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Project Title: Functional Analysis of Arabidopsis Sucrose Transporters

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Introduction

Sucrose is the main photoassimilate that is transported in the vascular tissue of plants from the site of synthesis (source leaves) to sink tissues such as flowers, seeds and roots. Sucrose transporters (SUTs or SUCs) are proton-coupled symporters and have two main functions in plants: the active loading of sucrose into the phloem and post-phloem uptake of sucrose into sink cells (Patrick and Offler, 2001). The activities of SUTs are essential for carbon transport to storage tissues and understanding SUT function and regulation is necessary to enable molecular breeding projects that target higher yield and quality. Therefore, this research topic is central to the mission of the Energy Biosciences Program. The goals of this project are to analyze the basic transport kinetics and substrate specificity of SUTs using heterologous expression and electrophysiology, determine if dimerization of SUTs is important for function or regulation, and to identify regulatory mechanisms functioning at the protein level.

Analysis of SUT transport functions

The first objective for this project is to analyze in detail the transport properties of at least one SUT in each of the three distinct clades of the phylogenetic tree of SUTs (Aoki et al., 2003). Initially, using the genetic model plant Arabidopsis for this purpose was attractive. Three sucrose transporters were selected as representatives of each clade: AtSUC2, AtSUT2, and AtSUT4. We characterized AtSUC2 in detail (Chandran et al., 2003). The activities of AtSUT4 and AtSUT2 when expressed in oocytes were too low for detailed analysis. Therefore, we tested the activities of related sucrose transporters from different species. A cDNA encoding barley HvSUT1 was obtained from Dr. U. Wobus, Gatersleben, Germany. HvSUT1 is a close homolog of AtSUT2 and was previously demonstrated to be functional by expression in yeast and hypothesized to have a function in sucrose uptake into the barley grain (Weschke et al., 2000). Analysis of HvSUT1 was completed (Sivitz et al., 2006) and results show that HvSUT1 is more selective for α-linked glucosides (such as sucrose) compared to AtSUC2 (Chandran et al., 2003).

We collaborated with Dr. Christopher Grof (University of Newcastle, Australia) to analyze sugarcane ShSUT1 (Rae et al., 2005), also a homolog of AtSUT2. Dr. Grof

visited our lab at the University of Minnesota for two weeks during the summer of 2004 to initiate the experiments and learn electrophysiological techniques. This collaboration resulted in the first analysis of a SUT from sugarcane (Reinders et al. 2006).

Analysis of SUT interaction

Analysis of sucrose transporter interaction in yeast showed that SUT1 from tomato (Reinders et al., 2002) and SUC2 from Arabidopsis (Schulze et al., 2003) are capable of forming homodimers and heterodimers with other sucrose transporters. Our objective was to test if SUT dimerization was important for function. We used several approaches as outlined in the following and all results were consistent with a monomer being the functional unit for transport activity. 1) A dominant negative approach was used in which a non-functional mutant of AtSUC2 (H64D) was coexpressed in oocytes with the wild type AtSUC2 transporter. The activity was identical to wild type AtSUC2 expression alone. 2) As described above, AtSUT2 and AtSUT4 have very low activity when expressed in oocytes. When expressed in yeast, these transporters have activity distinct from AtSUC2 (Weise et al., 2000; Schulze et al., 2000). Coexpression of AtSUT4 or AtSUT2 with AtSUC2 in oocytes produced activities identical to wild type AtSUC2 expression alone. This indicates that AtSUT2 or 4 do not modify AtSUC2 activity. 3). Coexpression of AtSUT2 and AtSUT4 in yeast and in oocytes does not produce significant sucrose transport activity. In summary, based on the results showing SUT interaction in yeast and expression of AtSUC2, SUT2 and SUT4 in companion cells in Arabidopsis (Schulze et al., 2003) it is possible that SUT protein physically interact in the plant. However, our results did not reveal any change of transport function that can be attributed to SUT interaction.

Regulation of sucrose transporters

There have been several reports describing regulation of sucrose transporters at the expression level. However, there is almost no information on regulation at the level of the protein. Using a proteomics approach, Nühse et al (2004) identified four potential phosphorylation sites within the N-terminus of AtSUC5. As shown in Fig. 1 three of these sites (S or T) are conserved in AtSUC2. To test whether phosphorylation of these sites in AtSUC2 affects transport activity we are generating mutant versions with S or T replaced by A or D. Replacement of serine or threonine with alanine provides a non-phosphorylated site while replacement with aspartic acid simulates a constitutively phosphorylated site. we have analyzed 8 of the possible mutant versions of AtSUC2 by expression in yeast and ^{14}C sucrose uptake. We found no effect on transport activity.

We used several approaches to study SUT regulation that were not successful. These experiments are briefly summarized in the following: 1) We hypothesized that AtSUT2 and AtSUT4 contained autoinhibitory domains in the cytosolic N- and C-termini. We generated mutants for both transporters with truncated N- and C-termini, but containing all membrane spans. These mutants did not show increased sucrose transport activity. 2) We tested the hypothesis that AtSUT2 and AtSUT4 require a regulatory protein for full activity when expressed heterologously. Expression of AtSUT2 or AtSUT4 in the yeast strain SuSy7 does not allow the strain to grow on sucrose (because of low sucrose transport activity). We transformed SuSy7 (expressing SUT2 or SUT4)

with an Arabidopsis cDNA library and looked for transformants that were able to grow on sucrose. This effort to clone activators of SUT2 or SUT4 was not successful.

Functional analysis AtPLT5, a sugar alcohol permease homolog

Arabidopsis encodes approximately 100 members of the Major Facilitator Superfamily (MFS) that are likely to be transporters for sugars and other metabolites. Sucrose transporters are distantly related to the MFS. Until recently the only well characterized plant MFS transporters were those in the STP subfamily (STPs have a specificity for monosaccharides). We found that the sugar alcohol permease homolog AtPLT5 was functional in the oocyte expression system and we analyzed its basic transport function by electrophysiology. AtPLT5 transports a wide range of monosaccharides and sugar alcohols and is a proton-coupled uptake transporter with a sink-specific expression pattern (Reinders et al., 2005).

Summary

Strong progress was made in the analysis of transport functions for three sucrose transporters and one sugar alcohol permease. Research on regulation of sucrose transporters proceeded quickly but results were negative. We show that functional impacts of dimerization of SUTs were not detected. We also show that modification of several phosphorylation sites did not affect transport activity. It is possible that interactions and phosphorylation may regulate other aspects of function such as stability/degradation or subcellular targeting in the plant.

Results from this project show that type I SUTs such as AtSUC2 have a wide substrate specificity: they transport a range of glucosides besides sucrose. On the other hand, type II SUTs are very selective for sucrose. This difference in substrate specificity will facilitate analysis of the substrate binding site of SUTs by site directed mutagenesis in the future.

Publications from project.

Chandran, D. Reinders, A. and Ward, J.M. (2003) Substrate specificity of the Arabidopsis thaliana sucrose transporter AtSUC2. J. Biol. Chem **278**: 44320-44325.

Reinders, A., Panshyshyn, J. A. and Ward J. M. (2005) Analysis of transport activity of Arabidopsis sugar alcohol permease homolog AtPLT5. J Biol Chem. **280**, 1594-1602.

Sivitz, A. B. Reinders, A. and Ward, J. M. (2005) Analysis of the Transport Activity of Barley Sucrose Transporter HvSUT1. Plant Cell Physiol **46**: 1673-167).

Reinders A., Sivitz, A. B., Hsi, A. Grof, C. P. L., Perroux, J. M. and Ward J. M. (2006) Sugarcane ShSUT1: analysis of sucrose transport activity and inhibition by sucralose. Plant Cell Env. 29: 1871-1880

Other References Cited

Aoki N, Hirose T, Scofield GN, Whitfeld PR, Furbank RT (2003) The sucrose transporter gene family in rice. Plant Cell Physiol **44:** 223-232

Nühse, T. S., Stensballe, A., Jensen, O. N. and Peck, S. C. (2004) Phosphoproteomics of the Arabidopsis Plasma Membrane and a New Phosphorylation Site Database. Plant Cell 16, 2394-2405.

Patrick JW, Offler CE (2001) Compartmentation of transport and transfer events in developing seeds. J Exp Bot **52:** 551-564

Rae, A. L., Perroux, J. M. and Grof, C. P. (2005) Sucrose partitioning between vascular bundles and storage parenchyma in the sugarcane stem: a potential role for the ShSUT1 sucrose transporter. Planta 220, 817-825.

Reinders, A., Schulze, W., Kuehn, C., Barker, K., Schulz, A., Ward, J.M., and Frommer, W.B. (2002) Protein-protein interactions between sucrose transporters with differing affinities in the same enucleate sieve element. Plant Cell 14:1567-1577.

Schulze W, Weise A, Frommer WB, Ward JM (2000) Function of the cytosolic N-terminus of sucrose transporter AtSUT2 in substrate affinity. FEBS Lett **485:** 189-194

Schulze, W. X., Reinders, A., Ward, J., Lalonde, S. and Frommer, W.B. (2003) Interactions between co-expressed Arabidopsis sucrose transporters in the split-ubiquitin system. BMC Biochem. 4:3.

Weise A, Barker L, Kühn C, Lalonde S, Buschmann H, Frommer WB, Ward JM (2000) A New Subfamily of Sucrose Transporters, SUT4, with Low Affinity/High Capacity Localized in Enucleate Sieve Element of Plants. Plant Cell **12:** 1345-1355

Weschke W, Panitz R, Sauer N, Wang Q, Neubohn B, Weber H, Wobus U (2000) Sucrose transport into barley seeds: molecular characterization of two transporters and implications for seed development and starch accumulation. Plant J **21:** 455-467