Sucrose Uptake by Developing Soybean Cotyledons

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ABSTRACT

Sucrose uptake by excised developing soybean cotyledons shows a biphasic dependence on sucrose concentration. At concentrations less than about 50 millimolar external sucrose, uptake can be described as a carrier-mediated process, with a $K_m$ of 8 millimolar. At higher external sucrose concentrations, a linear dependence becomes apparent, which suggests the participation of a nonnonsaturable component in total uptake. Sucrose absorption is dependent on the presence of an electrochemical potential gradient for protons since agents interfering with the generation or maintenance of this gradient (Na$_2$PO$_4$ or carbonylcyanide-$m$-chlorophenyl hydrazine) decrease sucrose transport to a level at or below that predicted from the operation of the noncarrier-mediated process alone. The saturable component of sucrose uptake is also sensitive to the sulfhydryl-modifying compounds N-ethylmaleimide and p-chloro-mercuribenzenesulfonate. The thiol-reducing agent diethyloethritol reverses fully the p-chloro-mercuribenzenesulfonate inhibition, but not that of N-ethyl maleimide. Sucrose transport is sensitive to external pH, being decreased at high pHs. Since sucrose-induced depolarization of the membrane potential and carrier-mediated sucrose influx show similar pH-dependence, inhibitor sensitivity, and values of $K_m$ for sucrose, a sucrose/proton cotransport process appears to operate in developing soybean cotyledon cells. Measurement of free space and intracellular sucrose concentrations in vivo suggests that the carrier-mediated process is fully saturated and that sucrose transport may be limiting for sucrose accumulation by the developing seed.

The ability of plant cells to transport carbohydrates and amino acids is well established. Evidence has been accumulating which suggests that nutrient uptake in plants is carrier-mediated and is coupled to proton flux (1) in a process similar to that which operates during $\beta$-galactoside transport in Escherichia coli. In addition to this carrier-mediated process, some tissues exhibit a passive nonsaturable component of nutrient uptake, especially at high extracellular concentrations (8, 15).

As the generation of the proton electrochemical gradient (the driving force for nutrient accumulation by a proton cotransport system) depends on cell metabolism, agents interfering with the generation or maintenance of this gradient should alter secondary active transport processes. Modification of membrane proteins, either those directly involved in mediation of solute transport or those governing membrane integrity can also affect nutrient transport.

Whereas sucrose is the main sugar translocated in the phloem of soybean, and sucrose appears to be the major sugar found in pod vascular bundles during initial distribution and subsequent redistribution of photoassimilate during seed-fill (27), either sucrose itself or its invert products (glucose and fructose) should be transported into the developing cotyledons. Starch is transiently accumulated in the plastids of the cotyledonary mesophyll, disappearing by maturation and seed desiccation as protein and lipid bodies are formed. Besides utilizing carbohydrates as carbon sources for amino acids and lipids, accumulated sugars are a means of osmotic adjustment, providing a driving force for water uptake, turgor maintenance, cellular enlargement, and substrate for cell wall components.

Soybean fruits act as principal sinks for photoassimilate during reproductive growth. A recent investigation describes sucrose uptake by the cotyledons as a necessary step in the maintenance of a source-sink concentration gradient (27).

An understanding of the mechanisms of sucrose movement into the developing fruit also is necessary to evaluate limitations to seed productivity and yield. The final weight of the seed, that is, the yield, is in part determined by its ability to draw nutrients from supply sources in the plant, i.e., by its sink strength. Sink strength, the product of sink size and sink activity, is defined as the net gain in dry weight per unit time per unit dry weight and necessarily includes factors relating to transport and metabolism of the assimilates. In source/sink relationships, an evaluation of the specific conductances at points along the pathway from the region of synthesis to that of accumulation can indicate which site(s) limit the movement of assimilate, and thus, may limit yield.

This type of analysis has been applied to wheat (12, 13) where assimilate movement has been extensively studied. In wheat, distinct reductions in sucrose concentration were noted between the vascular bundle in the grain furrow and the endosperm cells. Such concentration gradients imply that movement of sugar from sites of phloem unloading to the endosperm cavity (across the plasmalemma of the endosperm cell) meets considerable resistance and hence, may be limiting sucrose accumulation. Such a sharp profile in sugar concentration also exists between the liquid endosperm and the cotyledons in developing Phaseolus vulgaris seeds (26). Recent experiments (27) show that in soybeans there is a lag in sucrose appearance in the seeds, suggesting that the integument-embryo interface may be a control point for sucrose accumulation by the cotyledons.

MATERIALS AND METHODS

Preparation of Tissue. Soybeans (Glycine max Merr. cv. Chippewa 64) were grown and harvested at mid pod-fill as described previously (20). Freshly harvested embryos were used in flux measurements and in determinations of free space and intracellular sucrose content.

Flux Measurements. For measurements of sucrose influx, two to five excised embryos were secured in a cheesecloth bag and placed in a preincubation mixture containing (in mmol/liter): 0.5 K$^+$; 1.6 Cl$^-; 0.1$ Ca$^{2+}; 0.1$ Mg$^{2+}; 0.5$ Na$^+$; 1.0 buffer (for pH 5.0, 6.0, Mes; 7.0, Mops; 8.0, Tes; 9.0, Taps$^+$; 10.0 Caps). Inhibitors, when used, were introduced at this time. Preincubation time ranged from 15–45 min, depending on treatment schedule. The

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embryos were then blotted and transferred to uptake medium, containing various concentrations of sucrose or other sugars and uniformly labeled \(^{14}C\) sucrose (sp. activity of 0.1-1.0 Ci/mol; New England Nuclear). Inhibitors were present during the uptake period (generally 30 min) unless otherwise noted. For experiments in which the uptake period lasted longer than 30 min, 1 mg/ml gentamycin and 0.1 mg/ml penicillin were added to the uptake solution. At the end of the uptake period the embryos were transferred to unlabeled sucrose solution of the same concentration for 6 min, the time necessary to remove free space label as estimated from preliminary efflux experiments. The embryos were then placed into 1.0 ml of 80% ethanol and heated to 55°C for 2-24 h. After 30-min exposure of embryos to \(^{14}C\) sucrose at a concentration of 10 mM, greater than 95% of the label present in the embryos was extractable by 80% ethanol. Aliquots of the ethanol extract were counted in 5.0 ml Biofluor scintillation cocktail (New England Nuclear) in a Beckman LS-100C liquid scintillation counter at an efficiency of 75 to 90%. After correction for background and efficiency, results were computed as \(\mu\)mol of sucrose accumulated per gram fresh weight of tissue per hour.

Identification of Transported Sugars. The identity of the transported sugar was confirmed by TLC. Eighty per cent ethanol extracts of cotyledons exposed to \(^{14}C\) sucrose solutions were spotted on silica gel plates (pawed 24 h in 0.5 mM K2HPO4 and activated at 110°C for 1 h) along with glucose, fructose, and sucrose standards. The plates were developed in \(n\)-butanol acetone/water (4:5:1, v/v/v). The plates were then air dried, and standards were visualized with aniline diphenylamine spray (Sigma Chem. Co.) after developing for 10 min at 100°C. The plates were divided perpendicular to solvent movement into four zones, effectively isolating glucose and fructose from sucrose, and the silica gel scraped from the plates. The sugars were extracted in absolute ethanol and counted by liquid scintillation counting as previously described.

Sucrose Content of Free Space and Cotyledons. The sucrose content of the free space and of the cotyledon cells was determined as described previously (20).

RESULTS AND DISCUSSION

Effects of Sucrose and Protons on the Sucrose Uptake Rate. Sucrose uptake by developing soybean cotyledons is linear over a minimum of 25 min at external sucrose concentrations of 10 mM and 400 mM (data not shown). Analysis of 80% ethanolic extracts of cells incubated in radioactive sucrose for 30 min shows that 82 ± 7% of the label is retained in a fraction which cochromatographs with sucrose in the TLC system described in “Materials and Methods.” When cotyledons are exposed to various concentrations of sucrose, the uptake rate shows a biphasic dependence on sucrose concentration (Fig. 1), similar to that seen for uptake of a variety of organic (3, 9, 15) and inorganic nutrients (4, 19) in a range of plant tissues.

As the sucrose concentration is increased from 0 to 50 mM the sucrose uptake mechanism appears to approach saturation (Fig. 1, inset); examination of influx over a larger concentration range reveals that there is a linear dependence of uptake rate versus sucrose concentration at concentrations greater than 100 mM. This uptake in the high concentration range does not appear saturable. An Eadie-Hofstee plot of this data (Fig. 2) emphasizes the biphasic nature of this uptake, showing linear components at high and low sucrose concentrations, separated by a region of continuously varying \(K_m\) and \(V_{max}\) (Fig. 2). This type of pattern has been interpreted in a variety of ways: as a negatively cooperative carrier system (9, 18, 19); as diffusion into the cells or tissue superimposed upon carrier-mediated transport (3, 14); or as a dual mechanism of uptake seated at the plasma membrane (3). Since labeled free space sucrose is exchanged totally with unlabeled sucrose during the rinse period immediately following the uptake period (as calculated from preliminary efflux experiments), it would appear that the nonsaturable phase should not be a characterization of diffusion into the free space of the tissue itself and represents a movement into the cells. That diffusion into the tissue does not limit the influx of sucrose is suggested by the fact that uptake is linear with time and extrapolates to zero. Under conditions of a diffusion limitation there should be a lag in which labeled sucrose equilibrates with unlabeled free space sucrose before transport into the cell.

The apparent \(K_m\), with respect to sucrose, of the uptake process at low external sucrose can be calculated from the slope of the dashed line in Figure 2 at high v/c values. This value obtained through regression analysis is 5 mM, and compares favorably with the \(K_m\) for sucrose uptake in minor veins of Beta vulgaris source leaves (8) and in cotton hypocotyl segments (10).

Proton concentration in the external solution also affects uptake (Fig. 3). The velocity dependence of cotransport processes on external proton concentration has been shown in algae and higher plants, for example in Chlorella (24), B. vulgaris (8), pea and maize root segments (3), oat coleoptiles (6) tomato internodes (29) and barley leaf strips (25). A notable exception is Ricinus cotyledons where there is little influence of pH on uptake (11, 14). The variation of uptake with pH can be predicted by examination of the driving force for proton cotransport systems where decreasing proton concentration decreases the energy available for uptake, although a nonenergetic effect of pH on enzyme activity cannot be ruled out. The lack of a dramatic pH dependence in the soybean cotyledons may be due to the contribution by the linear phase of sucrose uptake which constitutes 30 to 50% of uptake at this concentration and which may be independent of pH over the range of pH 5 to 10. The absence of a larger pH effect may also be due in part to the high buffering capacity of the cell walls and their ability to exchange protons (14). While the uptake by cells immediately adjacent to the external solution (the epidermal cells of soybean cotyledons) would be influenced by the pH of the bulk solution, those deeper within the tissue may not be. This may explain the large pH effect seen in the sucrose-induced depolarizations measured within the first two or three cell layers (20) versus the smaller pH effect on uptake, which occurs throughout the entire tissue (Fig. 3).

Effect of Inhibitors. The influx of labeled sucrose is sensitive to

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** Dependence of sucrose uptake upon external sucrose concentration. External pH was 6.0. Data points between 0 and 50 mM sucrose are not shown except for selected values (see inset). Bars indicate SE for three experiments (n = 5 for each experiment). Inset, sucrose uptake over a concentration range of 0 to 50 mM.
The influx of sucrose into soybean cotyledons was studied to understand its role in photosynthesis and respiration. Inhibition of the uptake process was achieved by adding external 

\[ \text{CCCP} \] in a concentration-dependent manner (Fig. 4). Inhibition is maximal at concentrations of 100 \( \mu \text{M} \) or greater. A similar result was found with the metabolic inhibitor and protonophore \( \text{NaN}_3 \) except that maximal inhibition in the light was achieved at concentrations of 3 mm or greater (data not shown). Both CCCP and \( \text{NaN}_3 \) inhibit influx at 10 mm external sucrose concentration to a level close to or below that predicted for operation of the nonsaturable system alone. The contribution of the linear phase in Figure 4 was calculated from the additional rate of transport superimposed on the carrier-mediated component of uptake at high external sucrose concentrations. By translation of a straight line having the same slope as the line representing uptake in the nonsaturable uptake range (100 to 400 mm sucrose) through the origin, a rate of uptake due to this linear component alone at each sucrose concentration can be predicted. This can be represented by an Eadie-Hofstee transform of sucrose uptake data over the concentration range of 0.1 to 400 mm (Fig. 5) which shows that the portion of the graph representing the saturable system is virtually eliminated. The displacement of the CCCP-treated points toward the origin reflects a decreased maximal velocity with respect to the control at low external substrate concentrations. The displacement of corresponding points (in terms of sucrose concentration) of the treated versus nontreated tissue to an extent greater than predicted from the elimination of the carrier-mediated transport alone suggests, as explained here, that CCCP may have additional effects on sucrose uptake. Both azide and CCCP have been found to inhibit cotransport of amino acids (6) and sugars (8) in higher plants. These agents reduce influx by reduction of \( \Delta \mu_r \), the driving force for cotransport, either by eliminating the pH gradient or depolarizing the membrane potential, or both. In

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**Fig. 2.** Eadie-Hofstee transformation of sucrose influx data. The mean values of results presented in Figures 2 and 3 are replotted as sucrose uptake rate (v) versus uptake rate/sucrose concentration (v/c). The dashed line is used to calculate a \( K_m \) of 8 mm (see text).

**Fig. 3.** Effect of external pH on sucrose influx. Sucrose concentration was 10 mm. Values indicate means ± se of three experiments. The level of sucrose uptake predicted from the operation of the linear component alone is 0.5–0.8 \( \mu \text{mol sucrose/g fresh wt/h} \).

**Fig. 4.** Inhibition of sucrose influx by CCCP. Tissue was pretreated 15 min in CCCP at the indicated concentration; CCCP was also present during the uptake period. External sucrose concentration during uptake was 10 mm at pH 6.0. Hatched area represents the level of uptake predicted from the nonsaturable component alone (see text for details). Values shown are means ± se for at least five determinations. No difference is seen in level of inhibition ± darkness at 100 \( \mu \text{M} \) CCCP. Control uptake rate was 1.63 ± 0.15 \( \mu \text{mol sucrose/fresh wt/h} \) [mean ± se].

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Abbreviations: Caps, 3-(cyclohexylamino)propanesulfonic acid; CCCP, carbonylcyanide-m-chlorophenylhydrazone; DTE, diethioerythritol; Mops, 3-(4-morpholino)propanesulfonic acid; NEM, N-ethylmaleimide; PCMB, \( p \)-chloromercuribenzenesulfonate; Taps, \( N \)-tris(hydroxymethyl)-methyl-3-aminopropane-sulfonic acid.
addition, the acidification of the cell interior by these agents (16) probably increases carrier-mediated efflux by either direct protonation of the carrier protein, or by a more general effect on membrane structure or protein conformation (16).

It should be noted that in soybean cotyledons the concentration of these agents NaN₃ and CCCP, necessary to inhibit sucrose-induced depolarizations (Table I, in ref. 20) and sucrose uptake rate (Fig. 4), differs by a factor of 10. This probably reflects the fact that in measurement of sucrose-induced depolarizations, recordings are usually made from epidermal cells while maximal inhibition of sucrose influx occurs only when the cells of the entire cotyledon are poisoned.

Darkness causes a reduction in sucrose influx (at 10 mm external sucrose and an external pH of 6.0) to 75% of the rate in the light. This represents a decrease of 50% of the saturable rate. Amino acid uptake by barley (21) and pea (2) leaf slices is light stimulated, as is hexose cotransport in <i>Lemma</i> (28). There is no stimulation of uptake by light during treatment with NaN₃ or CCCP.

**Effect of Sulphydryl Reagents.** Sucrose influx is inhibited by the sulphydryl reagents NEM and PCMS (Figs. 6 A and B, respectively). Both inhibit uptake at 10 mm external sucrose concentration to a level predicted from the operation of the linear component alone, most probably through a direct modification of the carrier protein or additionally, in the case of NEM, by a disruption of the generation of ΔγH⁺ (20). As with CCCP-treated tissue, cotyledons inhibited by PCMS show minimal carrier-mediated activity as judged by the shape and displacement of the Eadie-Hofstee plot of influx data (Fig. 7) and also show a reduction in the linear component. This effect, as with CCCP, may result from a general effect on membrane structure and integrity by these agents as well as a specific or selective inhibition. Both PCMS and NEM have been found to be inhibitory in carrier-mediated influx and efflux of nutrients in both <i>Ricinus</i> cotyledons (17) and <i>Beta vulgaris</i> minor veins (7, 9).

PCMS inhibition of sucrose uptake is reversed by the sulphydryl reductant DTE (Fig. 8). DTE (10 mm) alone causes no increase in sucrose uptake rate, and DTE does not reverse the inhibition of sucrose uptake imposed by 10 mm NEM. The results are consistent with NEM forming a covalent alkylation of proteins while PCMS binds reversibly (7).

**Ion Specificity.** Generally, complete removal of Na⁺ or addition of K⁺ over a twenty-fold concentration range had little effect on sucrose uptake from 10 mm sucrose solution at pH 6.0 (Table I). A slight enhancement of sucrose uptake rate was seen upon removal of Na⁺ (judged significant at the 5% level using Student's

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**Fig. 5.** Eadie-Hofstee transformation of sucrose uptake data in the presence and absence of CCCP. CCCP concentration was 100 μm; external pH was 6.0. Sucrose concentration ranged from 0.1 to 400 mm. Values represent the mean of two experiments. The average of the absolute percentage difference between values for uptake for the two experiments was less than 15%.

**Fig. 6.** A, Effect of sulphydryl reagents on sucrose absorption. Tissue was pretreated 15 min in NEM at the concentration shown prior to uptake. External sucrose concentration was 10 mm at pH 6.0. Hatched area represents the level of uptake predicted from operation of the linear component alone (see text for details). Values shown are means ± se for two experiments. The average of the absolute percentage difference between uptake values for the two experiments was less than 10%. Five determinations at each concentration were performed for each experiment. Rate of absorption by the control embryos was 1.41 μmol sucrose/g fresh wt·h⁻¹. NEM was not present during uptake. B, Effect of PCMS on sucrose uptake. Tissue was treated 15 min in PCMS at the concentrations indicated, then washed 15 min in buffer prior to uptake. Sucrose concentration during uptake was 10 mm; external pH was 6.0. Hatched area represents the level of uptake predicted from operation of the nonsaturable component alone (see text for details). Results are presented as means ± se (n = 5). Different symbols indicate separate experiments.

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**Table I.** Effect of sulphydryl reagents on sucrose uptake. Values shown are means ± se for two experiments. The average of the absolute percentage difference between uptake values for the two experiments was less than 10%. Five determinations at each concentration were performed for each experiment. Rate of absorption by the control embryos was 1.41 μmol sucrose/g fresh wt·h⁻¹. NEM was not present during uptake. B, Effect of PCMS on sucrose uptake. Tissue was treated 15 min in PCMS at the concentrations indicated, then washed 15 min in buffer prior to uptake. Sucrose concentration during uptake was 10 mm; external pH was 6.0. Hatched area represents the level of uptake predicted from operation of the nonsaturable component alone (see text for details). Results are presented as means ± se (n = 5). Different symbols indicate separate experiments.
The movement of soybean that cavity endosperm sperm cells, which is cissed without giving mm (12, 13) further substantiates the contention that transport may be a direct or primary role in sucrose uptake. Thus, as with sucrose-induced depolarizations (20), the identity of the cotransported ion appears to be the proton since the only clear dependence of transport rests with external hydrogen ion concentration (Fig. 5; Table I).

Effect of Hexose on Sucrose Uptake. Glucose, over a concentration range of 5–25 mM, had no effect on sucrose uptake at external sucrose concentrations of 0.5–10.0 mM (Table II). This further substantiates the contention that sucrose uptake can proceed without prior inversion in legumes (23, 27).

Relationship of in Situ Conditions to Measurements with Excised Seeds. In freshly harvested soybean cotyledons, assuming a uniform cellular distribution free space sucrose concentration is estimated at 37 mM and intracellular sucrose concentration at 7 mM giving an in vivo accumulation ratio of 2. Jenner and Ratjen (12, 13) has suggested, on the basis of distinct drops in concentration across the interface between the vascular tissue and the endosperm cavity and from the endosperm free space into endosperm cells of wheat, that transport may be a limiting process during grain fill. In an analogous manner the plasma membrane of soybean cotyledonary cells is suggested to be a barrier to free movement of sucrose into sites of assimilation and storage. It follows that plasma membrane transport may be a limiting process in the accumulation of sucrose in developing soybean cotyledons.

Table I. Effect of Sodium and Potassium on Sucrose Uptake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sucrese Uptake Rate (μmol/g h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 mM Na⁺/0.5 mM K⁺</td>
<td>1.81 ± 0.12 a</td>
</tr>
<tr>
<td>Na⁺-free/0.5 mM K⁺</td>
<td>2.08 ± 0.08 b</td>
</tr>
<tr>
<td>1.0 mM Na⁺/K⁺-free</td>
<td>1.87 ± 0.09 a</td>
</tr>
<tr>
<td>1.0 mM Na⁺/5.0 mM K⁺</td>
<td>1.89 ± 0.07 a</td>
</tr>
<tr>
<td>1.0 mM Na⁺/10.0 mM K⁺</td>
<td>1.87 ± 0.06 a</td>
</tr>
</tbody>
</table>

Table II. Effect of Glucose on Sucrose Uptake Rates

<table>
<thead>
<tr>
<th>Glucose Concentration</th>
<th>Sucrese Uptake Rate (μmol/g fresh wt h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>10</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td>25</td>
<td>1.26 ± 0.10</td>
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</table>

CONCLUSIONS

The uptake of sucrose into developing soybean cotyledons requires metabolic energy as it is inhibited by NaN₃, CCCP, and partially by darkness. Transport is nonsaturable with respect to sucrose concentration; appropriate kinetic transformations reveal that uptake consists of a carrier-mediated component operating at low substrate concentrations and a component showing a linear concentration dependence at high substrate concentrations. Sucrose transport shows a limited dependence on external proton concentration and is decreased at high pH. Sulphhydril-reactive
protein modifiers decrease sucrose transport to a level predicted by the operation of the nonsaturating process alone. Sucrose uptake shows no dependence on external alkali cations and is unaffected by glucose concentrations up to 25 mM. A summary of data from this paper and a previous publication (20) is presented in Table III. These results are consistent with the operation of a proton/sucrose cotransport system. Although the magnitude of $\Delta_{H^+}$ should allow a sucrose accumulation of over a thousand-fold from dilute external sucrose concentrations, the sucrose accumulation ratio in vivo remained less than 1.0 suggesting that free sucrose is transformed rapidly within the cotyledon. According to the analysis of Jenner and Rathjen (12, 13), sucrose transport into developing soybeans may be a rate-limiting step for sucrose assimilation.

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LITERATURE CITED

22. Rottenberg H 1976 The driving force for proton(s) metabolites cotransport in bacterial cells. FEBS Lett 66: 159-163
29. Van Bel AJE, HP Hermans 1977 pH dependency of the uptake of glutamine, alanine and glutamic acid in tomato internodes. Z Pflanzenphysiol 84: 413-418