Characterization of Rice Functional Monosaccharide Transporter, OsMST5

Budsaraporn Ngampany,1,2 Anna Sobolewska,3 Taito Takeda,3 Kyoko Toyofuku,3 Jarunya Narangajavana,2 Akira Ikeda,1,4 and Junji Yamaguchi1,5

1Graduate School of Science, Hokkaido University, Kita-ku N10-W8, Sapporo 060-0810, Japan
2Department of Biotechnology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand
3Bioscience Center, Nagoya University, Chikusa-ku, Nagoya 464-0860, Japan
4CREST, JST (Japan Science and Technology Corporation)

Received August 29, 2002; Accepted October 15, 2002

cDNA of a monosaccharide transporter in rice, OsMST5 (Oryza sativa monosaccharide transporter 5) was cloned and its sugar transport activity was characterized by heterologous expression analysis. The amino acid sequence and topology were similar to the sequences and topology of other plant monosaccharide transporters. Yeast cells co-expressed with OsMST5 cDNA transported some monosaccharide substrates. The transport rate increased when ethanol as an electron donor was added, so the transporter was an energy-dependent active one. Most of the OsMST5 was expressed in panicles before pollination, indicating that it is associated with pollen development in rice.

Key words: energy-dependent active transport; heterologous yeast expression; Oryza sativa L.; pollen development; sink cell

As much as 80% of the carbon assimilated during photosynthesis is exported from the leaf for the metabolic needs of nonphotosynthetic cells. A central feature of this resource-partitioning step is phloem loading, and in many plants this process depends on an active sugar transporter. Two families of plant sugar transporters have been identified: sucrose (or disaccharide) transporters and monosaccharide transporters. A number of sucrose and monosaccharide transporters related to several biological processes during plant growth and development have been analyzed.11,12 Several sink cells can derive sucrose and other photoassimilates by direct access through symplastic connections to the phloem. However, other sinks are symplastically isolated; therefore, sucrose is either imported directly from apoplasts via a sucrose transporter or taken up by a monosaccharide transporter after the sucrose is hydrolyzed to glucose and fructose by cell-wall-bound invertases.3 Various plant monosaccharide transporters have been cloned and characterized by heterologous expression in yeast cells.3–5 The expression pattern of genes encoding monosaccharide transporters suggested that the transporters are involved in hexose uptake in sink tissue6 and are highly regulated after pathogen infection or wounding.6 In that is pollen grain developing, sinks need carbohydrates for maturation, germination, and growth7 and monosaccharide transporters seem to be in this physiological task. Indeed, genes for Pmt1 and AtSTP2, monosaccharide transporters in petunia and Arabidopsis thaliana, are up-regulated after pollen mitosis and are involved in the growth of pollen tubes.7–8 Male gametophytes and pollen grains have no intercellular connections (plasmodesmata) to sporophytic tissue, so the uptake of nutrients into the cells is exclusively dependent on an external supply of nutrients. Import of carbon and nitrogen is necessary for development of pollen grains in anthers. Pollen thus seems to be an ideal subject in studies of the role and regulation of nutrition transport.9

In rice, sucrose transporters OsSUT110–13 and OsSUT214 as well as monosaccharide transporters OsMST1, 2, and 315 have been cloned. Of monosaccharide transporters in rice, only OsMST3 has been found to have a physiological role involving the accumulation of monosaccharides for cell-wall synthesis during cell thickening in vegetative organs.15 Other than OsSUT2, there have been no reports since 2000 about such aspects of rice; that is a wide field. Here, we investigated sugar transport during rice flowering and grain developing. The monosaccharide transporter OsMST5 and its corresponding cDNA were cloned.
New Functional Monosaccharide Transporter in Rice

Materials and Methods

OsMST5 clone. OsMST5 cDNA was obtained from the Rice Genome Research Program (GenBank accession number X55350; full-length clone). The cDNA sequence was identified by dideoxy-chain termination with an ABI373A DNA sequencer (Perkin-Elmer Co., NJ).

Alignment of sequences. A phylogenetic tree (not shown) of monosaccharide transporters from yeasts, mammalian species, and plant species other than rice was prepared on the basis of the deduced amino acid sequences, in a comparison of OsMST5 with other known transporters.

Functional analysis of OsMST5 in yeast cells. For testing of the function of OsMST5, an expression plasmid was constructed with a GAL expression system in multicopy plasmid pTV3e. LBY416 (MATa hxt2::LEU2 snf3::HIS3 gal2 lys2 ade2 trp1 his3 leu2 ura3), a mutant strain of Saccharomyces cerevisiae in which high-affinity glucose transport activity is low because the monosaccharide transporter-related genes HXT2, GAL2, and SNF3, have been interrupted was used as the heterologous expression system. A plasmid with the open reading frame of OsMST5 introduced to replace that of GAL2 in a pTV3e cassette vector between the EcoRI and ClaI sites was introduced into LBY416. OsMST5 is expressed under the control of the GAL2 promoter in the presence of galactose. The transport activity of subcloned cDNA was compared with that of another monosaccharide transporter in rice, OsMST3 (see Fig. 3), because this other transporter had more activity than OsMST1 and OsMST2.

Transport activity assay. The monosaccharides including D-glucose, 3-O-methyl glucose (3-OMG); the nonmetabolizable substrate analogs for D-glucose, and D-xylose were used as transport substrates to analyze transport activity and for estimation of the energy dependence of the transport system of OsMST5. Uptake of glucose and other monosaccharides were assayed by procedures described previously. Yeast cells were grown to an OD_{600} of 0.2–0.4 in a synthetic medium containing 2% galactose. Cells were collected by centrifugation, washed three times with a medium for transport assays: 50 mM 2-(N-morpholino)ethanesulfonic acid in NaOH (pH 6.0) containing 2 mM MgSO_{4}. Uptake assays were started by the addition of 20 μl of radiolabeled sugars to 180 μl of a cell suspension, and stopped by the addition of transport assay medium containing 0.5 mM HgCl_{2} in stead of 2 mM MgSO_{4}. After incubation at 30°C, cells were collected by filtration under reduced pressure onto a glass fiber filter (GF/F, Whatman), and washed with 20 ml of cold assay solution containing HgCl_{2}. The radioactivity retained in the filter was measured by liquid scintillation counting. The initial rate of glucose transport was assessed by the transport of 0.1 mM D-[U^{14}C]glucose (0.5 μCi, CFB96; Amersham Pharmacia Biotech), or D-[U^{14}C]xylose (0.5 μCi, CFB96; Amersham), or 3-O-methyl D-[U^{14}C]glucose (0.5 μCi, CFB96; Amersham), for 5 s to 25 min at 30°C. Most assays were done in three or four independent experiments.

RNA extraction and northern blotting. Because the OsMST5 clone obtained from the Rice Genome Project was isolated from a panicle cDNA library, Northern blotting was done to detect OsMST5 mRNAs in several stages during flowering RNA was extracted from panicles at various stages with the aurin triacarboxylic acid method of Skadsen, with minor modifications. Total RNA (15 μg) of a sample was electrophoresed on formaldehyde gel and blotted onto a nylon membrane (Hybond N+; Amersham). Membranes were hybridized at 65°C in PerfectHyb hybridization solution (Toyobo, Osaka, Japan). A radiolabeled probe was prepared from gel-purified cDNA fragments (5-end of OsMST5, 1200 bp) by random primer labeling with α-[32P]dCTP. We confirmed that there was no cross-hybridization with this radiolabeled probe by Southern blotting (not shown). Hybridization and washing were done by the protocol of the manufacturer. Membranes were exposed with a Fujix BAS2000 Bio-Imaging Analyzer (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Results

OsMST5, a monosaccharide transporter in rice
The putative amino acid sequence of OsMST5 was 30.5–66.2% identical to the sequences of OsMST1, 2, and 3 of rice. OsMST5 was 518 amino acids long, with a calculated molecular mass of 55.9. Analysis of the deduced amino acid sequence by TMHMM (Version 2.0) to predict transmembrane helices in the OsMST5 protein gave two sets of putative transmembrane domains separated by a central long hydrophilic region (Fig. 2A). The first set was five domains and one putative domain inside the cell and the second set was of six domains. The amino acid sequence of the third putative domain in the first set was almost identical to one reported previously as the third transmembrane domain in OsMST1, 2, and 3.
Fig. 1. Amino Acid Sequences of OsMST1, 2, 3, and 5 Aligned with Monosaccharide Transporters of Other Organisms.

The alignment of the predicted amino acid sequences of OsMST1 (D251429), 2 (D46606), 3 (D40232), and OsMST5 (X55350) with the sequence of RcHEX6 (L08188) from caster bean, SopGlcT (AF215851) from spinach, and GLUT1 (KO3195) from humans is shown. Black boxes indicate identical amino acid residues. Asterisks below the sequences indicate conserved residues. Putative transmembrane domains are underlined. Multiple-sequence alignment was constructed by the DNASIS-Mac program, version 3.7 (Hitachi Software Engineering Co., Ltd., Yokohama, Japan).

Alignment of sequences

A phylogenetic tree (not shown) of monosaccharide transporters from yeasts, mammalian species, and plant species other than rice, in a comparison of OsMST5 with other known transporters showed a little different in transporters of monocots and dicots. However, SopGlcT, the plastidic glucose translocator in spinach,22) and GLUT1, from human were very different from the plant transporters (see Fig. 1). The conserved motifs in sugar transport proteins proposed by Henderson et al.23) were found in monosaccharide transporters of rice OsMST1, 2, 3, and 5, caster bean (RcHEX6), spinach (SopGlcT), and human (GLUT1). Many of the amino acid residues that are motifs of sugar transporters (indicated by asterisks in the figure) were conserved in OsMST5.

Functional analysis of OsMST5 in yeast cells

The glucose transport activity of OsMST5 was about one third that of OsMST3 and nearly twice that of the empty vector, pTV3e (Fig. 3). Transport activity was not found after the addition of the SH-group inhibitor HgCl2. The empty vector pTV3e had transport activity even in the absence of an inhibitor because of the activity of a minor sugar transporter(s).

Energy for sugar uptake of OsMST5

Transport activity for d-glucose (Fig. 4A) was approximately 8-fold and 14-fold, that of 3-OMG (Fig. 4B) and d-xylose (Fig. 4C). With the empty vector, pTV3e, the uptake level of d-xylose remained low level during the 5-min tracing with or without ethanol (Fig. 4D). Uptake upon energization, started by the addition of ethanol, was observed with all three substrates. LBY416 yeast cells harboring the pTV3e vector could not take up d-xylose, and showed a background level of transport. This finding was in contrast to those of cells containing OsMST5, which could take up d-xylose rapidly when ethanol was added (Fig. 4B and Fig. 5). This energization of the plasma membrane of yeast cells decreased when the uncoupler CCCP, an electron transport inhibitor, was used (Fig. 5). The energy generated by ethanol allowed a high rate of uptake of d-xylose into the cells, where it was rapidly metabolized. With CCCP act as a plasma membrane de-energization, the transport of d-xylose was decreased.

Kinetic properties

MSTs generally have broad substrate specificity, transporting a range of hexoses and pentoses with \( K_m \) values for the substrates being typically 10–100 mM. The \( K_m \) values for glucose transport of OsMST3 and OsMST5 were 0.3 and 0.5 mM, respectively. The \( V_{max} \) values were 450 and 471 pmol/10^7 min^−1 for OsMST3 and OsMST5, respectively.
Fig. 2. Hydropathy Profile and a Membrane-spanning Model of OsMST5. Calculation was done by an algorithm published elsewhere.20) Bold, lower, and upper line-blocks in the top indicate the transmembrane domain, peptides facing the cytoplasm inside, and peptides facing to the outside of the cell, respectively. Arrow indicates the third putative transmembrane.

Fig. 3. Glucose Transport in Yeast Cells with OsMST3 and 5. OsMST3 and 5 were expressed in S. cerevisiae with the GAL2 promoter in a multicopy plasmid, pTV3e, in LBY416 cells. The experiment done with the vector pTV3e shows value for transport. The final substrate concentration was 0.1 mM in all experiments. The transport activity in the presence of 0.5 mM HgCl₂ is shown as ‘‘+ HgCl₂’’.

Fig. 4. Transport of d-Glucose, 3-O-Methyl Glucose (3-OMG) and d-Xylose in Yeast Cells with OsMST5 (A, B, and C), and Transport of d-Xylose in Cells with the Only Vector (D). Transport in yeast cells with additional energization by 100 mM ethanol or without ethanol in the presence of 0.5 mM HgCl₂ or without HgCl₂ is shown.

Substrate specificity
With the OsMST3 transporter, the order of inhibition strong inhibition first was glucose, galactose, sucrose, xylose, fructose, and, interestingly, fructose, although the order for the last three sugars may be interchangeable. Galactose inhibited glucose by about 17% and glucose by about 30% (Fig. 6A). With OsMST5, none of the sugar including glucose caused inhibition (Fig. 6B).

Expression of OsMST5
A strong signal was detected only from panicles before heading; signals were not detected in developmental stages of rice seeds (Fig. 7). Signals not being detectable in the other tissues and organs tested, embryo-derived suspension cultured cells, leaf blades before and after heading, leaf sheaths, roots, dry seeds, and seedlings by Northern blotting (not shown) suggested that this monosaccharide transporter was associated with flower development.

Discussion
OsMST5 as a functional monosaccharide transporter in rice
The hydropathy profiles for all plant monosaccha-
Fig. 5. Effects of Ethanol and an Uncoupler (CCCP) on D-Xylose Transport in Yeast Cells with OsMST5.
The starting concentration of D-xylose in the medium was 0.1 mM, and the final concentration of CCCP in the medium was 50 μM. Arrows indicate the times of energization with the addition of ethanol to 100 mM or CCCP.

Fig. 6. Inhibition of Glucose Uptake of Yeast Cells with OsMST3 (A) or OsMST5 (B) by Different Sugars, Added to Activated Cells 1 min Before the Addition of D-Glucose [14C].
The starting concentrations of each unlabeled sugar and labeled D-glucose added were both 0.1 mM.

Fig. 7. Northern Blotting of OsMST5 mRNA.
Total RNA (15 μg) of a sample was electrophoresed on a formaldehyde gel and blotted onto a nylon membrane, and hybridized with a radiolabeled cDNA probe. After the membrane was washed, it was examined with a bio-imaging analyzer. Staining in the rRNA panel was with ethidium bromide. DAP, days after pollination.
transporter.

**Location and function of OsMST5**

Sugar transporters are found in various parts of plant cells and tissues. The expression pattern of the transporters reflect their physiological tasks. In rice, OsMST3 is to be found in leaf blades, leaf sheaths, calli, and sclerenchyma and xylem cells in the young roots. The abundant of mRNA in sclerenchyma and xylem cells indicated that OsMST3 is involved in the thickening of cell walls. The sequence of OsMST5 was similar to that of OsMST3, even when they were in different organs. OsMST5 mRNA was detected in panicles before heading. Bate and Twell proposed that the sequences AGAAA and TCCACCATA in the promoter region of the *lat52* gene in tomato are needed to code proteins expressed in pollen. We found the AGAAA sequence in 10 places and a sequence similar to TCCACCATA in a single place in the OsMST5 promoter (not shown). In addition, both sequences were detected in OsSUT2, which rice sucrose transporter is expressed only in the developing pollen. These results suggested that OsMST5 was a pivotal in early flower development, together with OsSUT2. Monosaccharide transporter involved in pollen development in petunia and Arabidopsis have been examined. AtSTP2 and PhPMTI are expressed after meiosis in pollen mother cells. The imported glucose (or carbohydrates) may need development of uninucleate microspores after supply of energy for pollen germination and pollen tube growth in those plant species. Takeda et al. mentioned that the OsSUT2 isolated from rice panicle cause the influx of sucrose into developing pollen for starch synthesis. Hence, OsMST5 might be associated with these developmental processes together with OsSUT2. However, additional sugar transporters may be involved in these physiological tasks.

**Acknowledgments**

We thank Dr. M. Kasahara of Teikyo University for providing us with a yeast expression system and Dr. T. Sasaki of the Japanese Rice Genome Program for providing us with expressed sequence tag clones. BN acknowledges a UDC scholarship supported by the Ministry of University Affairs, Thailand. This work also was supported by a Research for the Future grant (JSPS-00L01603) from the Japan Society for the Promotion of Sciences and CREST of Japan Science and Technology.

**References**


16) Nishizawa, K., Shimoda, E., and Kasahara, M., Sub-


