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Phloem Loading of Sucrose

pH DEPENDENCE AND SELECTIVITY[1]

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ABSTRACT

Autoradiographic, plasmolysis, and ^{14}C-metabolite distribution studies indicate that the majority of exogenously supplied ^{14}C-sucrose enters the phloem directly from the apoplast in source leaf discs of *Beta vulgaris*. Phloem loading of sucrose is pH-dependent, being markedly inhibited at an apoplast pH of 8 compared to pH 5. Kinetic analyses indicate that the apparent *Km* of the loading process increases at the alkaline pH while the maximum velocity, V_{max}, is pH-independent. The pH dependence of sucrose loading into source leaf discs translates to phloem loading in and translocation of sucrose from intact source leaves. Studies using asymmetrically labeled sucrose ^{14}C-fructosyl-sucrose, show that sucrose is accumulated intact from the apoplast and not hydrolyzed to its hexose moieties by invertase prior to uptake. The results are discussed in terms of sucrose loading being coupled to the co-transport of protons (and membrane potential) in a manner consistent with the chemiosmotic hypothesis of nonelectrolyte transport.

Phloem loading describes the processes attendant with sugar movement between the sites of photosynthetic assimilation in the mesophyll and the sites and mechanism(s) involved in the selective accumulation of sugars into the phloem prior to translocation (12). Studies by Geiger and co-workers (13) have shown that sugars are not transported along a diffusion gradient between the mesophyll cells and the phloem, but instead a distinct concentration gradient of solutes exits at the mesophyll-phloem interface. Evidence has been presented that sugars are accumulated into the phloem from the free space prior to translocation (14) and that the uptake resembled an enzyme-mediated process (33). Giaquinta (15) employed chemical probes to characterize phloem loading at the membrane level further. That study showed that sugars are accumulated into the phloem from the apoplast by an energy-dependent process which was associated with membrane-bound sulfhydryl groups.

The mechanism of energization of the metabolism-dependent transport of sugar into the phloem, whether it be by direct utilization of ATP (33) or by some other process, is not known. Recently, it was suggested (15) that the characteristics of the phloem cells were favorable for sugar uptake to be coupled to the co-transport of protons into the phloem. Chemical composition studies have documented the relatively low proton concentration of the phloem (about pH 8-8.5) along with a high potassium ion and sucrose concentration (5). Assuming the xylem or apoplast solution to be approximately pH 5.5 (that is, high proton concentration), conditions exist whereby the electrochemical potential gradient of protons established across the phloem membranes could be coupled to sugar influx. This hypothesis is attractive for phloem loading not only because of the existing prerequisites but also because nonelectrolyte transport (that is, sugars and uncharged amino acids) coupled to the co-transport of ions is well documented in a variety of organisms and may be a universal feature of nonelectrolyte membrane transport (8, 9 21-23, 29, 30-32, 34).

Little, if any, information is available on the process of phloem loading at the membrane level. Information is needed on the sugar selectivity of the loading process, the mechanism(s) which supplies the driving force for loading, and the regulation of the loading process. This study attempts to characterize phloem loading of sucrose in terms of the energization of the carrier mechanism involved in sucrose transport. Evidence is presented that is consistent with the selective and energy-dependent accumulation of sucrose into the phloem being coupled to proton fluxes in a manner consistent with the chemiosmotic theory of nonelectrolyte transport.

MATERIALS AND METHODS

Beta vulgaris L. (monohybrid A-1) were grown in a controlled environment under conditions described previously (15). Accumulation of ^{14}C-sugar was measured in 0.385-cm^2 discs obtained from source leaves of 5- to 6-week-old plants. Previous studies have shown that similar leaves constitute an exporting source of assimilates (10, 16). Discs were taken from the upper one-third of the leaf lamina in order to insure that the samples always reflected true source characteristics (10). Prior to collecting the discs, an area of selected source leaf was gently abraded with carborundum 300 to enhance solute entry through the cuticle (14, 15). After a brief washing in 1 mM $CaCl_2$, discs in triplicate were generally incubated on ^{14}C-sugar for 30 min with the specific treatment conditions being given in the figure and table legends. Discs were then exodiffused in three changes of 1 mM $CaCl_2$ for 5 to 10 min each which removed greater than 95% of the free space label. Leaf discs were transferred to liquid scintillation vials (1 disc/vial) containing 0.1 ml of 70% (v/v) perchloric acid plus 0.1 ml of 30% (v/v) H_2O_2, tightly capped, and heated in a water bath at 60 C for 20 to 30 min with occasional agitation. This procedure completely digested and decolorized the leaf material with no loss of ^{14}C label. The radioactivity (dpm) was determined by liquid scintillation spectroscopy using Handifluor cocktail (Mallinckrodt) with a resulting counting efficiency of 82 to 84%.

Steady-state translocation using exogenous ^{14}C-sucrose (14) and autoradiography of leaf tissue exposed to ^{14}C-sugar (10, 17) were as described earlier.

For the metabolite distribution studies, leaf discs were incubated on ^{14}C-sugar for 30 min, washed of free space label, and extracted in 80% (v/v) ethanol for 6 hr in a Soxhlet apparatus.

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The alcohol solubles were further fractionated by ion exchange column chromatography into basic (amino acids), acidic (organic acids and sugar phosphates), and neutral (sugars) fractions (1). Sugars in the neutral fraction were separated by one-dimensional descending paper chromatography using butanol-acetic acid-water (3:3:2, v/v). Sugars were localized and identified with Sigma aniline diphenylamine reagent and appropriate standards. The visualized spots were isolated, combusted to $^{14}CO_2$ by an Intertechnique Oxymat, and the $^{14}CO_2$ recovered in a phenethylamine-toluene liquid scintillation cocktail (15). In some cases, the sucrose region was eluted from unsprayed chromatograms, enzymically hydrolyzed to the hexoses by incubation with invertase (10–20 units/μmol sugar) at 50 C for 2 hr at pH 5, rechromatographed, and the distribution of ^{14}C between the hexoses determined.

RESULTS AND DISCUSSION

Since phloem translocation is an integrated whole plant process, it is important to recognize that the study of isolated or detached portions of the translocation system can introduce possible experimental artifacts. If an understanding of the basic processes associated with phloem loading is to be gained, the translocation system has to be simplified. There is much precedence in the literature for the use of detached or isolated portions of the plant in translocation studies (5). With the exception of a few studies (15, 33), little information is available on the ability of the detached portions to translate to the whole plant process of translocation. Since this study uses leaf discs obtained from source leaves to study phloem loading, it was of importance to determine how well sugar uptake into the discs reflects sugar accumulation into the phloem in relation to translocation. Three approaches were employed to study the suitability of using source leaf discs to monitor the phloem loading process. First, using autoradiography, the cellular localization of ^{14}C-sucrose in leaf discs was compared to that found in intact source leaves (12, 14). Second, using plasmolysis techniques, the extent of nonphloem sugar uptake could be determined and compared to comparable data obtained on the intact plant. Third, differences in mesophyll *versus* phloem accumulation of sugars could also be studied by determining the subsequent metabolism of the accumulated sugar.

The recent experiments of Geiger *et al.* (12, 14) using intact sugar beet plants have shown by autoradiography that most, if not all, of the sucrose presented to intact source leaves enters the phloem directly from the apoplast. The rates of translocation using exogenous sucrose supplied through the apoplast equaled the rates obtained for assimilates derived from $^{14}CO_2$. Additionally, source leaves in which the mesophyll cells were plasmolyzed (0.8 M osmoticum) were capable of translocating at rates equal to nonplasmolyzed plants when exogenous ^{14}C-sucrose was employed (14). The following experiments are designed to determine whether leaf discs of sugar beet source leaves behave similarly to intact source leaves in these respects. Figure 1A shows a tissue autoradiograph of source leaf disc tissue exposed to 1 mM ^{14}C-sucrose for 30 min. The radioactivity is localized mainly in the minor vein network of the phloem, with the mesophyll areas showing little radioactivity. This indicates that like intact leaves (12, 14), most of the sucrose is entering the minor veins directly from the apoplast. That the sucrose is not accumulated by the mesophyll cells and then transferred to the phloem via the symplasm is indicated by Figure 1B. Since mesophyll cells do not accumulate appreciable sucrose compared to glucose (4), any sucrose entering the mesophyll would presumably be first hydrolyzed to the hexoses with the hexoses then being accumulated. Figure 1B shows an autoradiograph of source leaf disc tissue incubated on 1 mM glucose for 30 min. In contrast to sucrose accumulation, the ^{14}C label is more uniformly distributed throughout the tissue, indicating substantial mesophyll accumulation. The distinctly different labeling pattern observed between sucrose and glucose uptake (Fig. 1, A and B) indicates that sucrose is entering the phloem directly from the apoplast and not via the symplasm and that glucose *per se* is not loaded into phloem. The slight labeling of the minor veins after glucose uptake probably arises from sucrose synthesized from glucose (see below) in the mesophyll and subsequently transferred to the phloem via the apoplast.

Plasmolysis followed by freeze substitution and electron microscopy have revealed that the various cell types in sugar beet source leaves respond differently to the osmotic potential of the bathing medium. It has been shown (13) that mesophyll and vascular parenchyma cells were at incipient plasmolysis in a 0.5 M mannitol solution and were severely plasmolyzed at 0.9 M osmoticum. In contrast, the phloem tissue (sieve tubes and transfer cells), due to their high solute content, showed no signs of plasmolysis at these high osmotic concentrations (0.95 M). If substantial uptake into the mesophyll cells was occurring during sucrose accumulation into the discs, plasmolyzing the tissue in 0.9 M osmoticum would decrease their contribution (13, 14) and indicate the relative contribution of nonphloem cells to the total accumulation. Source leaf discs which were first equilibrated in 0.30 or 0.95 M sorbitol prior to and during ^{14}C-sucrose accumulation showed similar rates of sucrose uptake over a range of sucrose concentrations of 1 to 100 mM (data not shown). The rate of uptake actually increased in the presence of osmoticum. Under conditions where the mesophyll but not the phloem is severely plasmolyzed (ascertained by light microscopy and a 35% reduction in leaf area), there was no inhibition of ^{14}C-sucrose accumulation into the discs and autoradiographs of plasmolyzed tissue showed a pattern similar to Figure 1A (data not shown). The data presented in Table I further support the contention that sucrose uptake into leaf discs is measuring sucrose uptake into the minor veins of the phloem. The distribution of ^{14}C label in the sugar fraction following a 30-min uptake of ^{14}C-sucrose or ^{14}C-glucose is shown in Table I. In source leaf discs supplied with ^{14}C-sucrose, 76% of the label was in the neutral fraction, 85% of which was in sucrose. The basic (amino acids) and acidic (sugar phosphates and organic acids) fractions contained 11 and 13% of the label, respectively. Discs incubated in ^{14}C-glucose, showed only 37% of the label in the neutral fraction (64% in sucrose), with 26 and 37 in the basic and acidic fractions, respectively. Also, the tissue incubated in ^{14}C-glucose had a greater amount of the label (22%) in the insoluble fraction compared to the ^{14}C-sucrose-treated tissue (8%). This increased metabolism of glucose into the various metabolic pools is most

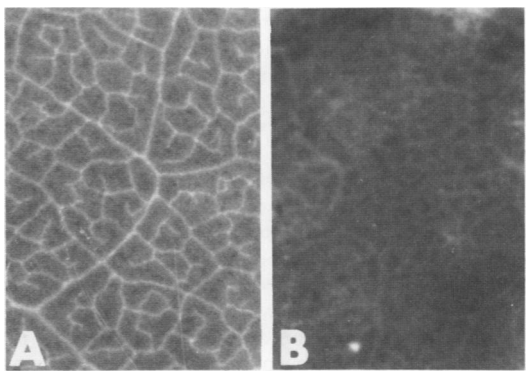

FIG. 1. Autoradiography of source leaf discs after accumulation of ^{14}C-sucrose and ^{14}C-glucose. Tissues were incubated on either 1 mM (A) sucrose or (B) ^{14}C-glucose for 30 min. After removal of free space label, tissues were rapidly frozen and lyophilized according to reference 10. White area denotes ^{14}C localization. Area of disc is 0.4 cm.

Table I. Metabolite Distribution of ^{14}C after Accumulation of Exogenously Supplied ^{14}C-Sucrose and ^{14}C-Glucose.

Source leaf discs were incubated on 2 mM ^{14}C-sugar (15 Ci/mole) for 30 min. Solvent extraction, ion exchange column and paper chromatography were as described in 'Materials and Methods' and Ref. (1).

Fraction	Sugar Supplied ^{14}C-Sucrose	^{14}C-Glucose
	% Distribution	
Insolubles	8	22
Lipid	2	2
Water Solubles	90	76
Basic[1]	11	37
Acidic	13	26
Neutral	76	37
Sucrose[2]	85	64
Glucose	10	22
Fructose	5	14
Total Radioactivity Incorporated dpm x 10^{-6}	3.04	2.96

[1] % ^{14}C in water-soluble extract

[2] % ^{14}C in neutral fraction

likely responsible for the uniform pattern of ^{14}C (mesophyll uptake) in the autoradiograph shown in Figure 1B. The above data for source leaf discs using autoradiographic, plasmolysis, and metabolite distribution techniques show that sucrose uptake into leaf discs reflects mainly sucrose loading into the phloem from the apoplast and closely resembles the intact plant data for these parameters (12). The more simplified and experimentally controlled source leaf discs can be used with reasonable confidence in the study of phloem loading.

Several lines of evidence have indicated that phloem loading is both selective and dependent on metabolism (11, 14).

As mentioned in the introductory section, virtually nothing is known about phloem loading at the membrane level other than that sulfhydryl groups are involved in the transport process (15). The following experiments attempt to characterize the loading process in terms of the feasibility of the hypothesis that sucrose uptake into the minor veins of the phloem is coupled to proton transport. If a proton gradient of approximately 3 pH units exists between the apoplast and phloem as the data indicate (see introductory section), and if this proton gradient is coupled to sucrose uptake, then reducing the magnitude of the pH gradient across the membrane would be expected to decrease the rate of sucrose loading into the phloem. By using various nonpermeant zwitterionic buffers (19), it is possible to change the proton concentration of the free space relative to the metabolic space of the phloem. Figure 2 shows the pH dependence of sucrose accumulation into source leaf discs from a 1 mM ^{14}C-sucrose solution containing nonpermeant buffers of the appropriate pK_a. The sucrose accumulation rate displays a pH optimum of approximately 5 to 5.5, with increases in pH of the apoplast causing a pronounced decrease in the rate of uptake, being 65% inhibited at pH 8 to 8.5, the approximate pH of the phloem. In order to relate sucrose uptake with proton involvement, it was important to establish that the inhibition of sucrose uptake by increasing pH (low proton concentration) was due to proton modulation of the sucrose carrier at the phloem membranes rather than from pH-induced secondary effects such as buffer effects, loss of membrane integrity, or inhibition of invertase activity. The pH 8-induced inhibition of sucrose accumulation (from 1 mM sucrose solution) was not dependent on the buffer used. The extent of inhibition at pH 8 compared to pH 5 was essentially independent of the buffer type. There was no difference between the inhibition caused by the ionic phosphate buffer at pH 8 compared to zwitterionic buffers (Tricine, TES, HEPES) at a similar pH. Neither did the chelating buffers (Tricine and phosphate) differ from nonmetal-binding buffers (HEPES, TES, tris). Tris buffer, a potent inhibitor of acid invertase activity (5, 24), inhibited sucrose accumulation to the same extent as the other buffers at pH 8, suggesting that hydrolysis of sucrose was not a prerequisite for active loading (see below). These results suggest that the difference in proton concentration is the major factor in inhibiting the sucrose accumulation rate and not buffer characteristics. This seemed important to establish since it has recently been shown that stimulatory effects of ATP on ion transport may be related to its chelating ability rather than to its energy-yielding property (26).

Since increasing pH can increase membrane permeability (6), the efflux of ^{14}C from leaf tissue preloaded with ^{14}C-sucrose was measured as a function of pH. pH 8 conditions, using a variety of buffer types, increased the amount of ^{14}C label that effluxed into the external media (5% efflux at pH 5 compared to near 10% at pH 8). The presence of 5 mM $CaCl_2$ in the pH 8 medium completely prevented the alkaline-induced efflux. If the apparent pH 8 inhibition of sucrose uptake was due to an increase in membrane leakiness to sucrose (greater than 90% of the effluxing label was in sucrose), then addition of $CaCl_2$ would prevent the efflux and negate the apparent inhibition. Calcium chloride (5 mM) had no effect on the pH 8-induced inhibition of the sucrose accumulation rate (from a 1 mM sucrose solution), indicating that the inhibition was not due to an increase in sucrose efflux. The magnitude of the difference in the amount effluxed and amount of inhibition in the uptake rate also argues against this possibility.

The extent of inhibition of sucrose accumulation at pH 8 is dependent on the sucrose concentration (Fig. 3). At low sucrose concentrations (0.1–5 mM), there is greater than a 50% inhibition in the rate of uptake at pH 8 compared to pH 5. The extent of inhibition progressively decreases with increasing sucrose concentration until at 100 mM the rates at pH 5 and 8 are approximately equal, 43 and 40 μmol/hr·dm^2, respectively. In some experiments, the rate of uptake at pH 8 slightly exceeded that at pH 5 at 100 mM sucrose. A comparison of the kinetic parameters (Fig. 4) at the two pH values revealed that the apparent affinity, K*m*, of the carrier for sucrose increases at pH 5 (high protons), whereas the maximum velocity, V_{max}, is essentially pH-independent. That sucrose uptake is inhibited to the same extent over the entire sucrose concentration (0.1–100 mM) by dinitrophenol, NEM[2] and low temperature (Fig. 5) eliminates the possibility of a nonmetabolic component of uptake (such as diffusion) intervening at the higher sucrose concentrations resulting in a similar V_{max} at pH 5 and 8. That pH 5 and pH 8 conditions have similar V_{max} indicates that we are not dealing with a simple pH optimum or denaturation of the uptake process; if this were the case, a different V_{max} would be expected. The results can be interpreted as indicating that the carrier for sucrose at the phloem membrane surface binds sequentially with the uncharged sucrose molecule and protons (H^+), the charged ternary complex (carrier-sucrose-H^+) can then be driven across the membrane by the electrochemical potential gradient of protons (proton motive force). The carrier complex presumably dissociates at the inner membrane surface and returns as the uncomplexed carrier. In a variety of nonanimal cells (bacteria, fungi, and algae), the electrical field component (membrane potential, interior negative) of the electrochemical potential seems to be of prime importance in driving this cycle (30). When one of the co-substrates is limiting, that is protons under pH 8 conditions, the carrier is in the unprotonated form with a lower affinity for sucrose (K*m* of 57 mM, Fig. 4); increasing the concentration of the second substrate, sucrose, can overcome the decrease in affinity at alkaline pH and result in a similar V_{max}. Increasing the proton concentration of the apoplast, re-

[2] Abbreviations: NEM: N-ethylmaleimide; DNP: 2,4-dinitrophenol; PCMB: *p*-chloromercuribenzoate; PCMBS: *p*-chloromercuribenzenesulfonic acid; CHES: cyclohexylaminoethane sulfonic acid.

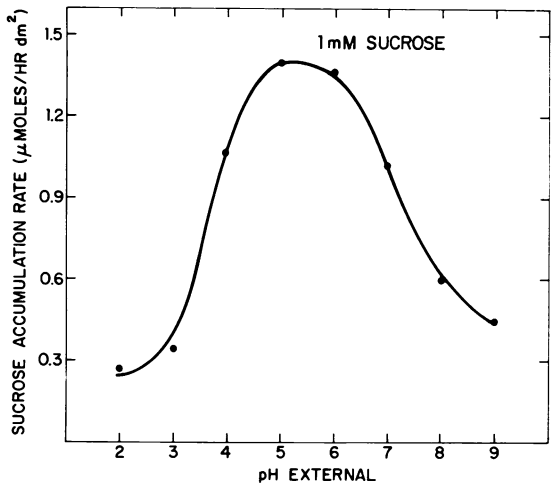

FIG. 2. pH dependence of sucrose accumulation into source leaf tissue. Discs (in triplicate) were preincubated in 1 ml of 50 mM buffer at the desired pH for 20 min. The discs were transferred to 1 mM ^{14}C-sucrose (1 Ci/mol) containing the appropriate buffer at 50 mM for 30 min. Citrate-phosphate buffer was used at pH 2 to 4; MES at pH 5 and 6; and MOPS, Tricine and CHES at pH 7, 8, and 9, respectively.

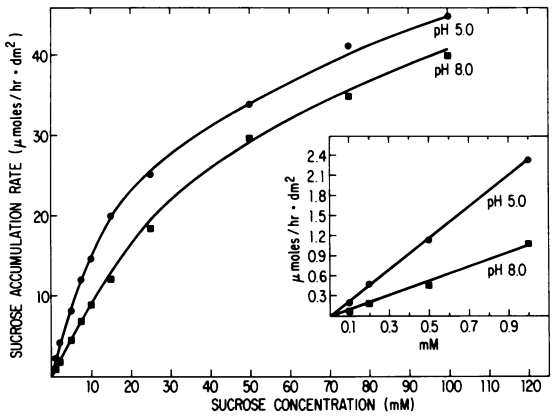

FIG. 3. Concentration dependence of sucrose accumulation at pH 5 and 8. Source leaf discs were incubated for 30 min on various sucrose concentrations containing 1 mM $CaCl_2$ and 20 mM of either MES (pH 5) or Tricine (pH 8) buffer. Sorbitol (0.8 M) was the osmoticum.

sults in protonation of the carrier with a resulting higher affinity for sucrose (*Km* of about 25 mM, Fig. 4). The *Km* of 25 mM at pH 5 agrees reasonably well with the *Km* of translocation from whole leaves of sugar beet (33) and from sucrose uptake into minor veins of tobacco leaves (4) (see figure legend).

Using the analysis of Komor and Tanner (21, 22), it is possible to estimate the relative proportion of the high affinity (protonated) carrier. This is shown in Fig. 6 where at low sucrose concentrations (1 mM), when the proton concentration is plotted as a function of the velocity of the sucrose accumulation rate, a Michaelis-Menten response is obtained with a *Km* for protons of 0.01 μM. That is, the carrier is half-maximally protonated near pH 8.

The type of mechanism proposed above for phloem loading whereby neutral molecules are co-transported with protons or ions is well documented in numerous studies in a variety of organisms (see introductory section). In particular, the studies by Komor and Tanner (21–23) using the alga, *Chlorella*, have shown that hexose transport is stoichiometrically coupled with the co-transport of protons. A similar pH profile for hexose uptake in *Chlorella* was noted as found here as well as pH-dependent changes in the *Km* values for hexose accumulation. Similarly, Slayman and Slayman (32) demonstrated a plasmalemma depolarization during the active transport of glucose into *Neurospora* which was related to a membrane potential-driven co-transport of sugar and hydrogen ions. All of the above models are based on Mitchell's chemiosmotic theory of nonelectrolyte transport (symport) (27).

The pH dependence of sugar uptake into the phloem appears to be relatively selective for sucrose. Glucose uptake into leaf discs of source leaf tissue (mainly into the mesophyll; Fig. 1B and Table I) shows only a marginal inhibition at pH 8 (20%) compared to sucrose accumulation (mainly phloem) which shows a 56% inhibition at pH 8, further indicating that these events

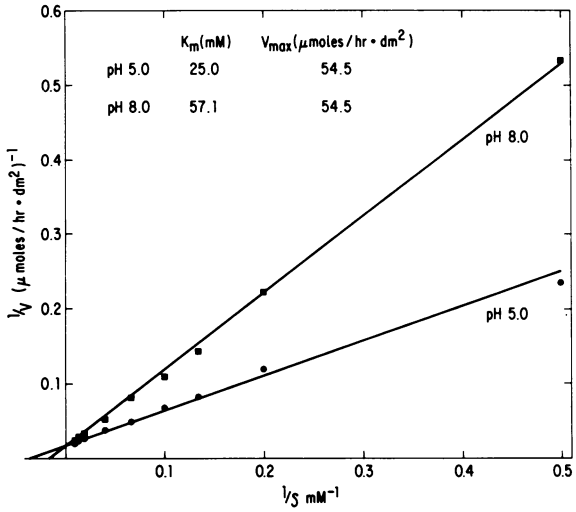

FIG. 4. Lineweaver-Burk analysis of sucrose accumulation rate at pH 5 and 8. Data from Figure 3. Three experiments gave *Km* and V_{max} values of 15 to 25 mM and 30 to 55 μmol/hr·dm², respectively, at pH 5. The *Km* values agree with the *Km* of 16 mM reported for translocation of sucrose from sugarbeet leaves (33) and sucrose uptake into isolated vein nets (4). The V_{max} values reported here agree with those for sucrose translocation in sugarbeet, 30 μmol/hr·dm² (33).

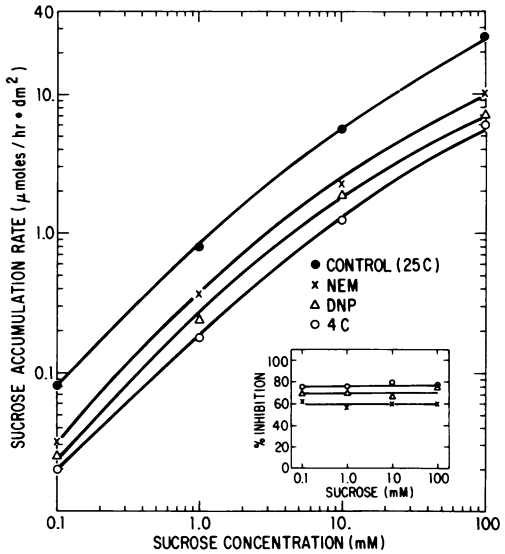

FIG. 5. Inhibition of sucrose accumulation into source leaf tissue by dinitrophenol, N-ethylmaleimide, and low temperature. Discs were incubated on ^{14}C-sucrose (1 Ci/mol) 5 mM KPO_4 (pH 5.5) for 30 min. Concentrations of DNP and NEM were 2 and 1 mM, respectively. DNP at 5 mM resulted in a 95% inhibition of sucrose uptake, indicating that the entire process is metabolic.

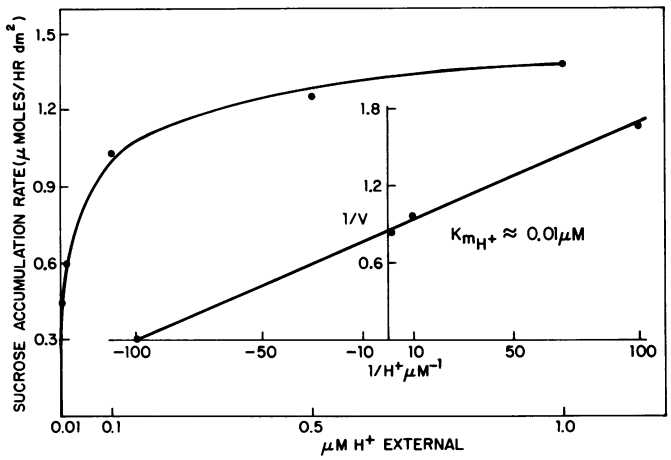

FIG. 6. Proton concentration dependence of sucrose accumulation rate into source leaf tissue. Details as in Figure 2.

characterize the phloem loading of sucrose. The 20% inhibition of glucose uptake may represent inhibition of uptake of sucrose into the phloem which was synthesized from glucose (Table I and Fig. 1B).

Since the electrochemical potential gradient (or membrane electrical potential) has to be maintained for the steady-state influx of sucrose into the phloem, the maintenance of the electrochemical proton gradient (that is, high protons outside the phloem) could be coupled to an ATP-driven electrogenic extrusion system. It has been documented by ultrastructural cytochemistry (18) that the plasma membranes of transfer cells and sieve elements of the phloem have substantial ATPase activity compared to mesophyll cells. If this membrane-associated ATPase complex operates as many do (20), then the hydrolysis of ATP by this enzyme could result in the vectorial displacement of protons exterior to the phloem with a concomitant release of hydroxyl ions within the phloem, thus maintaining the charge separation. Perhaps the known ATP stimulation of translocation (7, 14) can be attributed to hydrolysis of the exogenous ATP via the phloem membrane ATPase thus enhancing the electrochemical potential of H^+ across the phloem membrane. In this regard, Slayman *et al.* (31) have shown a positive correlation between cellular ATP concentration and an electrogenic ion pump in the plasma membrane of *Neurospora*. Further indication that a membrane ATPase may be involved in phloem loading is the cytochemical demonstration that ATPase activity increased during maturation of phloem cells in *Nicotiana tabacum* (18). It would be of interest to determine whether this increased enzyme activity of the phloem temporally coincides with the import-to-export transition of a young leaf (10) or to the appearance of cell wall invaginations (*i.e.* increase membrane surface area) in the transfer cells of certain species during development. Of importance is the observation that PCMB, a sulfhydryl-specific reagent, markedly inhibited ATPase activity of the phloem membranes (18). It is likely that the inhibition of phloem loading and translocation by the sulfhydryl group modifier, PCMBS (15), is due to inhibition of this phloem membrane-located at ATPase.

Komor and Tanner (22) demonstrated that protons are cotransported with hexoses in *Chlorella*. Additionally, they demonstrated that high concentrations of potassium chloride (200 mM) markedly reduced the rate of hexose uptake into the alga. These results, based on the electrical potential measurements of Barber (2), were interpreted as indicating that external high concentrations of positively charged ions created a diffusion potential, interior positive, reversing the original interior negative potential. In this regard, it is noteworthy that 100 to 500 mM KCl (under conditions where the osmotic potential of the control solutions is adjusted with sorbitol) inhibit sucrose uptake (from a 1 mM sucrose solution) into source leaf tissue by 40% (Giaquinta, unpublished data). These data indicate that both the presence of protons (increased affinity of the carrier to sucrose and the pH gradient) and the membrane potential (driving force) are necessary for sucrose loading into the phloem.

The pH dependence of sucrose uptake is not limited to the source leaf disc system. When two adjacent areas of an abraded sugar beet source leaf on an intact plant are treated with 0.5 mM ^{14}C-sucrose at either pH 5 (MES buffer) or pH 8.5 (CHES buffer) for 30 min, the discs cut from this area showed a sucrose accumulation rate of 0.45 and 0.21 μmol/hr·dm^2 at pH 5 and 8.5, respectively. The pH dependence of phloem loading into source leaf disc is also reflected in the translocation rate from the intact sugar beet plant. Using plants trimmed to a simplified source-path-sink system, 5 mM ^{14}C-sucrose at constant specific activity (1 Ci/mol) was fed to an abraded source leaf while monitoring the ^{14}C arrival rate in a developing sink with a thin window GM tube-rate meter-recorder assembly (14). After a steady-state rate of translocation was established at pH 5, the buffered sucrose solution was replaced with 5 mM sucrose at pH 8 of the same specific radioactivity. Within approximately 60 to 90 min, the translocation rate of sucrose decreased nearly 40% from 19.2 (at pH 5) to 12 nmol/hr·dm^2 at pH 8. These experiments show that the pH effects characterized on the leaf discs translate to phloem loading and subsequent translocation in the intact plant.

More insight into the selectivity of the carrier involved in the loading process can be attained by studying the uptake of asymmetrically labeled sucrose (^{14}C-fructosyl-sucrose). The Russian workers (3, 25) maintain that sucrose is hydrolyzed by a free space acid invertase to glucose and fructose which are then actively accumulated into the minor veins where they are resynthesized to sucrose. The major criticism of their work is that the observed invertase activity may be a result of the physical dissection of the phloem tissue and the long washing process (about 24 h) prior to the experiment (12). Also, depending on the developmental stage of the leaf used, there exist various source and sink zones within the same leaf lamina (10). It is not known whether their material contained sink tissue which could contribute to the invertase activity. The use of asymmetrically labeled sucrose to study the question of sucrose hydrolysis is well documented in plant tissues (24, 28). The rationale is that if the asymmetrically labeled sucrose is enzymically hydrolyzed by a free space invertase and then resynthesized intracellularly, the resynthesis process would, because of isomerase activity, introduce randomization of the radiocarbon among the hexose moieties. Retention of the ^{14}C label in the fructosyl moiety of sucrose is indicative of sucrose entering the cell intact. Table II shows that approximately 95% of the label was retained in the fructose position of sucrose (G/F = 0.05) when ^{14}C-fructosyl-sucrose was accumulated. As a control, when uniformly labeled sucrose was

Table II. Glucose/Fructose Ratio of Sucrose Accumulated from Various ^{14}C-Sugars.

Sugars were supplied to source leaf discs at 1 mM (15 Ci/mole) for 30 min. Tissue was extracted and fractionated by ion exchange chromatography according to methods given in the 'Materials and Methods' Section. The G/F ratios of the stock ^{14}C(U) sucrose and ^{14}C(F) sucrose were <1.01 and 0.01, respectively.

Sugar Supplied	G/F Ratio of Accumulated Sucrose
^{14}C(U) Sucrose	1.05
^{14}C(fructosyl) Sucrose	0.05
^{14}C Glucose	0.99

accumulated into source leaf tissue, the expected G/F ratio of unity was observed. When ^{14}C-glucose is accumulated by source leaf discs, the resulting sucrose is equally labeled in both hexose moieties, indicating active isomerase activity with subsequent synthesis to sucrose. These results indicate that sucrose *per se* is selectively accumulated into the phloem and is not hydrolyzed prior to phloem loading.

The observation that there is little invertase activity involved with phloem loading in source leaf tissue (Table II) further argues that the pH effects are not due to inhibition of invertase activity. The data indicate that sucrose is loaded as such by a sucrose-specific carrier.

From the data presented here and elsewhere (12, 15), it seems that photosynthetically derived sucrose enters the apoplast (presumably at the phloem region), where it is selectively accumulated into the phloem as such by an energy-dependent process involving membrane sulfhydryl groups. The accumulation of sucrose is possibly coupled to the co-transport of protons where the electrochemical potential of protons (the membrane potential) generated by a membrane-bound ATPase drives sucrose transport into the phloem.

Although the above data are consistent with this hypothesis, further confirmation is needed in terms of microelectrode measurements of the electrical potential of the transfer cells and sieve elements as well as measurements of proton fluxes in the regions between phloem membranes and their cell walls. Nevertheless, the characteristics of the phloem and the precedence of such a mechanism operating in other organisms make this an attractive as well as feasible hypothesis for phloem loading.

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LITERATURE CITED

1. Atkins CA, DT Canvin 1971 Photosynthesis and CO_2 evolution by leaf discs: gas exchange, extraction, and ion exchange fractionation of ^{14}C-labeled photosynthetic products. Can J Bot 49: 1225–1234
2. Barber J 1968 Measurement of the membrane potential and evidence for active transport of ions in *Chlorella pyrenoidosa*. Biochim Biophys Acta 150: 618–625
3. Brovchenko MI 1967 Some proofs of the splitting of sucrose during its movement from mesophyll into the fine bundles of sugar beet leaves. Sov Plant Physiol 14: 415–424
4. Cataldo DA 1974 Vein loading: the role of the symplast in intercellular transport of carbohydrate between the mesophyll and minor veins of tobacco leaves. Plant Physiol 53: 912–917
5. Crafts AS, CE Crisp 1971 Phloem Transport in Plants. W. H. Freeman & Co, San Francisco
6. Dilley RA, A Rothstein 1967 Chloroplast membrane characteristics. Biochim Biophys Acta 135: 427–443
7. Doman DC, DR Geiger 1976 Effect of nucleoside-phosphates on rate of export from leaves of *Beta vulgaris*. Plant Physiol 57: S-28
8. Eddy AA, JA Nowachi 1971 Stoichiometrical proton and potassium ion movements accompanying the absorption of amino acids by the yeast *Saccharomyces carlsbergensis*. Biochem J 122: 701–711
9. Etherton B, J Nuovo 1974 Rapid changes in membrane potentials of oat coleoptile cells induced by amino acids and carbohydrates. Plant Physiol 53: S-49
10. Fellows RJ, DR Geiger 1974 Structural and physiological changes in sugar beet leaves during sink to source conversion. Plant Physiol 53: 877–885
11. Fondy BR, DR Geiger 1976 Kinetics of several leaf sugars during phloem loading in *Beta vulgaris*. Plant Physiol 57: S-77
12. Geiger DR 1975 Phloem loading. *In* M H Zimmermann, J A Milburn, eds, Transport in Plants. I. Encyclopedia of Plant Physiol, New Series Vol I. Springer-Verlag, New York pp 395–431
13. Geiger DR, Giaquinta RT, RJ Fellows 1973 Solute distribution in sugar beet leaves in relation to phloem loading and translocation. Plant Physiol 52: 585–589
14. Geiger DR, SA Sovonick, TS Shock, RJ Fellows 1974 Role of free space in translocation in sugar beet. Plant Physiol 54: 892–898
15. Giaquinta R 1976 Evidence for phloem loading from the apoplast. Chemical modification of membrane sulfhydryl groups. Plant Physiol 57: 872–875
16. Giaquinta RT, DR Geiger 1973 Mechanism of inhibition of translocation by localized chilling. Plant Physiol 51: 372–377
17. Giaquinta RT, DR Geiger 1976 Mechanism of cyanide inhibition of phloem translocation. Plant Physiol 59: 178–180
18. Gilder J, J Cronshaw 1974 A biochemical and cytochemical study of adenosine triphosphatase activity in the phloem of *Nicotiana tabacum*. J Cell Biol 60: 221–235
19. Good NE, GD Winget, W Winter, TN Connolly, S Izawa, R Singh 1966 Hydrogen ion buffers for biological research. Biochemistry 5: 467–477
20. Hodges TK 1973 Ion absorption by plant roots. Adv Agron 25: 163–207
21. Komor E, W Tanner 1974 The hexose-proton co-transport system of *Chlorella*. pH dependent change in *Km* values and translocation constants of the uptake system. J Gen Physiol 64: 568–581
22. Komor E, W Tanner 1974 The hexose-proton symport system of *Chlorella vulgaris*. Specificity, stoichiometry and energetics of sugar-induced proton uptake. Eur J Biochem 44: 219–223
23. Komor E, W Tanner 1975 Simulation of a high- and low-affinity sugar-uptake system in *Chlorella* by a pH-dependent change in the *Km* of the uptake system. Planta 123: 195–198
24. Kriedemann P, H Beevers 1967 Sugar uptake and translocation in the castor bean seedling. II. Sugar transformations during uptake. Plant Physiol 42: 174–180
25. Kursanov AL, MI Brovchenko 1969 Free space as an intermediate zone between photosynthesizing and conducting cells of leaves. Sov Plant Physiol 16: 965–972
26. Lüttge U, EV Schöch, E Ball 1974 Can externally applied ATP supply energy to active ion uptake mechanisms of intact plant cells? Aust J Plant Physiol 1: 211–220
27. Mitchell P 1967 Translocations through natural membranes. Adv Enzymol 29: 33–87
28. Sacher JA, MD Hatch, KT Glasziou 1963 Sugar accumulation cycle in sugarcane. III. Physical and metabolic aspects of cycle in immature storage tissues. Plant Physiol 38: 348–354
29. Seaston A, C Inkson, AA Eddy 1973 The absorption of protons with specific amino acids and carbohydrates by yeast. Biochem J 134: 1031–1043
30. Slayman CL 1974 Proton pumping and generalized energetics of transport: a review. *In* U Zimmermann, J Dainty, eds, Membrane Transport in Plants. Springer-Verlag, New York pp 107–119
31. Slayman CL, WS Long, CYH Lu 1973 The relationship between ATP and the electrogenic pump in the plasma membrane of *Neurospora crassa*. J Membr Biol 14: 305–338
32. Slayman CL, CW Slayman 1974 Depolarization of the plasma membrane of *Neurospora* during active transport of glucose: evidence for a proton-dependent co-transport system. Proc Nat Acad Sci USA 71: 1935–1939
33. Sovonick SA, DR Geiger, RJ Fellows 1974 Evidence for active phloem loading in the minor veins of sugar beet. Plant Physiol 54: 886–891
34. West I, P Mitchell 1972 Proton-coupled β-galactoside translocation in non-metabolizing *Escherichia coli*. J Bioenergetics 3: 445–462