Substrate Specificity of the Arabidopsis thaliana Sucrose Transporter AtSUC2*

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The Arabidopsis sucrose transporter AtSUC2 is expressed in the companion cells of the phloem (specialized vascular tissue) and is essential for the long distance transport of carbohydrates within the plant. A variety of glucosides are known to inhibit sucrose uptake into yeast expressing AtSUC2; however, it remains unknown whether glucosides other than sucrose could serve as transported substrates. By expression of AtSUC2 in Xenopus oocytes and two-electrode voltage clamping, we have tested the ability of AtSUC2 to transport a range of physiological and synthetic glucosides. Sucrose induced inward currents with a K_m of 1.44 mM at pH 5 and a membrane potential of −137 mV. Of the 24 additional sugars tested, 8 glucosides induced large inward currents allowing kinetic analysis. These glucosides were maltose, arbutin (hydroquinone-β-D-glucoside), salicin (2-(hydroxymethyl)phenyl-β-D-glucoside), α-phenylglucoside, β-phenylglucoside, α-parani trophenylglucoside, β-parani trophenylglucoside, and parani trophenyl-β-thioglucoside. In addition, turanose and α-methylglucoside induced small but significant inward currents indicating that they were transported by AtSUC2. The results indicate that AtSUC2 is not highly selective for α- over β-glucosides and may function in transporting glucosides besides sucrose into the phloem, and the results provide insight into the structural requirements for transport by AtSUC2.

EXPERIMENTAL PROCEDURES

Cloning—The coding region of AtSUC2 was amplified using ExTaq polymerase (Panvera, Madison, WI) and the following primers, 5'-ATGGTGCAAGCCATCTCAATGGA and 5'-TCAATGGAATCCCTATGTCAG. An A. thaliana ecotype Col-0 seedling library was used as a template. The PCR product was cloned into pCR2.1 (Invitrogen) and sequenced. AtSUC2 was subcloned into the EcoRI site of pOO2 (16), an expression vector containing Xenopus β-globin 5'- and 3'-untranslated regions and a 92-bp poly(A) tail. This construct was linearized using PmaCI (Panvera), and 1 μg was used as template for cRNA synthesis using the mMessage mMachine kit (Ambion, Austin, TX).

Heterologous Expression—Xenopus oocytes, stages V and VI, were isolated by incubation in 10 mg/ml collagenase A (Boche Applied Science) in Barth’s medium (88 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO_3)_2, 0.41 mM CaCl_2, 0.82 mM MgSO_4, 2.4 mM NaHCO_3, 10 mM HEPES, pH 7.6, 10 mg/ml penicillin, and 10 mg/ml streptomycin) for 2–3 h until separated. The oocytes were then washed five times in 1 mg/ml bovine serum albumin in Barth’s medium. The oocytes were injected with 50 nl (1.1 ng/μl) of AtSUC2 cRNA and incubated at 15 °C in Barth’s medium supplemented with 0.1 mg/ml gentamicin. Electrophysiological experiments were performed 3–5 days following the RNA injection.

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AtSUC2 Substrate Specificity

**Electrophysiological Methods**—Oocytes were bathed in modified Ringer’s solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 1 mM NaHCO₃, 10 mM HEPES, pH 5.0, and 10 mM MgCl₂) with continuous perfusion at 1 ml/min. Recording pipettes, filled with 1 M KCl, with resistances between 1.5 and 3 megaohms were used. Currents were measured using the two-electrode voltage clamp technique with a Dagan TEV 200A amplifier (Dagan Corp., Minneapolis, MN). The holding potential was −40 mV in sodium-Ringer solution at pH 5.0. Substrates were applied at 10 mM in the same solution where indicated, and downward deflections indicate inward proton (coupling ion) current. Suc, sucrose; Arb, arbutin; Glc, glucose; α-pnp-glc, α-paranitophenylglucoside; β-p glc, β-phenylglucoside; α-p glc, α-phenylglucoolcoside; Malt, maltose.

**RESULTS**

The *Arabidopsis* sucrose transporter AtSUC2 was expressed in *Xenopus* oocytes, and transport properties were analyzed by voltage clamping. Consistent with a H⁺-coupled transport mechanism for AtSUC2 (4), at a holding potential of −40 mV, sucrose induced inward currents in AtSUC2-injected oocytes (Fig. 1, 87 ± 6.7 nA, n = 45 oocytes). Other physiological glucosides including arbutin (hydroquinone-β-D-glucoside) and maltose, and the synthetic glucosides α-paranitophenylglucoside and α- and β-phenylglucoside induced inward currents, whereas glucose did not. No sucrose-induced currents were observed for uninjected oocytes (0.7 ± 0.5 nA, n = 5 oocytes). None of the substrates induced currents in uninjected oocytes (Fig. 1, n = 3 or more oocytes/substrate).

To analyze the voltage dependence of sucrose-induced currents, voltage pulses of 150 ms were made from 57 to −157 mV. Steady-state currents were observed following capacitive transients and were averaged between 110 and 140 ms following the onset of voltage pulses. The current/voltage relation of steady-state currents before, during, and after perfusion with 10 mM sucrose is shown in Fig. 2A. Background currents averaged 33 ± 2.1 nA (n = 6 oocytes) at −40 mV in sodium-Ringer solution at pH 5.0. Sucrose-dependent currents, obtained by subtracting background currents (Fig. 2B), were inward at all potentials (between 57 and −157 mV).

For kinetic analysis, currents were measured at sucrose concentrations between 0.05 and 10 mM. Currents were saturable (Fig. 2C) with an apparent affinity for sucrose ($K_{0.5}$) of 1.44 ± 0.19 mM (n = 3 oocytes) at −137 mV. $K_{0.5}$ values were voltage-dependent (Fig. 2D) with higher apparent affinity at more negative potentials and ranged between 1.44 ± 0.19 mM at −137 mV and 6.13 ± 1.9 mM at −1.1 mV.

To assay the structural requirements for transport by AtSUC2, potential substrates were applied to AtSUC2-expressing oocytes at a final concentration of 10 mM in sodium-Ringer solution at pH 5.0. Average steady-state currents at −137 mV are presented in Fig. 3. Of the sugars tested, only α-paranitophenylglucoside and α-phenylglucoside produced larger inward currents than sucrose. Consistent with the ability of maltose to block sucrose uptake into yeast expressing SoSUT1 (12) or AtSUC1 and AtSUC2 (4), maltose also induced inward currents (39% of sucrose-induced currents) indicating that it is a transported substrate. Glucose, trehalose, palatinoce (isomaltulose), cellubiose, isomaltose, melibiose, melezitose, raffinose, gentiobiose, and D-amylgluclidin did not induce detectable currents. The plant glucosides salicin (2-(hydroxymethyl)phenyl-β-D-glucoside) and arbutin both induced large inward currents. Consistent with their ability to block sucrose uptake into yeast expressing AtSUC2 (4), phenylglucosides with both α- and β-linkage also induced large inward currents. Although glucose, 3-O-methylglucose, and 2-deoxyglucose were not transported, α-methylglucoolcoside induced inward currents.

Kinetic analysis was performed for substrates that induced large inward currents. The apparent affinities for all transported substrates were voltage-dependent (Fig. 4) with a lower $K_{0.5}$ observed at more negative membrane potentials. The voltage dependence of $K_{0.5}$ for all transported substrates was similar to that of sucrose (Fig. 2D) indicating a common binding site. $K_{0.5}$ values for transported substrates at −137 mV are presented in Table I. The $K_{0.5}$ for maltose transport was 7.8-fold higher than for sucrose indicating that AtSUC2 is highly selective for sucrose over maltose. The apparent affinities for the plant glucosides salicin and arbutin were similar to that of sucrose. The ability of AtSUC2 to transport synthetic glucosides allows a determination of the effect of α- versus β-linkage on substrate affinity (Table I). The $K_{0.5}$ for α-phenylglucoside (0.94 mM) was lower than for β-phenylglucoside (1.18 mM). Similarly, the $K_{0.5}$ for α-paranitophenylglucoside (0.40 mM) was lower than for β-paranitophenylglucoside (2.70 mM). These results indicate that AtSUC2 binds α-linked glucosides at higher affinity than β-linked glucosides. The apparent affinities for paranitophenyl-β-thioglucoside and β-paranitropheg-
ylglucoside were similar indicating that AtSUC2 does not discriminate between O-linked and S-linked glucosides.

**DISCUSSION**

Previous studies have shown that glucosides such as maltose and α- or β-phenylglucoside inhibit [14C]sucrose uptake into yeast expressing plant sucrose transporters (4, 7, 12–15). To address the question of whether these and other glucosides are transported substrates or transport inhibitors, we expressed AtSUC2 in *Xenopus* oocytes and applied two-electrode voltage clamping to measure transport activity.

Sucrose and other glucosides were found to induce inward currents consistent with a H⁺-coupled transport mechanism (17). The *Kₘ* for sucrose transport at pH 5.0 by AtSUC2-expressing yeast was reported previously to be 0.8 mM (4). This is significantly lower than the value obtained in this study (1.44 ± 0.19 mM, at pH 5.0 and −137 mV). When the apparent affinity of AtSUC2 for sucrose was measured by uptake into yeast, it is likely that higher concentrations of sucrose caused membrane depolarization lowering the driving force for sucrose uptake, as well as the affinity of the transporter for sucrose, and therefore the observed *Vₘₐₓ*. Under these conditions, the observed *Kₘ* for sucrose would be lower than when the membrane potential is clamped, as in this study. This idea is supported by data from Sauer and Stolz (4) who found higher *Kₘ* values for sucrose uptake into yeast expressing AtSUC2 in the presence of glucose than in the absence of glucose, which would support a more negative membrane potential.

The voltage dependence of the *Kₜₕₐₜₗₜ* for sucrose of AtSUC2 (Fig. 2) was generally consistent with results for StSUT1 from potato (17) and AtSUC1 from *Arabidopsis* (18). For all of these transporters, the *Kₜₕₐₜₜₜ* for sucrose is lower and less voltage-dependent at a lower pH. At pH 5.0, the *Kₜₕₐₜₜₜ* for sucrose of StSUT1 is not significantly voltage-dependent (17). However, as shown in Fig. 2D, the *Kₜₕₐₜₜₜ* for sucrose of AtSUC2 at pH 5.0 is lower at more polarized potentials. This represents a difference between StSUT1 and AtSUC2 activities. The dependence of the apparent affinity for sucrose on extracellular pH and membrane potential within the physiological range is impor-
tant for the function of sucrose transporters in plants. The results of this study and others (17, 18) indicate that activation of the plasma membrane H\textsuperscript{+}/H\textsubscript{2}ATPase, which generates a negative membrane potential and acidifies the extracellular space relative to the cytoplasm, would enhance sucrose transport not only through increasing proton motive force but by lowering the $K_m$ of sucrose transporters.

Glucosides such as maltose and $\alpha$- and $\beta$-phenylglucoside, which had been demonstrated previously to inhibit $[\text{14C]}$sucrose uptake into yeast expressing AtSUC2 (4), induced inward currents indicating that they serve as transported substrates. Furthermore, of the 25 sugars tested, 8 induced large inward currents (Fig. 3) in addition to sucrose allowing determination of apparent affinities (Table I and Fig. 4). In addition, both $\alpha$- and $\beta$-linked phenyl (and nitrophenyl) glucosides were transported by AtSUC2 with $\alpha$-linked glucosides consistently showing lower $K_{0.5}$ values. It is particularly interesting that salicin and arbutin ($\beta$-linked glucosides) are transported with $K_{0.5}$ similar to sucrose. Although arbutin has not been detected in Arabidopsis, homologs of the plant glycosyltransferase arbutin synthase (19) exist in Arabidopsis (At4g01070 and At1g01420). This suggests that arbutin or related glucosides are present in Arabidopsis and potentially may be transported into the phloem by AtSUC2. There is evidence that the transport of glucosides other than sucrose by the phloem is physiologically important. For example, glucosinolates are transported in the phloem in Arabidopsis (20).

In uptake studies using isolated plant protoplasts, sucrose transporters were found to interact specifically with the glucosyl hydroxyls 3, 4, and 6 of sucrose (9). Similarly, phenyl-$\beta$-galactopyranoside did not induce inward currents, whereas $\beta$-phenylglucoside was transported with a $K_{0.5}$ of 1.18 mM indicating strong selectivity for the glucoside, which differs in side group orientation at position 4 compared with the galactoside. The conclusion by Hitz et al. (9) that the fructosyl moiety of sucrose does not interact specifically with the transporter but presents a hydrophobic surface that interacts with the binding site is also supported by our results. Although glucose did not induce inward currents in oocytes expressing AtSUC2, $\beta$-methylglucoside served as a substrate (Fig. 3) indicating that even small hydrophobic groups at position 1 of glucose are sufficient for interaction with the transporter. For compounds with larger side groups, orientation or linkage position was critical in determining whether interaction with AtSUC2 occurred. For example, trehalose, which is an $\alpha$-glucose [1$\rightarrow$1] $\alpha$-glucoside and is similar to maltose except that glucose is linked at position 1, was not transported. Along the same lines, palatinose was not transported although it is similar to sucrose except that fructose is $\alpha$-linked at position 6.

**Fig. 3. Substrate specificity of AtSUC2.** Substrate-dependent currents were recorded from Xenopus oocytes expressing AtSUC2 under voltage-clamp conditions. Substrates were applied at 10 mM in sodium-Ringer solution, pH 5.0. Currents were recorded at a membrane potential of $\pm$137 mV. Substrate-dependent currents were normalized to currents recorded with 10 mM sucrose to control for differences in AtSUC2 expression between oocytes. Mean currents for 3 oocytes $\pm$ S.E. are presented.
Fig. 4. Voltage dependence of substrate affinity for AtSUC2. $K_{0.5}$ values for transported substrates were measured as explained in the legend to Fig. 2 for substrate concentrations between 50 $\mu M$ and 10 mM (30 mM for maltose). $K_{0.5}$ values (mean for 3 oocytes $\pm$ S.E.) are plotted as a function of membrane potential ($V_m$) for the following substrates: A, maltose; B, salicin; C, arbutin; D, $\alpha$-phenylglucoside; E, $\beta$-phenylglucoside; F, $\alpha$-paranitrophosphylglucoside; G, $\beta$-paranitrophosphylglucoside; H, paranitrophosphyl-$\beta$-thioglucoside.
(compared with position 2 in sucrose). It is widely assumed that the sucrose isomers turanose and palatinose are not transported into plant cells (21), although this has not been tested directly. Our results show that turanose, but not palatinose, serves as a substrate for AtSUC2 (Fig. 3). This is significant because turanose and palatinose both induce extracellular invertase expression similarly to sucrose, and although sucrose inhibits expression of photosynthetic genes, turanose and palatinose do not (21). Differences in the perception of turanose and palatinose in comparison to sucrose have been explained in terms of the inability of plant cells to transport the sucrose analogs, and this conclusion may need to be reevaluated in light of the current results. However, it should be pointed out that plants encode multiple sucrose transporters, and differences in transport activity have been reported. Although substrate specificity has not been analyzed for other plant sucrose transporters, in Arabidopsis AtSUT2 and AtSUT4 show a low affinity for sucrose (7, 8) compared with AtSUC2. It is possible that plant sucrose transporters have different substrate specificities. For example, for yeast expressing AgSUT1, a sucrose transporter from celery, raffinose competed more effectively than maltose for \([^{14}\text{C}]\text{sucrose} \) uptake (15). This was not the case for AtSUC2 (4), and raffinose did not induce inward currents in oocytes (Fig. 3).

In conclusion, the results show that AtSUC2 has a weak selectivity for \(\alpha\)-linked glucosides but will transport \(\beta\)-glucosides such as arbutin and salicin with a \(K_{\text{m,5}}\) equal to or lower than for sucrose. AtSUC2 did not show selectivity between thio- and \(O\)-linked glucosides. This is potentially of physiological significance because glucosinolates (\(S\)-linked glucosides) are known to be transported in the phloem (20). The broad specificity of AtSUC2 indicates that glucosides other than sucrose may be transported as physiological substrates. For example, several plant hormones, such as auxins and cytokinins, are glucosylated (22, 23), and the ability to transport low concentrations within the phloem may be important. Insertional mutants of AtSUC2 show severe growth phenotypes (6) that have been assumed to be caused by an inability to load sucrose into the phloem. Additional work will be required to determine whether AtSUC2 function in the plant includes transport of glucosides in addition to sucrose.

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