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Sugar transport across the plasma membranes of higher plants

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Abstract

The fluxes of carbohydrates across the plasma membranes of higher-plant cells are catalysed mainly by monosaccharide and disaccharide-H^+ symporters. cDNAs encoding these different transporters have been cloned recently and the functions and properties of the encoded proteins have been studied extensively in heterologous expression systems. Several of the proteins have been identified biochemically in these expression systems and their location in plants has been shown immunohistochemically or with transgenic plants which were transformed with reporter genes, expressed under the control of the promoters of individual transporter genes. In this paper we summarize the current knowledge on the molecular biology and biochemistry of higher-plant sugar transport proteins.

Introduction

Photosynthetic CO_2 fixation in the mature leaves of higher plants and the controlled partitioning of assimilated carbon between photosynthetically inactive organs, developing tissues or storage compartments are two highly interconnected processes. Photosynthetic tissues possess only a limited capacity for the storage of assimilates and most of the carbohydrates that are not needed for the leaf's own metabolism enter the sieve elements of the vascular system. The role of higher-plant long-distance phloem transport is therefore not only the supply of assimilates to all kinds of sink tissues but also the removal of fixation products from the source tissues to avoid accumulation of metabolites and feed back inhibition on the photosynthetic machinery. This allocation system seems to be carefully balanced, but mechanisms controlling its regulation are still poorly understood.

Two fundamentally different models are discussed for the way used by photoassimilates to get into or out of the sieve elements of the phloem. The symplastic model depends on the existence of symplastic connections between photosynthetically active mesophyll cells and sieve elements, the plasmodesmata, and similar connections would also be needed at the sink end of the phloem. According to this model carbohydrates synthesized in the mesophyll would diffuse from one cell type to the other through these plasmodesmata, never leaving the symplastic space and reaching their final destination without a single membrane translocation step. The apoplastic model of phloem loading and unloading, on the other hand, depends on the existence of specific transporters, catalysing the membrane penetration of sugars or amino acids, and several types of transporters might be necessary to allow this apoplastic pathway. The first type of transport protein is responsible for the efflux or export

of assimilates, such as the efflux of sucrose from the mesophyll cells or the efflux of sucrose from the phloem at the sink end of the sieve elements. The second type of transporters is needed for the catalysis of substrate import, for example the loading of sucrose into the phloem or the transport of monosaccharides into the cells of a specific sink tissue.

Results have been published supporting either the apoplastic, carrier-mediated way or the symplastic transport via plasmodesmata. In recent years cDNAs and/or genes have been cloned for a number of higher-plant sugar transporters. The identification of DNA encoding such proteins not only provided strong evidence for the apoplastic model, but it also allowed studies of individual transporters and determination of their location *in planta*.

Identification of clones encoding sugar transporters and homology to other transport proteins

The first plant sugar transporter cDNA was cloned from the lower plant *Chlorella kessleri* by differential screening of a cDNA library from cells which were induced for monosaccharide transport [32]. The obtained cDNA clone (*HUP1*) was used for the screening of several cDNA and genomic libraries from higher plants and the cloning of the first higher-plant monosaccharide transporter from *Arabidopsis thaliana* was published in 1990 [28]. Both the *Chlorella* HUP1 and the *Arabidopsis* STP1 protein showed homology to transporters from mammals, yeast, and bacteria [19] and were unequivocally characterized as glucose-H^+ symporters by heterologous expression in *Schizosaccharomyces pombe* ([27, 28]; see below). The availability of probes for monosaccharide transporters and the fact that plant plasma membrane proteins could be functionally expressed were the basis for the cloning of many other transporters.

A sink-specific monosaccharide transporter (MST1) was cloned from *Nicotiana tabacum* [29], and families of at least 7 to 12 monosaccharide transporters (or monosaccharide transporter-like proteins) were shown to exist in *Ricinus communis* [45], *Chenopodium rubrum* [26], and *Arabidopsis thaliana* ([26], Baier and Sauer, unpublished data). Not all of these putative transporters have been fully characterized by heterologous expression and it may turn out that the substrate specificities of some of these proteins differ. In the lower plant *Chlorella kessleri*, for example, a second gene encoding a monosaccharide-H^+ symporter was cloned (*HUP2*) which gene turned out to be a galactose-H^+ symporter [37]. Up to now there is no indication, however, that any of the higher-plant transport proteins isolated by heterologous screening with monosaccharide transporter probes might transport disaccharides such as sucrose or maltose.

The cDNA of the first higher-plant sucrose transporter was cloned by Riesmeier *et al.* in 1992: a yeast strain which was deficient in both sucrose transport and invertase activity was transformed with a plant-derived sucrose synthase to allow sucrose degradation in the yeast cytoplasm. The resulting strain was then used for the expression of a spinach cDNA library and transformed yeast cells were screened for their capability to grow on sucrose as single carbon source which should only be possible when either a sucrose transporter or a secreted invertase is expressed. Shortly after the spinach sucrose transporter (*SoSUT1* [24]) a sucrose transporter from potato was isolated also by complementation cloning in *Saccharomyces cerevisiae* (*StSUT1* [23]) and both plants seem to have only one single gene encoding a sucrose transport protein. In contrast, cDNAs for two different sucrose transporters were identified by heterologous screening in cDNA libraries constructed from whole plants of *Arabidopsis thaliana* (*AtSUC1* and *AtSUC2* [30]) or from isolated vascular bundles of *Plantago major* (*PmSUC1* and *PmSUC2* [8], Gahrtz and Sauer, unpublished data).

The different higher-plant sucrose-H^+ symporters show a high degree of homology on the amino acid level with 63% (PmSUC1 versus AtSUC1) to 77% (AtSUC1 versus AtSUC2) of identical amino acids in their translated sequences and similar degrees of identity can be calculated

for the monosaccharide-H^+ symporters (e.g. 79% for MST1 versus STP1 or 63% for STP1 versus STP4). A comparison of disaccharide transporters versus monosaccharide transporters, on the other hand, suggests that these two types of transport proteins are less closely related with only 20% of identical amino acids in the average. Despite this low homology between mono- and disaccharide transporters, however, the tertiary structures of the proteins in the membrane might not be so different. On the basis of hydrophilicity plots and in agreement with results from bacterial and mammalian sugar transporters [5, 21, 35], 12 putative transmembrane helices were predicted for all cloned plant sugar transporters; it is assumed that, as in these other transporters, N- and C-termini of the plant proteins are located on the cytoplasmic side of the membrane.

It may seem erroneous to use data on the structure of bacterial or mammalian sugar transporters to explain the structure of higher-plant sugar-H^+ symporters. However, as Marger and Saier [19] pointed out, the plant monosaccharide-H^+ symporters belong to a large superfamily of transmembrane facilitators which possibly evolved from one single ancestral transport protein. The structurally related members of this superfamily catalyse the transport of quite different substrates such as organic acids, drugs, organic phosphates or oligo- and monosaccharides. A typical feature of these 12 membrane transporters is a rather high degree of similarity between the first and the second half of the protein [12, 18, 33]. Such conserved sequences can also be found in the two halves of higher-plant mono- and disaccharide transporters [30], showing that the sucrose-H^+ symporters may also be members of this superfamily.

Heterologous expression and kinetic characterization of plant transporters

First hints towards an energy-dependent sugar-H^+ symport in higher plants came from the work of Sovonick *et al.* [36], Giaquinta [10] and Komor *et al.* [14] but the first unequivocal proof for sugar-H^+ symport in plants came again from *Chlorella kessleri* [13]. Since then proton-coupled mono- and disaccharide transporters have been identified and studied in many different tissues of higher plants (reviewed by Bush [4]). Only the cloning of the respective genes and cDNAs, and their expression in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Xenopus laevis* oocytes allowed the investigation of one specific higher-plant transporter at a time, separated from all other transporters possibly present in the same cell or plasmalemma preparation. Studies of sugar transport into tissue slices, cell suspensions, or even into lipid vesicles prepared from plant plasma membranes or reconstituted from detergent extracts thereof may always deal with the concerted reaction of a set of similar transporters. Thus the proton-coupled hexose carrier from *Chenopodium rubrum* [11] has turned out to consist of at least three different monosaccharide transporters [26] and also the inducible *Chlorella* glucose-H^+ symporter [39] has been shown to be composed of three different inducible proteins, with one of them being a galactose-H^+ symporter [37]. Since two sucrose-H^+ symporters have been identified in *Arabidopsis* [30] and *Plantago* [8] one cannot be sure whether the earlier results published on sucrose transporters in other plants represent the kinetic properties of only one or possibly two sucrose transporters.

Ideally, a transporter should be studied in a unicellular, stable expression system with no or only a negligible background of endogenous transporters. The system should be eukaryotic to allow possible modifications and it should be easy to handle. Fission yeast *Schizosaccharomyces pombe* and baker's yeast *Saccharomyces cerevisiae* fulfil all of these criteria: they can utilize several monosaccharides as sole carbon source and the uptake of these sugars is catalysed by sugar uniporters (or facilitators) with K_m values in the millimolar range. Furthermore there are yeast mutants deficient in sugar transporters, and the expression of many sugar uptake systems is repressed in the presence of glucose.

The *Arabidopsis thaliana* STP1 protein was the first higher-plant sugar transporter to be ex-

pressed in yeast cells [28, 38]. It could be shown by membrane fractionation and with immunohistochemical methods that 100% of the recombinant STP1 protein reach the yeast plasma membrane (Stadler and Stolz, personal communication). The protein was characterized as monosaccharide transporter with a K_m for *D*-glucose of 20 μM. Other sugars such as *D*-xylose or *D*-galactose, which are normally not transported by yeast cells, are also substrates of the STP1 protein allowing transport studies with no interference by yeast endogenous transporters. The STP1 protein is sensitive to incomplete uncouplers of the energy gradient, causes accumulation of non-metabolizable sugar analogs. This accumulation by STP1 depends both on the change in pH across the plasma membrane and on the energy status of the cells since starved yeast cells are unable to energize their plasma membranes. This suggests that STP1 is an energy-dependent monosaccharide-H^+ symporter. Several other monosaccharide transporters have been studied in yeast systems: the MST1 protein from tobacco [29], the HEX3 protein from *Ricinus* [45], and three transporters from *Arabidopsis* (STP2, STP3, and STP4; Baier and Sauer, unpublished data). All of them have similar kinetic properties as the STP1 protein described above.

For a better understanding of the energy dependence during active sugar transport by STP1-plasma membranes were isolated from transgenic, STP1-expressing yeast cells and fused with proteoliposomes containing cytochrome-*c*-oxidase from beef heart mitochondria [38]. In the presence of the electron donor ascorbate this system generates a proton motive force (*pmf*) which should be high enough to energize sugar accumulation inside the vesicles if STP1 is a monosaccharide-H^+ symporter. The same reconstitution system had already been used successfully for *in vitro* characterization of the *Chlorella* HUP1 protein [22] and, in fact, Stolz *et al.* [38] could demonstrate that a *pmf* generated by cytochrome-*c*-oxidase can drive the accumulation of *D*-glucose by STP1. Similar results could also be obtained with STP1 protein solubilized from yeast plasma membranes with octylglucoside after reconstitution in cytochrome-*c*-oxidase-containing proteoliposomes [38].

STP1 has also been studied in *Xenopus* oocytes [1] where it caused hexose-elicited depolarization of the oocyte membrane. Sugar uptake by STP1 was stimulated by low external pH and the determined K_m values and pH optima were in good agreement with results from the yeast system.

The cDNAs of the spinach SoSUT1 and the potato StSUT1 sucrose transporters were isolated by complementation cloning in yeast [23, 24]. The K_m values for sucrose uptake into transgenic yeast cells were shown to be 1.5 and 1 mM, respectively. These values correlate well with K_m values determined *in planta* (for review see [6]). Of all the substrates tested only maltose competed with sucrose for transport by SoSUT1 and StSUT1. The K_m values for maltose were about 5 and 10 mM, respectively. In contrast, Buckhout [2] and Bush [3] had previously published that sucrose transport in sugar beet is highly specific for sucrose with maltose causing no inhibition at a 10-fold higher concentration than sucrose. Sucrose uptake by SoSUT1 and StSUT1 was shown to depend on the energy status of the yeast cells, but a possible accumulation of the substrate inside the cells could not be shown, since sucrose was readily metabolized. This and the uncoupler sensitivity of sucrose uptake suggested that sucrose transport might be a H^+ symport.

Using *SoSUT1* cDNA as hybridization probe Sauer and Stolz [30] cloned two cDNAs from *Arabidopsis thaliana* (*AtSUC1* and *AtSUC2*) which were studied in a *S. cerevisiae* wild-type strain. Most kinetic parameters of the two *Arabidopsis* transporters were very similar to those determined for SoSUT1 and StSUT1 with one single exception: the activity of AtSUC1 was constant from pH 4 to 6 and decreased only to about 50% at pH 7, whereas the activity of AtSUC2, SoSUT1, and StSUT1 increased drastically with decreasing external pH values, suggesting different functions of the two *Arabidopsis* sucrose transporters. The same results were obtained when the two sucrose transporters PmSUC1 and PmSUC2 from *Plantago* were expressed in *S. cerevisiae*. PmSUC1

exhibited a pH dependence like AtSUC1, and the pH-dependence of PmSUC2 was like that of AtSUC2 ([8], Gahrtz and Sauer, unpublished data). Expression of the *Plantago* PmSUC2 transporter in an invertase-deficient strain of *Saccharomyces cerevisiae*, which is unable to split sucrose, revealed that sucrose can be accumulated more than 200-fold in this strain. Isolation of plasma membranes from this PmSUC2 expressing yeast strain and fusion of these membranes to cytochrome-*c*-oxidase-containing proteoliposomes (see above) proved unequivocally that *pmf* is the driving force for this accumulation and that sucrose transport by PmSUC2 is a H^+ symport [8].

Identification of the proteins

Only two sugar transport proteins had been identified biochemically: the *Chlorella* HUP1 protein [31] and a sucrose transporter from sugar beet [15]. Both proteins had an apparent molecular mass of about 42 kDa when separated in sodium dodecyl sulfate (SDS)-polyacrylamide gels. This molecular mass on SDS gels is clearly smaller than the molecular masses calculated from the cDNA sequences which are in the range of 55k Da for all transporters. Antibodies raised against C-terminal fusions of various monosaccharide transporters with *Escherichia coli* β-galactosidase showed that the *Arabidopsis* STP1 protein and the tobacco MST1 protein have apparent molecular masses of about 42 kDa in SDS gels when isolated from transgenic yeast cells [29, 38] and the same molecular mass was found for STP1 isolated from *Arabidopsis thaliana* plasma membranes [38]. This difference between apparent and calculated molecular mass is not due to N- or C-terminal modifications, since protein sequencing of the N-terminus of purified STP1 protein yielded the same sequence that had previously been determined by cDNA sequencing and a C-terminal histidine tag which had been used for purification was still present [38].

Histidine tagging has also been used for identification of the *Arabidopsis* AtSUC1 sucrose transporter protein in plasma membranes of transgenic yeast cells (45k Da including the tag of 6 histidines) and the subsequent purification of the protein [30]. Addition of a C-terminal histidine tag did not interfere with the transport activity of STP1 or AtSUC1 and may be an important tool for the large-scale purification of plant sugar transporters [30, 38]. The *Plantago* PmSUC2 sucrose-H^+ symporter is expressed at very high levels in *Saccharomyces cerevisiae*. About 10% of the protein in purified yeast plasma membranes is PmSUC2 protein. The apparent molecular mass of this transporter on SDS gels is only 35 kDa. For this transporter it has been shown that deletion of the cytoplasmic N-terminus has hardly any effect on the transport activity of PmSUC2 and on the sorting of the protein towards the yeast plasma membranes (Stadler and Sauer, unpublished data).

Plant sugar transporters do not seem to be N-glycosylated. Neither the *Chlorella* HUP1 glucose-H^+ symporter nor the *Arabidopsis* STP1 monosaccharide-H^+ symporter possess consensus sequences for N-glycosylation [28, 32]. The sucrose-H^+ symporters do have potential N-glycosylation sites. None of these sites, however, is at a conserved position in all sucrose transporters sequenced so far and in most of these transporters the consensus sequences are located on the putative cytoplasmic side of the protein (according to the 12-helix model) and would therefore never face the lumen of the endoplasmic reticulum (ER [8, 24, 30]). Despite the lack of N-glycosylation which would proof that these transporters use the secretory pathway in plants, it is very likely that the proteins reach the plasmalemma via this classical way, since similar transporters in yeast and mammals are glycosylated in their first extracellular loop which means that they have to be synthesized at the ER. Introduction of an artificial N-glycosylation consensus sequence into this loop of the STP1 protein might be a way to prove this hypothesis.

Localization in the plant

After the identification and characterization of mono- and disaccharide transporters it was im-

portant to determine the tissue and/or cell type specificity of expression of the respective proteins and, at least for one or two of them, the localization in the plasma membrane. Only recently it became obvious that sequence homology alone is not sufficient for the assignment of transporters to a specific membrane: GLUT7, a human glucose uniporter, shares 68% identical amino acids with the liver plasma membrane glucose transporter GLUT2 but it is located in liver microsomes, most likely the endoplasmic reticulum [44]. The fact that a protein is sorted to the plasma membrane in a heterologous expression system provides strong evidence but is no proof for the same localization *in planta*. Only for the *Chlorella* HUP1 protein the localization in the plasmalemma has unequivocally been determined using anti-HUP1 antibodies on *Chlorella* thin sections [37].

The existence of higher-plant sugar transporters, especially of monosaccharide transporters, has frequently been explained with the retrieval of effluxed solutes back into the cells by these proton-dependent carriers (for review see [17]). In view of the large numbers of monosaccharide transporters found in higher plants, however, this simple function is very unlikely. According to the apoplastic theory one would expect to find monosaccharide transporters mainly in the sink tissues where they catalyse the import of glucose and fructose into the respective cells after sucrose hydrolysis by cell wall bound invertases. Northern blot analysis of the organ-specific expression of the tobacco MST1 monosaccharide-H^+ symporter showed that this protein is expressed predominantly in roots, the probably strongest sink of these plants [29]. Lower levels of MST1 expression were also found in tobacco leaves with a clear decrease during leaf development and higher expression levels in young (sink) leaves than in mature (source) leaves.

A more precise answer on the location of a specific transporter *in planta* can be obtained using transgenic plants expressing the β-glucuronidase reporter gene under the control of the promoter of the corresponding gene. The expression of four monosaccharide-H^+ symporters from *Arabidopsis thaliana* (STP1 to STP4) has been studied with this method and the results were surprising: all of them seem to be expressed in a very restricted area of the plant with STP1 being found in the ovaries of *Arabidopsis* flowers, STP2 in the anthers, STP3 in the mesophyll cells of the green leaves and sepals, and STP4 in the anthers and the roots (Truernit and Sauer, unpublished). Thus, the expression of STP1, 2, and 4 seems to be strictly sink-specific, since the tissues of their expression are heterotrophic, non-green parts of the plant depending on carbon supply from the leaves. STP1 is the only transporter which is not expressed in a sink tissue and the function of STP3 in the green leaves of *Arabidopsis* is still unclear. Maybe STP1 has in fact a function in the retrieval of effluxed monosaccharides from the leaf apoplast as mentioned above.

Similar studies were performed with promoter/β-glucuronidase constructs of the *Arabidopsis thaliana SUC2* gene. Sucrose transporters would be expected in the phloem cells of the vascular system catalyzing the import of sucrose from the apoplastic space into the phloem companion cells and/or the sieve elements. The results obtained with transgenic *Arabidopsis plants* confirm these expectations showing that *SUC2* expression is found in the phloem practically all over the plant with one single exception, the petals [40]. Expression of *SUC2* is clearly limited to the vascular bundles and cross sections through *Arabidopsis* stems reveal that this expression is restricted to the phloem. Surprisingly *SUC2* expression was found also in the phloem of sink tissues such as the roots, the ovaries, and the filaments, all organs where phloem loading is not expected to occur. This observation may be taken as evidence that SUC2 is possibly not only responsible for phloem loading, but also for unloading. An almost identical expression pattern has been published for the *Arabidopsis thaliana* plasma membrane H^+-ATPase AHA3 [7] which therefore may represent the primary active pump for the AtSUC2 secondary active transporter. Expression of SUC2 in *Arabidopsis* leaves is regulated during growth starting at the tip of young rosette leaves und proceeding towards the leaf's basis

during development finally reaching all the way down into the stem of fully developed leaves. This agrees with data published by Turgeon and Webb [42] showing that the sink/source transition of *Cucurbita* leaves goes from the tip towards the basis (for review see [41]). The tissue specificity of AtSUC1 expression has not yet been determined.

Using the method of *in situ* hybridization on thin sections of potato leaves, Riesmeier *et al.* [23] demonstrated that the potato StSUT1 sucrose transporter is expressed in the minor veins of potato which also provides strong evidence for phloem loading by StSUT1. These results were confirmed with potato plants expressing *StSUT1* antisense RNA. These plants have reduced levels of StSUT1 protein resulting in increased levels of soluble carbohydrates in the leaves and increased starch content [25]. Due to impaired sugar export from the leaves these plants show reduced root growth and tuber yield.

Gahrtz and coworkers isolated vascular bundles from *Plantago major* and northern blots and RNase protection experiments revealed that both PmSUC1 and PmSUC2 are predominantly expressed in the vascular bundles ([8]; Gahrtz and Sauer, personal communication). Antibodies raised in rabbits against PmSUC2 protein which were purified from transgenic *S. cerevisiae*, were used for a more precise analysis of PmSUC2 expression and thin sections through the basal part of *Plantago* leaves showed that PmSUC2 is in fact expressed in phloem of *Plantago* and in no other part of the vascular bundles (Stadler, Brandner and Sauer, unpublished data).

The presented data confirm the theory of apoplastic phloem loading and unloading in the investigated plants, with sucrose transporters being expressed only in the phloem and monosaccharide transporters being found mainly in the sinks of these plants.

Outlook

The results summarized in this article show that the field of plant sugar transport, carbohydrate partitioning and long-distance transport has quickly developed over the past years. With the help of molecular biology cDNAs and genes for many transporters have been cloned, the encoded proteins have been studied in detail in various expression systems, the location of some of these transporters has been determined in the plant, and mutants with reduced amounts of sucrose transporter have been generated. We are now starting to collect first insights into how plants manage to distribute the photosynthates to their different heterotrophic organs and storage tissues, but we are far from understanding the regulatory mechanisms controlling this important step.

On first sight it may seem that phloem loading is fully explained by the identification of sucrose-H^+ symporters and their localization in the phloem. But there are still many open questions. Where and how is sucrose leaving the mesophyll cells? Is sucrose entering the companion cells, the sieve elements, or both? How is the situation in plants transporting sorbitol, mannitol, or raffinose? And, last but not least, are there plants with symplastic phloem loading as suggested by data obtained mainly with members of the Cucurbitaceae [9, 16, 34]?

The situation in the sink tissues, where the phloem is unloaded, is comparable: the large number of monosaccharide-H^+ symporters identified in higher plants and the sink-specific expression of many of these proteins favour the idea of apoplastic unloading of sucrose from the phloem, followed by extracellular hydrolysis. Mutants with decreased or artificially increased levels of cell wall invertase undergo phenotypic changes which also supports an apoplastic step [20, 43]. But why do plants have so many monosaccharide transporters compared to only one or two disaccharide transporters, and how is sucrose leaving the phloem?

We are still far from understanding the actual step of membrane penetration, and we have nothing more than weak, preliminary models of how the sugars might pass the transport proteins, or which amino acid residues line the channel or pore. The possibility to express transporters in systems accessible to electrophysiological methods will be helpful in answering some of these

questions; purification and three dimensional resolution of the structure of at least one of these proteins will be essential.

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