Transport Mechanism of the Cloned Potato H⁺/Sucrose Cotransporter StSUT1*

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The transport mechanism of the potato StSUT1 H⁺/ sucrose cotransporter expressed in Xenopus oocytes was investigated using the 2-electrode voltage clamp and radiotracer flux methods. Sucrose induced inward currents through the transporter that were dependent on the extracellular sucrose and H⁺ concentrations and the membrane voltage. The activation of StSUT1 by H⁺ and sucrose displayed Michaelis-Menten-type kinetics suggestive of a 1:1 H⁺:sucrose stoichiometry. This was confirmed by simultaneously measuring inward currents and sucrose flux in voltage-clamped oocytes. The apparent affinities Kₐ,S for H⁺ and sucrose were voltage-dependent. At −150 mV Kₐ,S was 0.5 ± 0.07 mM at 10 μM H⁺, and Kₐ,H was 0.1 ± 0.05 mM at 20 mM sucrose. StSUT1 exhibited presteady-state transient currents, which relaxed with time constants between <1 and 4 ms and fitted to the Boltzmann equation: maximum charge transfer Qₘₐₓ ∼ 1.8 nanocoulombs; apparent valence e ∼ 2; potential for 50% charge transfer Vₐₜₐₜ ∼ −15 mV at 0.032 μM H⁺ and −45 mV at 10 μM H⁺. The steady-state data were used to formulate a kinetic model for sucrose transport, and computer simulations were performed to obtain rate constants for the partial reaction steps. Our model is consistent with protons binding to StSUT1 before sucrose with both ligands transported simultaneously across the membrane.

Sucrose is the major mobile carbohydrate in the majority of higher plants. Sucrose is loaded into the phloem against a large concentration gradient and is transported to heterotrophic tissues where it is used for metabolism or storage. Phloem loading is catalyzed by specific transport proteins, which couple the uptake of sucrose to the electrochemical potential gradient for protons generated by the H⁺-ATPase (Buckhout, 1989, 1994; Bush, 1988, 1990, 1993; Williams et al., 1990, 1992).

Recently, cDNAs encoding putative H⁺/sucrose cotransporters have been isolated from spinach (SoSUT1; Riesmeier et al., 1992), potato (StSUT1; Riesmeier et al., 1993a), Arabidopsis thaliana (SUC1 and SUC2; Sauer and Stolz, 1994), and Plantago major (PmSUC2; Gahrtz et al., 1994). They are all exons of membrane potential and external sucrose and H⁺ concentrations on the transport kinetics of STSUT1. We show that STSUT1 is electrogenic with membrane potential affecting the maximal rate of transport and apparent affinities for H⁺ and sucrose. The kinetic properties of StSUT1 can be explained by an 8-state ordered simultaneous model with H⁺ binding to the transporter before sucrose.

EXPERIMENTAL PROCEDURES

Molecular Biology Methods—To obtain sucrose transport activity in oocytes, the NosI fragment of StSUT1 was inserted into the NosI site of pKB1, a vector with a poly(A) tail of 70 adenosine residues (Boorer et al., 1996). The resulting plasmid pKNSTUT1 was linearized with KpnI, and capped cRNA was transcribed in vitro using T7 RNA polymerase and an RNA transcription kit (Ambion, Austin, TX).

Preparation of Oocytes—Xenopus oocytes were isolated and injected with 50 ng (1 μg/μl) of cRNA encoding the potato StSUT1 transporter or with 50 nl of water and were maintained in Barth’s medium for up to 15 days post-injection as described previously (Boorer et al., 1994, 1996).

Electrophysiological Methods—The 2-electrode voltage clamp method was used to measure the kinetics of H⁺/sucrose cotransport (Loo et al., 1993; Boorer et al., 1994, 1996). Steady-state currents recorded 40 ms after the onset of the voltage pulse were obtained by taking the difference between steady-state currents in the presence and absence of sucrose.

The expression of the cloned potato H⁺/sucrose cotransporter, StSUT1, showed that it plays a vital role in phloem loading, assimilates partitioning, and is essential for the growth and development of potato plants (Riesmeier et al. 1993b).

Little is known about the molecular mechanisms of sucrose transport. Previous studies of cloned sucrose transporters utilized transfected Saccharomyces cerevisiae cells where sucrose transport was stimulated by increasing the extracellular proton concentration [H⁺]o, and reduced by protonophores (Riesmeier et al., 1992, 1993a; Sauer and Stolz, 1994). These observations indicate that the uptake of sucrose is dependent on a proton gradient. To understand the molecular mechanisms of sucrose transport in more detail, we expressed the potato StSUT1 transporter in Xenopus oocytes and used a combination of electrophysiological and radiotracer flux methods to determine the effects of membrane potential and external sucrose and H⁺ concentrations on the transport kinetics of STSUT1. We show that STSUT1 is electrogenic with membrane potential affecting the maximal rate of transport and apparent affinities for H⁺ and sucrose. The kinetic properties of StSUT1 are explained by an 8-state ordered simultaneous model with H⁺ binding to the transporter before sucrose.

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Antisense inhibition of the potato StSUT1 trans-

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25139
Mechanism of StSUT1 Expressed in Oocytes

**RESULTS**

Steady-state Kinetics—Fig. 1 shows the currents recorded from an oocyte expressing StSUT1 when the membrane potential was held at -50 mV and sucrose was added to the bathing medium. Addition of 20 mM sucrose at 0.032 and 10 mM H⁺ induced inward currents (~40 nA), which returned to base-line levels after removal of sucrose. Increasing the [H⁺], to 10 mM induced an inward current (~15 nA), which increased (~90 nA) on addition of 20 mM sucrose.

The current-voltage relationships were fitted to the Boltzmann Equation 5,

\[
Q = \frac{Q_{\text{max}}}{1 + \exp(z(V_m - V_{0.5})/F RT)}
\]  
(Eq. 3)

where \(Q_{\text{max}} = Q_{\text{dep}} - Q_{\text{app}}\), \(Q_{\text{dep}}\), and \(Q_{\text{app}}\) are \(Q\) at depolarizing and hyperpolarizing limits, \(z\) is apparent valence of movable charge, \(V_{0.5}\) is potential for 50% charge transfer, \(F\) is Faraday's constant, \(R\) is gas constant, and \(T\) is absolute temperature.

All fitting procedures were done using software from Sigma Plot (Jandel Scientific, San Rafael, CA) and Origin (Microcal Software, Inc., Northampton, MA). The error bars in Figs. 3 and 4 are the errors of the fit.

Determination of H⁺:Sucrose Coupling Ratio—The ratio of H⁺:sucrose transport was determined by simultaneously measuring inward fluxes of [14C]sucrose and net inward sucrose-induced currents. Substrate-induced currents were recorded on tape (Unitrade DAS 900). Oocytes were clamped at -50 mV and superfused with transport buffer at 0.032 or 10 mM H⁺ at a rate of 160 µl/min. Current traces were monitored until they reached a steady base line after which [14C]sucrose was superfused for 5-10 min while recording the sucrose-induced current. The oocyte was washed in the absence of sucrose until the current returned to base-line levels. The oocyte was quickly removed from the chamber, washed three times in 5 ml of ice-cold buffer, lysed in 10% sodium dodecyl sulfate, and the amount of radioactivity determined by liquid scintillation counting. The amount of radioactivity in water-injected oocytes was subtracted from oocytes expressing StSUT1. The tape was digitized at 1 Hz, and the total inward charge was calculated by integrating the area under the current versus time curve. The ratio of H⁺:sucrose transported is presented as pmol net inward charge:pmol sucrose transport.

The results are representative of experiments that were repeated with oocytes from different donor frogs. All experiments were carried out at 22 °C. Chemicals were purchased from Sigma.

The kinetics of StSUT1 were studied with a pulse protocol where the membrane potential was stepped from -50 mV to test values ranging from +50 to -150 mV. Fig. 2 shows current traces obtained when the voltage pulses were applied ("ON" currents) and removed ("OFF" currents). The rapid jumps in membrane potential induced capacitative transients with decay time constants between 0.5 and 0.8 ms. Oocytes expressing StSUT1 also exhibited presteady-state transient currents, which decayed slowly to steady-state levels and which will be described below. Addition of sucrose induced inward currents at every potential, as can be seen for 100 mM sucrose at 0.032 mM H⁺ (Fig. 2A) and for 20 mM sucrose at 10 mM H⁺ (Fig. 2C). Neither the presteady-state currents in the absence of sucrose nor the sucrose-induced steady-state currents were observed in water-injected oocytes (Fig. 2, B and D).

The steady-state currents generated by StSUT1 were determined as functions of the external sucrose and H⁺ concentrations and membrane voltage. To compare data from different oocytes, the sucrose-induced currents were normalized to the current induced by 20 mM (saturating) sucrose at 10 mM H⁺, and -150 mV (typically 100-300 nA). Fig. 3A shows the I/V relationships of the sucrose-induced currents at 0.032 mM H⁺, with 100 mM [sucrose], and at 10 mM H⁺, with 20 mM [sucrose]. Over the voltage range used in this study (+50 to -150 mV), the currents did not show reversal. At the lower H⁺, the current increased linearly with hyperpolarizing voltages, and at the high H⁺, (10 mM) the current was voltage-independent at large hyperpolarizing voltages.

Fig. 3B shows the activation of the transporter by sucrose. The experiment was performed at a holding potential of -50 mV with H⁺, maintained at 0.032 or 10 mM. The current-concentration curves were hyperbolic and suggest that 1 su-
Sucrose molecule binds to the transporter. Fig. 3C shows the dependence of the maximal current $i_{\text{max}}$ on voltage. The $i_{\text{max}}$ versus voltage curves were relatively independent of $[H^+]_o$ with a 300-fold increase in $[H^+]_o$, yielding a 1.4-fold increase in $i_{\text{max}}$ and convergence of the curves at potentials more negative than $-150$ mV (not shown). Fig. 3D shows the voltage dependence of the apparent affinity constant $K_{0.5}$. $K_{0.5}$ was greater and more voltage-dependent at $0.032 \mu M [H^+]_i$, $57 \pm 15 \mu M$ at $10$ mV and $21 \pm 4 \mu M$ at $-150$ mV than at $10 \mu M [H^+]_i$, $1.1 \pm 0.2 \mu M$ at $10$ mV and $0.5 \pm 0.07 \mu M$ at $-150$ mV. Thus, negative potentials and high $[H^+]_o$ increased the apparent affinity of StSUT1 for sucrose.

Fig. 4 shows the kinetic data obtained by varying $[H^+]_o$ at fixed sucrose concentrations (5 and 20 mM). At both concentrations of sucrose the IV relationships increased linearly to a 300-fold increase in $[H^+]_o$ steady-state I/V relationships obtained at $0.032$ and $10$ mM sucrose. At $0.032 \mu M H^+$ sucrose-induced currents increased linearly whereas at $10 \mu M H^+$ sucrose-induced currents saturated at more negative potentials.

Voltage dependence of the sucrose activation kinetics of StSUT1. Sucrose-induced currents were recorded at potentials between $-150$ and $50$ mV using the pulse protocol described in the legend to Fig. 2. The steady-state sucrose-dependent current/voltage (IV) relationships were obtained by subtracting the IV curves obtained in the absence of sucrose from those obtained in the presence of sucrose. A, steady-state IV relationships obtained at $0.032$ and $10 \mu M H^+$ with $10$ and $20$ mM sucrose, respectively. At $0.032 \mu M H^+$ sucrose-induced currents increased linearly as the membrane potential became more negative, whereas at $10 \mu M H^+$ sucrose-induced currents saturated at more negative potentials. B, concentration/current curves obtained at $-150$ mV with $0.032$ and $10 \mu M H^+$. The experimental data (symbols) were fitted to Equation 1 (lines) to yield the sucrose activation kinetic parameters. C, voltage dependence of $i_{\text{max}}$ at $0.032$ and $10 \mu M H^+$. $i_{\text{max}}$ was voltage-dependent and 1.3-fold higher at $10 \mu M$ than at $0.032 \mu M H^+$. D, voltage dependence of $K_{0.5}^{\text{suc}}$ at $0.032$ and $10 \mu M H^+$. Increasing $[H^+]_o$, increased $K_{0.5}^{\text{suc}}$ 40–70-fold.

Voltage dependence of the proton activation kinetics of StSUT1. A, concentration/current curves obtained by plotting the sucrose-induced currents at 5 and $20$ mM sucrose, against $[H^+]_o$, at $-150$ mV. The data (symbols) fitted to Equation 1 (lines). B, voltage dependence of $i_{\text{max}}$ at 5 and $20$ mM sucrose. Increasing [sucrose]$_o$, increased $i_{\text{max}}$. C, voltage dependence of $K_{0.5}^{\text{suc}}$ at 5 and $20$ mM sucrose. Increasing [sucrose]$_o$, increased the apparent affinity of StSUT1 for $H^+$.

As described earlier (Fig. 1) inward currents were induced in StSUT1-expressing and control oocytes when $[H^+]_o$ was increased in the absence of sucrose. Fig. 5 shows the $H^+$-dependent IV relationship due to StSUT1, which was obtained by subtracting the $H^+$-dependent IV curve of control oocytes (mean of three oocytes) from the $H^+$-dependent IV curve of StSUT1-expressing oocytes (mean of three oocytes). The error bars have been omitted for clarity. Uncoupled $H^+$ transport was voltage-dependent and, at $-150$ mV, was between 20 and 30% of the current induced at saturating $H^+$ ($10 \mu M$) and sucrose ($20$ mM) concentrations.

$H^+$:Sucrose Stoichiometry—The $H^+$:sucrose stoichiometry was determined by simultaneously measuring the sucrose-induced current and flux of $[^{14}\text{C}]$sucrose in the same oocyte. Fig. 6, A and B, shows typical current traces obtained from oocytes superfused at $0.032 \mu M H^+$ with $5$ mM $[^{14}\text{C}]$sucrose and $10 \mu M H^+$ with $1$ mM $[^{14}\text{C}]$sucrose. At $0.032 \mu M H^+$, the magnitude of the current was constant over time. However, at $10 \mu M H^+$, the magnitude of the current declined with time reaching a steady value between $5$ and $10$ min (see “Discussion”). When sucrose was removed, currents returned to the base-line levels recorded before sucrose was added. Fig. 6C shows the relationship between the total $[^{14}\text{C}]$sucrose flux and the inward charge. At $10 \mu M H^+$ and $1$ mM sucrose, regression analysis yielded a linear relationship with a slope of $1.26 \pm 0.09 (r^2 = 0.94)$, which is equivalent to a coupling ratio of $1 H^+:1.26$ sucrose. At $0.032 \mu M H^+$ and $5$ mM sucrose, the slope was $1 \pm 0.09 (r^2 = 0.93; data not shown) equivalent to $1 H^+:1$ sucrose. The regression lines intercepted the y axis, indicating that there is uptake of sucrose...
in the absence of an inward current (74 ± 14 pmol/oocyte at 10 μM H⁺ and 41 ± 19 pmol/oocyte at 0.032 μM H⁺). The uptake of sucrose into water-injected controls was between 5 and 10 pmol/oocyte.

**Presteady-state Kinetics**—Presteady-state transient currents associated with the expression of StSUT1 were isolated from the total currents shown in Fig. 2, A and C, by subtraction of the steady-state currents and the capacitive transients. Fig. 7A shows the transient currents at 30, −50, and −150 mV for the ON response at 0.032 and 10 μM H⁺ in the absence of sucrose. The asymmetry of the currents at 0.032 μM H⁺ compared with the symmetrical currents at 10 μM H⁺ is indicative of H⁺ influencing the distribution of charge and hence the conformation of StSUT1 in the membrane. Integration of the transient currents yielded the total charge Q at each membrane potential. Fig. 7B shows the Q versus Vm relationships of the ON response for an oocyte superfused at 0.032 and 10 μM H⁺ in the absence of sucrose. The data fitted to the Boltzmann equation with z = 1, V₀.5 = −45 mV, and Qmax = 1.8 at 10 μM H⁺; z ≈ 1, V₀.5 = −45 mV, and Qmax = 1.8 at 10 μM H⁺, C, the relaxation of each transient current was described by a single time constant τ. The time constants shown are for the ON currents. At 0.032 μM H⁺, τ was voltage-dependent and decreased from 4 ms at 50 mV to <1 ms at −150 mV. At 10 μM H⁺, τ was relatively voltage-independent (2–3 ms).

**Discussion**

Expression of the cloned potato StSUT1 sucrose transporter in Xenopus oocytes has several advantages over the use of isolated plasma membrane vesicles or yeast cells to study sucrose transport, including the ability to measure sucrose transport kinetics as a function of membrane voltage and H⁺ and sucrose concentrations in a single oocyte with extremely fast temporal resolution (1 ms), the determination of H⁺:sucrose...
stochiometry under voltage-clamped conditions, and the isolation of presteady-state currents associated with the transporter.

StSUT1 is electrogic as demonstrated by the sucrose-induced, inward, H+ -dependent currents which were accompanied by the influx of sucrose and which increased with increasing [H+]o, [sucrose]o, and membrane voltage. H+ /sucrose cotransport by StSUT1 exhibited hyperbolic kinetics, suggesting that 1 H+ and 1 sucrose bind to StSUT1 per transport cycle. A 1 H+ :1 sucrose stoichiometry was reported for sucrose transport into plasma membrane vesicles isolated from sugar beet (Bush, 1990; Slone and Buckhout, 1991). Measurement of sucrose influx and sucrose-induced inward current in the same oocyte under voltage-clamped conditions showed that the H+ : sucrose stoichiometry was dependent on [H+]o (1.1 and 1.126 at 0.032 and 10 μM H+, respectively). The increase in stoichiometry at 10 μM H+ is due to the increase in uncoupled transport of H+ when [H+]o is increased from 0.032 to 10 μM (see Fig. 5).

The apparent affinities for sucrose and H+ are relatively independent of the cell type in which they are expressed. When expressed in oocytes, the K1/2 for sucrose was between 0.5 and 1 mM at 10 μM H+ which is similar to K1/2 for sucrose in yeast (Riesmeier et al., 1993a), K1/2 of the spinach (Riesmeier et al., 1992), Arabidopsis (Sauer and Stolz, 1994), and Plantago (Gahrtz et al., 1994) sucrose transporters, and the K1/2 of sucrose transport into isolated plasma membrane vesicles (Bush, 1990; Buckhout, 1994). The apparent affinity for H+ was 0.1 μM (pH 7.0) at saturating [sucrose] and −150 mV, which is lower than the K1/2 for H+ of 0.7 μM (pH 6.2) obtained by Bush (1990) but similar to 0.23 μM (pH 6.6) obtained by Buckhout (1994). Differences in apparent affinities are probably due to variations in the membrane potentials of the experimental systems.

The positive cooperativity between H+ and sucrose, i.e. increasing [H+]o, and [sucrose]o increases the apparent affinity for sucrose and H+, respectively (Figs. 3D and 4D), suggests that StSUT1 operates via a simultaneous mechanism (Jauch and Läuger, 1986). Since the vmax/voltage voltage curves converge at potentials more negative than −150 mV whereas the i1 max/voltage curves do not, we conclude that H+ acts as an essential activator and binds to StSUT1 before sucrose.

In addition to the cotransport of H+ and sucrose, we also observed uncoupled H+ and sucrose transport by StSUT1 (Figs. 5 and 6). Uncoupled transport of cations is also part of the transport cycle of the H+/amino acid AAP1 cotransporter (Boorer et al., 1996) and the Na+/glucose cotransporters (Parent et al., 1992a, 1992b; Loo et al., 1993). Uncoupled sucrose transport is interesting in the light of previous studies, which isolated two components of sucrose transport in Vicia faba (Delrot and Bonnemain, 1981; Delrot, 1981) and sugar beet (Maynard and Lucas, 1982a, 1982b); leaf tissues, one H+ -dependent/saturable system and one linear system. It will be interesting to investigate the effect of [sucrose], on the uncoupled transport of sucrose by StSUT1 to determine whether a single carrier could be responsible for the biphasic kinetics observed in previous studies.

The voltage-dependent and -independent regions of the UV relationships show that there are voltage-dependent and -independent steps in the transport cycle of StSUT1. Hyperpolarizing potentials increased the apparent affinity of StSUT1 for sucrose and H+. Thus, StSUT1 is negatively charged with voltage driving protons into their binding site. Protons bind before sucrose, hence the voltage dependence of K1/2 influences the voltage dependence of K1/2.

Based on the steady-state kinetics, we propose a model of H+ /sucrose transport, which is summarized in Fig. 8A. One proton binds to the external face of the negatively charged empty transporter before sucrose. The fully loaded transporter undergoes a conformational change and releases the ligands at the cytoplasmic surface. The ligand-binding sites return to the external surface to complete the transport cycle. Uncoupled transport of H+ and sucrose is indicated by the dotted lines. This model is similar to that previously proposed for the Na+/glucose cotransporter in a single carrier [see Fig. 6A and 6B (1)].

The model was modified to account for a single cation binding step and a valence of −1. We have omitted the steps involved in the uncoupled transport of sucrose from the simulations. The derived rate constants describe the steady-state reaction steps in the model. The voltage-dependent steps in the reaction scheme are states 1 → 6, 6 → 1, 1 → 2, and 2 → 1, where k16 = k28(0.5 μ), k23 = k28(−0.5 μ), k12 = k18[H+]exp(−0.5 α μ), and k21 = k28exp(0.5 α μ). The coefficients α, α', and δ describe the fraction of the electric field sensed by the H+ binding to the external site (α') or internal site (α) and by the empty ion binding site on the carrier during membrane translocation δ, where α + α' + δ = 1 and μ is the electrical potential FV/RT. The rate constants describing the steady state reaction steps are k12 = 1 × 104 m−1s−1, k23 = 220 m−1s−1, k45 = 50 s−1, k45 = 50 s−1, k45 = 50 s−1, k45 = 50 s−1, k45 = 1 × 10−06 s−1, k20 = 3.10 × 10−06 s−1, and k12 = 1.70 × 10−03 m−1s−1, k45 = 5 s−1, k45 = 1.10 × 10−03 s−1, k45 = 1 × 10−03 s−1 with α = 0.4 and δ = 0.6. The model predictions were compared with the actual data shown in Figs. 3 and 4 (symbols). The rate constants predict the data both qualitatively and quantitatively. Model predictions are as follows: B, K1/2, C, K1/2, D, K1/2, and E, K1/2.
glucose cotransporter (SGLT1; Parent et al., 1992a, 1992b; Loo et al., 1993) except 1 H⁺ binds to the transporter instead of 2 sodium ions. To test the model, the computer program used for the simulation of SGLT1 was modified for a 1 cation:1 organic substrate stoichiometry, and the valence of the empty transporter was changed to -1. The rate constants for the partial reactions in Fig. 5A were varied until the predicted data best described the experimental data. To simplify this preliminary model, we have omitted the uncoupled transport of sucrose from the simulations. Fig. 8, panels B–E, shows the data derived from the computer simulations superimposed on the experimental data. The rate constants described in the legend to Fig. 8 reasonably predict the quantitative and qualitative characteristics of 1, 1, 1, 1, and 1. Essentially, the steady-state kinetic data of StSUT1 are qualitatively similar to the Na⁺/glucose cotransporter, the main distinction being the rate of cation binding: H⁺ binding to StSUT1 is 2 × 10^-6-fold faster than Na⁺ binding to SGLT1 (Parent et al., 1992a, 1992b) and is also 10-fold less than H⁺ binding to the human hPEPT1 dipeptide transporter (Mackenzie et al., 1996). Our model is in contrast to that proposed for the sucrose transporter in sugar beet leaves by Buckhout (1994), who concluded that the fully loaded carrier is positively charged and sucrose binds to the transporter before H⁺.

Using plasma membrane vesicles, Buckhout (1994) varied the internal concentrations of H⁺ and sucrose and showed that sucrose transport was inhibited by H⁺ and not sucrose. In the present study at 10 μM H⁺, there was a gradual reduction in the magnitude of the inward current with time before it reached a constant value, whereas at 0.032 μM H⁺ the current remained relatively constant (Fig. 6, panels A and B). The model predicts that the rate constant for H⁺ dissociation is 4.3 s⁻¹, which is >200-fold less than the predicted rate of sucrose dissociation (1 × 10⁶ s⁻¹). Thus, the slow rate of H⁺ dissociation predicts trans-inhibition of sucrose transport and may be a rate-limiting step in the transport cycle. This hypothesis can be tested further by varying the cytoplasmic [H⁺] and [sucrose] using the cut-open oocyte method (Taglialatela et al., 1992).

We assume that the presteady-state currents recorded after rapid jumps in membrane potential are due to StSUT1; they were not observed in water-injected oocytes, the maximal charge, Q_max, was directly proportional to the maximal current induced by sucrose, i_max, and increasing [H⁺], shifted the V_{0.5} to more negative values. Unlike the Arabidopsis H⁺/hexose STP1 (Boorer et al., 1994) and the Na⁺/glucose SGLT1 (Loo et al., 1993) cotransporters, the StSUT1 presteady-state currents were not altered quantitatively or qualitatively after the addition of saturating [sucrose]... Likewise, presteady-state currents associated with the norepinephrine transporter were not altered by norepinephrine (Galli et al., 1995). The nature of these effects is not understood. When [H⁺]o was increased from 0.032 to 10 μM, there was a change in the charge distribution across the membrane with V_{0.5} becoming more negative. Thus, at high [H⁺]o, more ligand-binding sites face the cytoplasmic surface at hyperpolarizing potentials. This observation is counterintuitive with respect to the observation that hyperpolarizing potentials increase the magnitude of the sucrose-induced currents. We assume that StSUT1 is negatively charged; thus, hyperpolarizing potentials would be expected to orientate the binding sites to the outside. At present, we are unable to correlate the effect of membrane voltage and H⁺ on the presteady-state kinetics with the steady-state results.

The relaxation time constants τ were fast compared to STP1 (3–14 ms; Boorer et al., 1994) and SGLT1 (5–20 ms; Loo et al., 1993). At low [H⁺]o, τ was voltage-dependent and decreased from 4 ms at 50 mV to >1 ms at −150 mV, whereas at high [H⁺]o, τ was relatively voltage-independent (2–3 ms).

Neither the membrane potential nor the apoplastic [H⁺] of the sieve element/companion cell complex from potato has been determined. However, membrane potentials between −100 and −130 mV have been measured in the sieve element/companion cell complex of other plant species (Fromm and Eschrich, 1989; van Bel and van Rijen, 1994), and estimates of apoplastic [H⁺] vary between 0.32 and 10 μM (Grignon and Sentenac, 1991). It is likely that membrane potentials more negative than −100 mV are maintained in potato sieve element/companion cell complexes due to the high levels of ATPase in these cells. Therefore, sucrose transport by StSUT1 in vivo is probably not limited by membrane potential. Due to the high affinity of StSUT1 for H⁺, the maximum rate of sucrose transport will be maintained as long as apoplastic [sucrose] is high. If apoplastic [H⁺] is high, changes in membrane potential will not affect τ. Therefore, the rate constants in sucrose transport in vivo will be either apoplastic [sucrose] and/or [H⁺].

In conclusion, we have shown that StSUT1 is an electrogenic, H⁺/sucrose transporter with a 1 H⁺:1 sucrose stoichiometry of 1:1. Protons act as an essential activator for sucrose transport, and negative potentials increase the maximal rate of transport and the affinity for H⁺ and sucrose. The steady-state kinetic properties can be accounted for by an 8-state, ordered, simultaneous model with H⁺ binding before sucrose. Further studies are required to refine the kinetic model including determination of the mechanism of the presteady-state currents and the internal reaction steps.

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