Plant Physiol. (1983) 72, 362–367
0032-0889/83/72/0362/06/$00.50/0

Pathway of Phloem Unloading of Sucrose in Corn Roots[1]

Received for publication October 29, 1982 and in revised form February 14, 1983

ROBERT T. GIAQUINTA, WILLY LIN, NANCY L. SADLER, AND VINCENT R. FRANCESCHI
E. I. du Pont de Nemours and Company, Experimental Station, Central Research and Development Department, Wilmington, Delaware, 19801

ABSTRACT

The pathway of phloem unloading and the metabolism of translocated sucrose were determined in corn (*Zea mays*) seedling roots. Several lines of evidence show that exogenous sucrose, unlike translocated sucrose, is hydrolyzed in the apoplast prior to uptake into the root cortical cells. These include (a) presence of cell wall invertase activity which represents 20% of the total tissue activity; (b) similarity in uptake and metabolism of [^{14}C]sucrose and [^{14}C]hexoses; and (c) randomization of ^{14}C within the hexose moieties of intracellular sucrose following accumulation of [^{14}C](fructosyl)sucrose. Conversely, translocated sucrose does not undergo apoplastic hydrolysis during unloading. Asymmetrically labeled sucrose ([^{14}C](fructose)sucrose), translocated from the germinating kernels to the root, remained intact indicating a symplastic pathway for unloading. In addition, isolated root protoplasts and vacuoles were used to demonstrate that soluble invertase activity (V_{max} = 29 micromoles per milligram protein per hour, K_m = 4 millimolar) was located mainly in the vacuole, suggesting that translocated sucrose entered via the symplasm and was hydrolyzed at the vacuole prior to metabolism.

In contrast to sucrose loading into the phloem of exporting source leaves, the cellular processes associated with the unloading and the subsequent transfer of assimilates in sink regions are poorly understood. Identifying the pathways and mechanisms involved in sucrose unloading and utilization is central to the eventual chemical or genetic regulation of assimilate partitioning into the harvestable *versus* nonharvestable regions of a crop.

There are several possible pathways for assimilate unloading in sink regions (Fig. 1). One possibility is that sucrose exits the phloem via either a passive, facilitated, or energy-dependent transfer step and enters the apoplast. Sucrose can then either be hydrolyzed by a cell wall invertase to hexoses which are then accumulated by hexose-specific carriers in adjacent consuming cells or sucrose can enter without hydrolysis via a sucrose-specific carrier. Alternatively, assimilates can be unloaded via a symplastic route through plasmadesmata. In the latter scheme, intracellular hydrolysis of sucrose could occur within the cytoplasm or vacuole depending on the intracellular location of invertase activity.

That experimental support exists for the above routes in various species indicates that different pathways and mechanisms exist in different plants and organs and, perhaps, even in the same organ at different developmental stages (2, 6, 13). For example, sucrose unloading and storage in sugarcane parenchyma involve sucrose transfer through the apoplast and hydrolysis by a cell wall invertase. The resulting hexoses are accumulated by the parenchyma cells and resynthesized to sucrose by a cytoplasmic sucrose phosphate synthetase prior to transfer to the vacuole for storage (7). In sugar beet storage roots, however, sucrose hydrolysis in the free space is not a prerequisite for unloading and storage (5, 15). Differences in the requirement for free space sucrose hydrolysis are also evident in developing seeds where no symplastic continuity exists between the maternal phloem terminals and embryonic consumer tissues. In corn kernels, sucrose hydrolysis in the apoplast appears to be necessary for active uptake into the endosperm (11), whereas no such obligatory sucrose hydrolysis occurs during phloem unloading and accumulation in developing wheat (8) or soybean seeds (12).

In vegetative tissues, such as young sugar beet leaves, a symplastic unloading route is suggested by the retention of asymmetry of labeling within [^{14}C](fructosyl)sucrose imported from abraded source leaves (3). The pattern of sucrose metabolism in these sink leaves was also consistent with unloading via the symplasm (3). Similarly, Dick and ApRees (1) have provided convincing evidence for symplastic movement of sucrose between the stele and cortex of *Pisum* roots.

The aforementioned studies reinforce the view that no ubiquitous mechanism or pathway exists for assimilate unloading and, therefore, the unloading pathway will have to be elucidated for the target organ and species under study. This study investigates the cellular pathway of sucrose unloading in roots of developing corn seedlings. Results show that in corn roots, translocated sucrose is unloaded from the phloem and transferred to the surrounding root cells via the symplasm and that sucrose is hydrolyzed by a vacuolar invertase prior to metabolism.

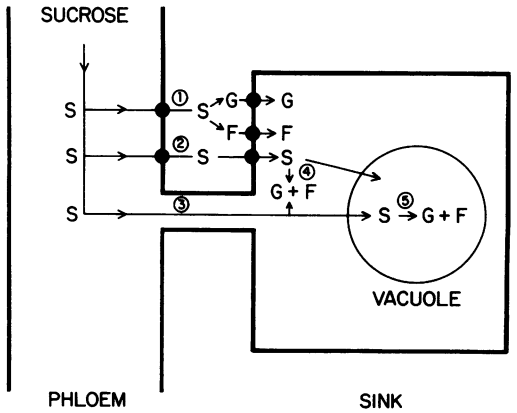

FIG. 1. Schematic of possible pathways of sucrose unloading. (1), Sucrose hydrolysis by cell wall invertase; (2), uptake by sucrose-specific carrier; 3, symplastic transfer via plasmodesmata; 4, cytoplasmic invertase; 5, vacuole invertase. It should also be noted that, in sugar beet roots, evidence indicates that sucrose freely permeates the plasma membrane of the storage parenchyma and is accumulated via an alkali cation/sucrose cotransport into the vacuole (10).

[1] Contribution No. 3139 from the Central Research and Development Department, Experimental Station, E. I. du Pont de Nemours and Company, Inc., Wilmington, DE 19801.

MATERIALS AND METHODS

Plant Material. All tissues for the metabolism, sugar uptake, enzyme, and protoplast studies were obtained from 3-d-old etiolated corn (*Zea mays*) seedlings (9) which were comprised of the germinating kernels, hypocotyl (3 cm), and developing roots (4–5 cm in length).

Soluble and Insoluble Invertase Activity from Intact Roots. One g of 2-cm root segments (without the terminal 1- to 2-mm tip) was homogenized in 5 ml of 85 mM Hepes (pH 8.0), 15 mM dithioerythritol, 15 mM EDTA, and 1% Polyclar AT in a mortar and pestle at 0 to 4°C. After centrifugation at 25,000*g* for 15 min, 2.5 ml of supernatant was passed through a Pharmacia Sephadex G-25 M column (9 ml bed volume) equilibrated with 5 mM Hepes (pH 7.0). Protein was eluted with equilibrating buffer in a 3.5-ml final volume. Aliquots (100 μl, approximately 25 μg protein) were assayed at 30°C for invertase activity in a 1-ml reaction mixture containing either 0.5, 1, 2, 5, 10, 15, or 20 mM sucrose in 25 mM citrate-phosphate buffer (pH 5.0). In some experiments, the pH of the assay was varied between 4 and 8 with citrate-phosphate. The reactions were terminated after 1 h by immersion in a boiling water bath (4 min). Glucose was determined enzymically by the addition of 3 ml of Statzyme reagent per assay tube (5).

The insoluble cell wall pellet was washed and centrifuged four times with distilled H_2O, resuspended in distilled H_2O (3 ml), and aliquots assayed for invertase activity in a 20 mM sucrose reaction mixture. The reactions were centrifuged and assayed for glucose as above. All assays were done in triplicate and corrected with heat-denatured enzyme controls, which always represented less than 5% of the experimental values.

Sugar Uptake and Metabolite Distribution in Roots. Root segments (1–2 cm, split lengthwise), were washed for 5 min in 0.2 mM $CaCl_2$ and then incubated for 30 min in 1 ml of either 1 mM [U-^{14}C]sucrose, [^{14}C]glucose, [^{14}C]fructose, or [^{14}C](fructosyl)-sucrose (9.3–10.6 μCi/μmol). Tissues were washed four to five times in 0.2 mM $CaCl_2$ (15 min total) and frozen with dry ice. Tissue was extracted for 3 h in 80% (v/v) ethanol at 80°C in a Soxhlet apparatus. The ethanol solubles were evaporated to dryness under a reduced atmosphere, resuspended in 1 ml of H_2O, and fractionated on ion-exchange columns (3). The neutral fraction was eluted with H_2O, evaporated to dryness, resuspended in less than 500 μl of 50% (v/v) ethanol, and chromatographed on paper. Sucrose was eluted from the chromatograms, hydrolyzed with invertase, and rechromatographed to determine the [^{14}C] glucose/fructose (G/F) ratio. The G/F ratios represent the ratio of ^{14}C in each hexose fraction. Processing of filter paper discs containing tracer amounts of [^{14}C](fructosyl)sucrose showed that no hydrolysis occurred during the preparative procedures.

The insoluble fractions were incubated in NCS solubilizer overnight prior to liquid scintillation counting.

Translocation of ^{14}C-Sugars. Hypocotyls (alternate sink) were removed from germinating corn seedlings to give a simple source-sink system (germinating kernel and developing roots, respectively). To establish exporting sources, the kernels were incubated in 1 ml of either 2.5 mM [U-^{14}C]sucrose, [^{14}C]glucose, or [^{14}C] (fructosyl)sucrose (45 μCi total), containing 50 mM phosphate buffer (pH 6.3) for 50 min at room temperature in a humidified atmosphere. The roots were not in contact with the labeled solutions. The outer seed coats of the kernels were removed to facilitate sugar absorption. After 50 min, the root sinks (which were not in contact with the label) were harvested and frozen. Kernels were washed to remove free space sugar, and then frozen for later extraction and assay. Tissues were extracted with 80% (v/v) ethanol at 80°C in a micro-Soxhlet apparatus. The ethanol-soluble fractions were evaporated to dryness under a reduced atmosphere, resuspended in 1 ml of H_2O, and passed through a 4-cm ion-exchange resin [2 cm of AG50Wx8 (H^+) separated from 2 cm of AG1x8 (formate) by a 2-mm zone of G-25 resin] to remove the basic and acidic metabolites. The neutral fractions were evaporated to dryness, resuspended in 100 to 400 μl of H_2O, and 50-μl aliquots were separated by HPLC. Sucrose fractions were collected, hydrolyzed with invertase, rechromatographed by HPLC, and the glucose/fructose ratios determined.

Sugar Uptake into Root Protoplasts. Protoplasts were isolated from corn roots according to the protocol described previously (9). Two procedures were used to measure sugar uptake. They gave similar results. In the first procedure, protoplasts (approximately 9×10^6) were suspended in 0.8 ml of 0.7 M mannitol, 25 mM Mes (pH 6), 0.2 mM $CaCl_2$, and 1 mM KCl. A 100-μl aliquot of the protoplast suspension (~10^6 protoplasts) was incubated in 1 ml of solution containing 1 mM ^{14}C-sugar (10 μCi/μmol) and the above suspending medium for 30 min at 30°C. The reaction mixture was then diluted with 35 ml of osmoticum, centrifuged at 100*g* for 5 min, and the supernatant aspirated. This was repeated twice, and the final pellet was resuspended and vortexed in hot water to lyse the protoplast prior to counting a 0.5-ml aliquot.

The second procedure involved incubating protoplasts in labeled sugar solutions followed by rapid centrifugation through silicone oil (9). Approximately 10×10^6 protoplasts (~40 μg protein) were incubated at 30°C in 5 ml of 1 mM sugar (2 μCi/μmol), 0.2 mM $CaCl_2$, 0.6 M mannitol, and 1 mM Hepes (pH 6). At 5, 10, 15, 20, and 30-min intervals, 300-μl aliquots (0.5×10^6 protoplasts) were collected and rapidly centrifuged through silicone oil in a microfuge. The tip of the microfuge tube containing the protoplast pellet was cut off, placed in 0.5 ml of boiling water, and the ^{14}C and protein determined. The protein content was used to estimate the number of protoplasts that passed through the silicone oil.

Invertase Activity in Protoplasts and Vacuoles. Protoplasts were isolated as described above and resuspended in 0.2 ml of 0.7 M mannitol, 25 mM Mes (pH 6), 0.2 mM $CaCl_2$, and 1 mM KCl (approximately 3×10^6 protoplasts). The protoplast suspension was then diluted to 2.5 ml with 5 mM Hepes buffer (pH 7) and vortexed vigorously to osmotically and physically lyse the protoplasts. The entire 2.5-ml volume was passed through a Sephadex G-25 column, and eluted with 5 mM Hepes in a total volume of 3.5 ml. Aliquots (200 μl, ~35–40 μg protein) were assayed for invertase as described above.

Vacuoles were isolated as described previously (14) and assayed for invertase activity.

RESULTS AND DISCUSSION

Our strategy to study unloading was to address the following questions: (a) is cell wall invertase present?; (b) if present, can it effectively hydrolyze sucrose present in the apoplast?; (c) is exog-

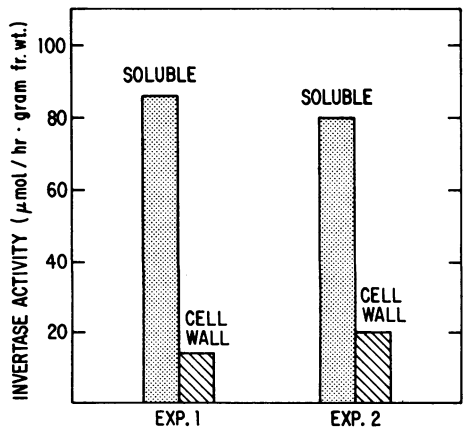

FIG. 2. Distribution of soluble and cell wall invertase in corn roots. Activities were assayed at 20 mM sucrose (see "Materials and Methods" and text for details).

Table I. *^{14}C-Metabolite Distribution in Corn Roots following the Accumulation of Various ^{14}C-Sugars*

One mM sugar was supplied for 30 min. The G/F ratio of stock [^{14}C](fructosyl)sucrose was <0.02.

Fraction	Supplied Sugar [^{14}C](Fructosyl)Sucrose	[U-^{14}C]Sucrose	[^{14}C]Fructose	[^{14}C]Glucose
		% distribution		
Insoluble	28	31	32	27
H_2O solubles	72	69	68	73
Basic/acidic[a]	24	25	30	30
Neutral	76	75	70	70
Glucose[b]	10	20	15	25
Fructose	12	8	20	10
Sucrose	78	72	65	65
G/F[d]	0.60	0.75	0.79	ND[c]

[a] % of H_2O-soluble fraction.
[b] % of neutral fraction.
[c] Not determined.
[d] G/F ratios are absolute values.

Table II. *^{14}C-Metabolite Distribution and Glucose/Fructose Ratio of Sucrose Translocated from Germinating Kernels to Corn Roots*

G/F ratios of sucrose have been corrected by 0.02, the G/F ratio of the stock [^{14}C](fructosyl)sucrose.

Fraction	Supplied Sugar					
	[^{14}C](Fructosyl) Sucrose		[U-^{14}C]Sucrose		[^{14}C]Fructose	
	Kernel	Roots	Kernel	Roots	Kernel	Roots
			% distribution			
Insoluble	4	14	5	21	10	20
H_2O solubles	96	86	95	79	90	80
Basic/acidic[a]	4	4	7	8	14	19
Neutral	96	96	93	92	86	81
G/F of sucrose[b]	0.05	0.06	0.96	0.89	0.87	0.84

[a] % of H_2O solubles.
[b] G/F ratios are absolute values.

enously supplied sucrose accumulated intact or does hydrolysis and resynthesis occur?; (d) is translocated sucrose accumulated in a manner similar to exogenously supplied sucrose?; (e) is there structural support for a symplastic *versus* apoplastic pathway; and (f) if sucrose is accumulated intact either from the apoplast or via the symplasm, does intracellular hydrolysis occur in the cytoplasm or the vacuole?

Distribution of Invertase Activity in Corn Root Segments. The distribution of invertase activity between the soluble (intracellular) and insoluble (cell wall) fractions shows that approximately 20% of the total tissue activity was tightly bound to the cell wall fraction (Fig. 2). This may represent a minimum level of invertase activity in the apoplast since the soluble fraction presumably contains both intercellular invertase and any invertase which is either loosely bound to the cell walls or which freely exists in the space between the cell wall and plasmamembrane of the cortical cells. It is also possible that the wall invertase activity originated from within the symplasm. The physiological data presented below, however, show that the invertase activity measured on the cell wall fraction represents *in vivo* cell wall invertase. The invertase activity in the soluble and insoluble fractions in Experiment 1 (Fig. 2) were 67 $\mu mol \cdot h^{-1} \cdot g^{-1}$ fresh weight (30.5 $\mu mol \cdot h^{-1} \cdot mg^{-1}$ protein) and 10.6 $\mu mol \cdot h^{-1}\ g^{-1}$ fresh weight, respectively. The activities of both the soluble and insoluble invertase from roots were optimal at pH 4.5 to 5.0 (at pH 8.0, soluble invertase activity was less than 10% of the activity at pH 5.0); therefore, they can be classified as acid invertases. The soluble invertase had a V_{max} of 71 $\mu mol \cdot h^{-1} \cdot mg^{-1}$ protein and a K_m for sucrose of 4 to 6 mM. Although these results show that cell wall invertase is present in corn roots, it is important to show that this level of invertase activity is sufficient to hydrolyze sucrose if it indeed enters the apoplast.

Hydrolysis and Metabolism of Exogenous Sucrose by Corn Roots. Sucrose and hexoses supplied directly to the apoplast in corn root segments were used to determine whether the cell wall invertase activity noted in Figure 2 was able to substantially hydrolyze free space sucrose. Two approaches were used to show that exogenous sucrose was hydrolyzed prior to accumulation into the root cells. First, the distribution of ^{14}C among the hexose moieties of internal sucrose following the uptake of asymmetrically labeled sucrose ([^{14}C](fructosyl)sucrose) was used to assess the extent of sucrose hydrolysis in the apoplast and its subsequent resynthesis in the cytoplasm. Second, the pattern of sucrose *versus* hexose metabolism in roots was used as substantiating evidence for hydrolysis in the apoplast. If asymmetrically labeled sucrose was hydrolyzed prior to uptake, then intracellular resynthesis would, because of isomerase activity, randomize the ^{14}C label within the hexose moieties of the resynthesized sucrose. Table I shows the glucose/fructose ratio of sucrose isolated from corn root segments following the accumulation of various ^{14}C sugars. The glucose/fructose ratio of 0.6 following [^{14}C](fructosyl)sucrose uptake indicates that substantial hydrolysis had occurred. As a control, the uptake of uniformly labeled sucrose gave a G/F ratio of 0.75. Similarly, the labeled sucrose which was synthesized following uptake of [^{14}C]fructose had a G/F ratio of 0.79, showing that adequate intercellular isomerase activity was present to substantially randomize the label prior to sucrose resynthesis.

That the distribution of ^{14}C among various metabolites is nearly identical following the uptake of [^{14}C]sucrose, [^{14}C]glucose, and [^{14}C]fructose, is consistent with sucrose hydrolysis in the free space prior to uptake and metabolism. Therefore, if *in vivo* 'translocated' sucrose entered the apoplast of the root, we would expect substantial hydrolysis to occur as measured by the ^{14}C glucose/fructose ratio.

Retention of Asymmetry after Translocation of [^{14}C](Fructosyl)Sucrose to the Roots. [^{14}C](Fructosyl)sucrose was supplied to the kernels of seedlings and the label was allowed to 'translocate' to the distal portions of the developing roots (approximately 3–5 cm from the source). [^{14}C]Sucrose was then isolated from the roots and kernels and its glucose/fructose ratio determined. Table II shows the ^{14}C metabolite distribution. [^{14}C](Fructosyl)sucrose fed to 'source' kernels for 50 min undergoes very little metabolism (8% of the label is in the combined insoluble, amino acid, and organic acid fractions, while 92% remains in the sugar fraction). Moreover, the glucose/fructose ratio of 0.05 indicates that [^{14}C](fructosyl)sucrose was accumulated in the source without extra-

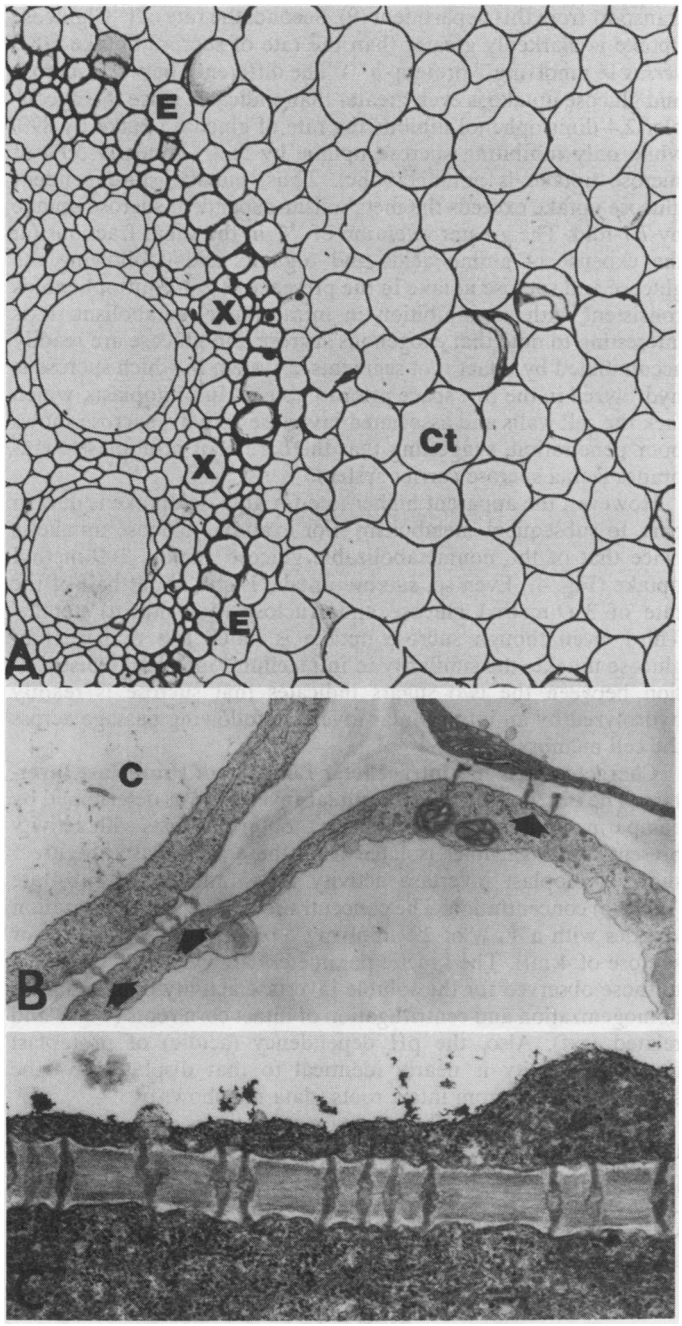

FIG. 3. Micrographs of corn root showing plasmodesmata connections between various cell types. A, Root cross-section showing cortex (Ct), xylem (X), and endodermis (E) (× 240). B, Electron micrograph showing interface between companion cell (C) and surrounding stellar cells. Note plasmodesmata connections (arrows) between cell types (× 13,800). C, Plasmodesmatal field between cortical cells (× 28,700). Tissues were fixed in glutaraldehyde/formaldehyde, postfixed in O_sO_4, and embedded in epoxy resin according to standard procedures.

cellular hydrolysis. ^{14}C label translocated to roots is metabolized to a slightly greater extent, particularly in the insoluble fraction (14% *versus* 4%). This is to be expected since cell wall and protein synthesis are occurring during active root growth.

Importantly, the [^{14}C]sucrose isolated from the roots has retained its asymmetry (G/F = 0.06) following translocation and unloading of [^{14}C](fructosyl)sucrose. Since the apoplast of the root contains invertase which is capable of hydrolyzing free space sucrose (Fig. 2; Table I), the retention of asymmetry in sucrose

Table III. *^{14}C-Metabolite Distribution following Accumulation of ^{14}C-Sugars into Corn Root Protoplasts*

Fraction	^{14}C-Sugar Supplied			
	[^{14}C]Glucose		[^{14}C]Sucrose	
	Control	+ 0.5 mM DNP[a]	Control	+ 0.5 mM DNP
	% distribution			
H_2O solubles				
Basic/acidic[b]	29 (30)[d]	13	23	5
Neutral	71 (70)	87	77	95
Sucrose[c]	70 (65)	12	80	
Glucose	25 (25)	81	15	
Fructose	5 (10)	7	5	
Total dpm incorporated ($\times 10^{-3}$)	65.9	7.31	10.99	4.76
Accumulation rate (nmol/h·mg^{-1} protein)	78.9	9	16	7

[a] DNP, 2,4-dinitrophenol.
[b] % of H_2O fraction.
[c] % of neutral fraction.
[d] Values in parentheses are the ^{14}C-metabolite distributions after glucose uptake into intact root segments (from Table I).

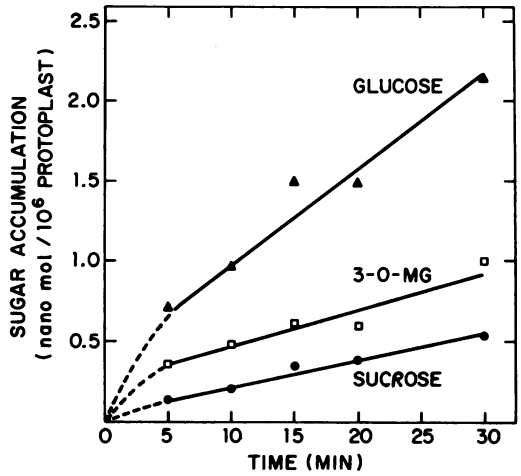

FIG. 4. Time course of sugar accumulation in corn root protoplasts. See "Materials and Methods."

indicates that sucrose was transferred from the phloem to the consuming cells via a symplastic route. The distribution of ^{14}C among the various metabolites following the accumulation and translocation of uniformly labeled sucrose in the seeds and roots was similar to that obtained for [^{14}C](frutosyl)sucrose except that the G/F ratios (0.96 and 0.89 for seed and roots, respectively) approached the expected value of unity. When [^{14}C]fructose was supplied to the source, about twice as much label was incorporated into the insoluble fraction than was observed after sucrose uptake (10% *versus* 5%). More label was also found in the amino acid/organic acid fraction (14%). Differences in labeling patterns between sucrose and hexose have been noted previously for sugar beet source and sink leaves (4). These differences in hexose and sucrose metabolism noted here, where sucrose is not hydrolyzed prior to uptake, is to be compared to the similarity in labeling patterns for exogenous sucrose and hexose noted in Table I where extracellular hydrolysis occurs. These results indicate that extracellular hydrolysis occurred when exogenous sucrose was supplied to the root.

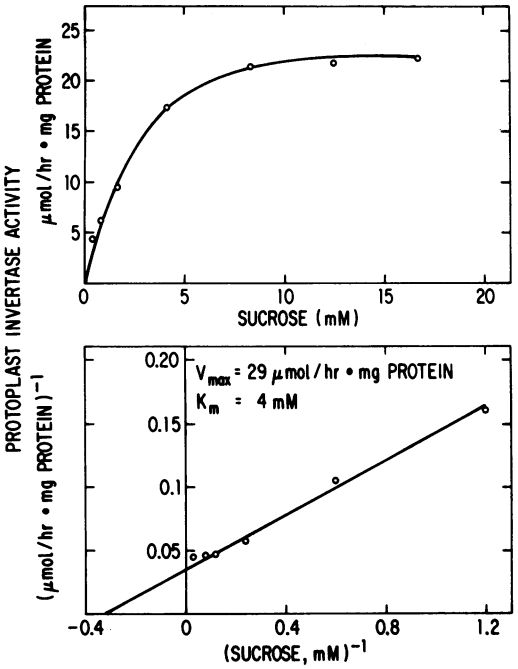

FIG. 5. Invertase activity in isolated corn root protoplasts. See "Materials and Methods."

Table IV. *Invertase Activity in Corn Root Protoplasts and Vacuoles*

In four experiments, the invertase activity in the vacuole fraction ranged from 70 to 95% of that of the protoplasts.

Compartment	Invertase Activity	Protoplast
	μmol/h·10⁶ protoplasts or vacuoles	%
Protoplasts	4.20	
Vacuoles	2.95	70

The glucose/fructose ratio of 0.87 in sucrose which was synthesized after [^{14}C]fructose uptake into the germinating seed shows that substantial isomerization had occurred and, therefore, we should and would have detected substantial randomization if hydrolysis occurred during [^{14}C](fructosyl)sucrose uptake. The results favor a symplastic route of unloading into the cortical cells.

Symplastic Continuity between the Phloem and Adjacent Cells. Electron micrographs show that plasmodesmata exist between the phloem companion cells and adjacent cells and between all the cortical cells (Fig. 3). It should be recognized that quantitative studies on the total cell wall area occupied by plasmodesmata are necessary before structural evidence, alone, can be used as proof for a symplastic route of transfer. Nevertheless, the presence of plasmodesmata noted here, is at least consistent with the physiological data presented above which support a symplastic transfer route.

Sugar Uptake and Metabolism in Root Protoplasts. Corn root protoplasts, free from cell wall invertase, were used to study the (a) uptake and metabolism of various sugars, (b) nature of intracellular invertase, and (c) intracellular distribution of invertase between the vacuole and cytoplasm.

Table III shows the ^{14}C-metabolite distribution following accumulation of [^{14}C]sucrose and glucose in root protoplasts. Several points are noteworthy. First, ^{14}C distribution in the water soluble components following [^{14}C]glucose uptake is essentially identical to that found in the intact root sections (values for intact roots are given in parentheses for comparison). This indicates that these protoplasts are metabolically competent and can be used with reasonable assurance for metabolism and sugar uptake studies. A similar protoplast preparation was used in recent studies on ion transport from this department (9). Second, the rate of [^{14}C]glucose uptake is markedly greater than the rate of sucrose uptake (78.9 *versus* 16 nmol·mg^{-1} protein·h^{-1}). The difference between sucrose and glucose uptake is even greater than indicated since the uncoupler 2,4-dinitrophenol inhibits the rate of glucose uptake by 89% while only inhibiting sucrose uptake by 56% (*ie* nearly 50% of sucrose uptake is nonmetabolic). Thus, metabolism-dependent glucose uptake exceeds the metabolism-dependent sucrose uptake by 7.7-fold. The greater retention of ^{14}C in the sugar fractions (at the expense of amino acids and organic acids) following the glucose and sucrose uptake in the presence of 2,4-dinitrophenol is consistent with an inhibition in intracellular metabolism. It is interesting to note that exogenous sucrose and glucose are readily accumulated by intact root segments, a system in which sucrose is hydrolyzed in the free space prior to uptake. In protoplasts, which lack the cell walls and associated invertase activity, sucrose shows poor penetration, suggesting that the corn root protoplast membranes lack a sucrose carrier system.

However, the apparent higher rate for glucose uptake is due, in part, to subsequent metabolism. For example, glucose uptake is twice that of the nonmetabolizable glucose analog, 3-*O*-methyl uptake (Fig. 4). Even so, sucrose uptake is still about half of the rate of 3-*O*-methyl glucose and fructose (not shown) uptake. Third, even though sucrose uptake is much less than that of glucose uptake, the similarity in intracellular metabolite distribution between the two sugars indicates that sucrose is readily hydrolyzed by an intracellular invertase following passage across the cell membrane.

Characteristics and Intracellular Location of Protoplast Invertase. The distribution of intracellular invertase was determined by comparing invertase activity in intact root protoplasts with activity present in the vacuoles isolated from these protoplasts. Figure 5 shows protoplast invertase activity as a function of substrate (sucrose) concentration. The concentration curve shows saturation kinetics with a V_{max} of 29 μmol·mg^{-1} protein·h^{-1} and a K_m for sucrose of 4 mM. The kinetic parameters are essentially identical to those observed for the soluble invertase activity obtained after homogenization and centrifugation of intact corn roots (Fig. 2 and related text). Also, the pH dependency (acidic) of protoplast invertase activity is nearly identical to that displayed by the soluble invertase from intact roots (data not shown).

Table IV compares the invertase activity in protoplasts with that found in vacuoles from protoplasts. Assuming one vacuole/protoplast, at least 70% of the activity was in the vacuole fraction. This represents a minimum value, since in four experiments the vacuoles contained from 70 to 95% of the total protoplast invertase activity.

SUMMARY

Several lines of evidence are presented which collectively support an *in vivo* symplastic route for sucrose unloading in corn roots. Based on the vacuolar location of invertase in the root cells, incoming sucrose appears to be hydrolyzed at the vacuole prior to metabolism associated with root growth.

Acknowledgments—The technical assistance of C. Arrabal and manuscript preparation by T. Sparre and C. Kremer are greatly appreciated.

LITERATURE CITED

1. DICK PS, T APREES 1975 The pathway of sugar transport in roots of *Pisum sativum*. J Exp Bot 26: 305-314
2. GEIGER DR, BR FONDY 1980 Phloem loading and unloading: pathways and mechanisms. What's New in Plant Physiol 11: 25-28
3. GIAQUINTA RT 1977 Sucrose hydrolysis in relation to phloem translocation in *Beta vulgaris*. Plant Physiol 60: 339-343
4. GIAQUINTA RT 1978 Source and sink metabolism in relation to phloem translocation. Carbon partitioning and enzymology. Plant Physiol 61: 380-385
5. GIAQUINTA RT 1979 Sucrose translocation and storage in the sugar beet. Plant

Physiol 63: 828–832
6. GIAQUINTA RT 1980 Translocation of sucrose and oligosaccharides. *In* J Preiss, ed, The Biochemistry of Plants, Vol III. Academic Press, New York, pp 271–320
7. GLASZIOU KT, KR GAYLER 1972 Storage of sugars in stalks of sugarcane. Bot Rev 38: 471–490
8. JENNER CF 1974 An investigation of the association between the hydrolysis of sucrose and its absorption by grains of wheat. Aust J Plant Physiol 1: 319–329
9. LIN W Inhibition of anion transport in corn root protoplasts. Plant Physiol 68: 435–438
10. SAFTNER RA, RE WYSE 1980 Alkali cation/sucrose transport in the root sink of sugar beet. Plant Physiol 66: 884–889
11. SHANNON JC, CT DOUGHERTY 1972 Movement of ^{14}C-labeled assimilates into kernels of *Zea mays* L. II. Invertase activity of the pedicel and placentochalazal tissues. Plant Physiol 49: 203–206
12. THORNE JH 1982 Characterization of the active sucrose transport system of immature soybean embryos. Plant Physiol 70: 953–958
13. THORNE JH, RT GIAQUINTA 1982 Pathways and mechanisms associated with carbohydrate translocation in plants. *In* DH Lewis, ed, Physiology and Biochemistry of Storage Carbohydrates in Vascular Plants. Soc Exp Bot Symp Series. Cambridge University Press. In press
14. WAGNER GJ, HW SIEGLMAN 1975 Large scale isolation of intact vacuoles and isolation of chloroplasts from protoplasts of mature plant tissue. Science 190: 1298–1299
15. WYSE RE 1979 Sucrose uptake by sugar beet (*Beta vulgaris* L.) taproot tissue. Plant Physiol 64: 837–841