtSUT4-GFP* constructs, transiently expressed in either Arabidopsis- or onion cells, clearly decorate the tonoplast and not the plasma membrane [43]. Secondly, transcripts coding for *AtSUT4* specifically accumulate in mesophyll cells and not in the smaller companion cells, or in the chloroplast-free sieve element cells [43]. Mesophyll cell vacuoles represent the main store for sucrose synthesized during photosynthesis. However, since both, *HvSUT4* and *AtSUT4* catalyze a H⁺/sucrose cotransport when recombinantly synthesized in yeast [44,45] we have to assume that (due to the existing proton gradient, see above and Fig. 1) these carriers are involved in sucrose export from the vacuole into the cytosol.

Besides sucrose the monosaccharides glucose and fructose are typically present the vacuole [46]. In some tissue, e.g. grape berries [47], glucose represents the main storage sugar and both, facilitated diffusion as well as energized proton/sugar antiport mechanisms have been described for glucose uptake into isolated vacuoles, or into tonoplast vesicles prepared from various plant species [48,49] (Fig. 1).

PCR amplification using primers directed against conserved domains allowed to identify a so far unknown putative hexose carrier from sugar beet [50]. By use of two independent peptide specific antibodies it was possible to locate the corresponding protein, exhibiting a calculated molecular mass of 54 kDa, in enriched vacuolar membranes from both, transgenic tobacco (expressing the sugar beet gene) and native sugar beet tissue [50]. However, since all attempts to synthesize a functional recombinant transport protein in yeast failed unfortunately [50], the exact transport activity of this carrier remains to be analyzed.

The Arabidopsis genome contains about 60 genes coding for monosaccharide transporter isoforms and more than ten of these proteins have been characterized as plasma membrane located monosaccharide importers [51,52]. Within these large monosaccharide family a small group comprising only three isoforms exhibits unique structural characteristics. These carriers, named *Arabidopsis thaliana* tonoplast monosaccharide transporter, *AtTMT* [53], exhibit about 12 predicted transmembrane domains, as known from other carriers belonging to the major facilitator superfamily [54], but harbour an extraordinarily long, hydrophilic loop connecting trans-membrane domains six and seven [53]. This loop comprises up to 320 amino acids in length and is therefore 4–5 times longer than the corresponding loop domain in other so far known monosaccharide transport proteins. A specific function of this loop domain is so far unclear, but it appears remarkable that in the central portion of this loop a strong negative cluster, built up by 11 aspartate residues in a stretch of only 20 amino acids, is present [53]. Interestingly, such negatively charged clusters (also built by aspartate residues) of unknown function are also present in a wide number of mitogen-activated protein kinases (data not shown).

*AtTMT-GFP* fusion proteins of all three isoforms are directed into the vacuolar membrane after heterologous transient expression in either tobacco- or onion cells and promoter-reporter data revealed that *Attmi* genes are active in sugar storing mesophyll cells [53] (Fig. 1). These results are completely in line with proteomic data made on vacuolar membranes from Arabidopsis mesophyll cells revealing the presence of *AtTMT1*.
and AtTMT2 [27]. The absence of AtTMT3 in the detectable proteome of Arabidopsis tonoplasts [27] concurs with the generally extremely low expression level of the third Attm1 gene under every condition tested [53].

A detailed expression analysis of all three Attm1 genes showed that both, Attm1 and Attm2 represent cold induced genes, which are also responsive upon drought and salt stress [53]. Interestingly, these three stress stimuli usually lead to an accumulation of solutes, including sugars, in Arabidopsis leaves [55–57] and since most of the sugars in mesophyll cells accumulate in the vacuole [1,25], it is very likely that AtTMT proteins contribute to the molecular response of Arabidopsis upon osmotic stress. The observation that vacuoles isolated from cold induced Arabidopsis wild type leaves exhibit a markedly increased glucose transport rate when compared with the properties of vacuoles from plants grown under moderate temperatures [53] further supports this assumption.

Clear evidence for the involvement of AtTMT proteins in vacuolar monosaccharide transport has been gained by comparing the glucose uptake properties of vacuoles isolated from either, wild type plants or from knock out lines. The absence of a functional Attm1 gene reduced the cold stimulated glucose transport to about 1/3 of the transport observed on wild type vacuoles [53]. As cold stimulated glucose transport into wild type vacuoles is inhibited by fructose and the uncoupler compound ammonia it is very likely, that both types of monosaccharides are transported by AtTMT in a proton/sugar antiport mode of transport.

The latter observations are somehow surprising given that relatively close structural AtTMT homologs reside in the plasma membrane of cyanobacteria like Synechocystis spec. [58] or Nostoc punctiforme (data not shown). The transporter from Synechocystis spec., named SmGTR, is characterized to exert a comparable high degree of specificity for glucose and to catalyze a proton coupled sugar import into the bacterial cell [58]. Thus, it will be interesting in the near future to identify the structural determinants for such altered transport characteristics. However, in this context it has to be considered that even small structural modifications in a transport protein might affect the mode of transport (e.g. antiport to uniport) drastically [59].

### 3. What’s next to do?

As outlined above, first transport proteins involved in the movement of monosaccharides across the tonoplast have been identified. These transporters, belonging to the TMT group, obviously mediate a proton-coupled antiport mechanism, but the tonoplast contains in addition a glucose facilitator protein of unknown molecular structure. On the basis of the available proteomic information, this facilitator protein and further carbohydrate transport proteins might become identified in the near future. Another challenging topic is the establishment of a suitable recombinant system allowing the identification of the biochemical properties of the carriers in more detail. However, this aim seems currently difficult to approach since the level of recombinant tonoplast proteins in heterologous systems appear to be extremely low. Moreover, it is totally unknown whether coherent modifications occurring in the ER or Golgi compartment are required for function. A third challenging task is the analysis of post-translational regulation of sugar transporters. Corresponding processes might involve protein/protein interactions which have to be analyzed by use of genetic and biochemical methods. It is very likely, that research on the vacuolar membrane will lead to novel carrier proteins and uncover so far unknown regulatory properties.

### Acknowledgement

The author gratefully acknowledges the financial support by the Deutsche Forschungsgemeinschaft.

### References


