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Evidence for the Presence of a Sucrose Carrier in Immature Sugar Beet Tap Roots[1]

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ABSTRACT

The objectives of this work were to determine the path of phloem unloading and if a sucrose carrier was present in young sugar beet (*Beta vulgaris* L.) taproots. The approach was to exploit the characteristics of the sucrose analog, 1′-fluorosucrose (F-sucrose) which is a poor substrate for acid invertase but is a substrate for sucrose synthase. Ten millimolar each of [^{3}H]sucrose and [^{14}C]F-sucrose were applied in a 1:1 ratio to an abraded region of an attached leaf for 6 hours. [^{14}C]F-sucrose was translocated and accumulated in the roots at a higher rate than [^{3}H]sucrose. This was due to [^{3}H]sucrose hydrolysis along the translocation path. Presence of [^{3}H]hexose and [^{14}C]F-sucrose in the root apoplast suggested apoplastic sucrose unloading with its subsequent hydrolysis. Labeled F-sucrose uptake by root tissue discs exhibited biphasic kinetics and was inhibited by unlabeled sucrose, indicating that immature roots have the ability for carrier-mediated sucrose transport from the apoplast. Collectively, in vivo and in vitro data indicate that despite sucrose hydrolysis by the wall-bound invertase, sucrose hydrolysis is not entirely essential for sugar accumulation in this tissue.

The pathway of sucrose unloading in various sink organs has been extensively studied. In species such as corn and sugar cane where sucrose unloading occurs by way of the apoplast and a cell-wall acid invertase is present, sucrose hydrolysis is believed to be essential, because hexoses are the major transport sugars (10, 19).

In mature sugar beet taproot, sucrose unloading is apoplastic and uptake and accumulation in the vacuole occurs without hydrolysis (23). In immature roots, however, the pathway of sucrose unloading and its movement into the sink parenchyma cells has not been determined. Furthermore, due to the presence of wall-bound invertase activity in this tissue, the relative importance of invertase and the presence of a sucrose-specific carrier at the plasmalemma, i.e., the requirement for hydrolysis prior to uptake, are not known. In the case of apoplastic sucrose unloading in immature roots, sucrose may follow a different fate than that in the mature tissue in which wall-bound invertase activity is negligible.

The objectives of the present work were twofold. First, to determine whether sucrose unloading in immature sugar beet taproot is apoplastic or symplastic and, second, to determine whether a sucrose carrier is present at this stage of growth. For this purpose we used young sugar beet roots at two different stages of growth: 4 weeks after emergence when there is high wall-bound acid invertase activity and 7 weeks after emergence when the invertase activity is negligible. To facilitate the study, we used a synthetic analog of sucrose F-sucrose.[2] This analog is a poor substrate for the acid invertase but is subject to sucrose synthase action. Furthermore, F-sucrose is phloem translocated, and it is recognized and transported by the sucrose carrier (2, 11).

Evidence suggested that sucrose unloading in this tissue is most likely apoplastic. Furthermore, root cells have the ability to transport sucrose (analog) despite high wall-bound invertase activity indicating the presence of a sucrose-specific carrier. Therefore, it is suggested that sucrose hydrolysis is not entirely essential for sugar accumulation in this tissue. In immature roots, sucrose and hexose are transported by their respective carriers.

MATERIALS AND METHODS

Plant Material. Sugar beets (*Beta vulgaris* L., cv Mono Hy) were greenhouse grown in 10 L pots filled with soil. Plants were fertilized twice a week with a 20-20-20 Peters nutrient mixture and weekly with Peters micronutrients (Foglesville, PA). Daylight was extended to 14 h with fluorescent lamps. Light intensity at the canopy level was 400 μmol m^{-2} s^{-1}. Day/night temperature in the greenhouse was set at 25/18°C.

Analog Synthesis. F-sucrose was synthesized from 1′-fluorofructose and labeled UDP-glucose according to procedures described by Card and Hitz (2) with the following modifications. To purify sucrose synthase, wheat germ was defatted in three changes of hexane, ground in a chilled mortar and pestle in the buffer of Card and Hitz (2), and filtered through cheesecloth. The homogenate was centrifuged at 12,000 *g* for 10 min and the supernatant centrifuged at 42,000*g* for 20 min. The resulting supernatant was brought slowly to 35% saturation in $(NH_4)_2SO_4$ on ice. The pelleted protein was centrifuged at 20,000*g* for 10 min, redissolved in a minimal amount of reaction buffer (500 μl), desalted on Sephadex G-25, and freeze-dried. Enzyme activity was retained for two weeks when stored at −20°C.

Results presented here were obtained with a generous gift of [^{14}C]F-sucrose provided by Dr. W. D. Hitz of the DuPont Experimental Station. We also synthesized [^{3}H]F-sucrose (specific activity 218.3 MBq/mmol) using [^{3}H](glucosyl)-UDP glucose and 1′-fluorofructose which was used in a second set of experiments, yielding identical results.

Sugar Uptake. In vitro F-sucrose uptake rates were determined in both mature (6 months old) and immature (4 weeks old) taproots. Discs 5mm in diameter and 1 mm thick were cut from mature roots whereas immature taproots were cut with a razor blade into 1 mm thick slices. Mature tissue discs were preincubated for 90 min in 3 ml of a solution containing 300 mM mannitol

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[2] Abbreviations: F-sucrose, 1′-fluoro-1′-deoxy-sucrose; PCMBS, *p*-chloromercuribenzene sulfonate.

(osmoticum), 1 mM $CaSO_4$, 2 mM $CaCl_2$, 2 mM KCl, and 5 mM $MgCl_2$, buffered at pH 5.5 with 20 mM MES. Immature roots were preincubated for 1 h in identical solutions which contained 150 mM mannitol. For each tissue type, incubation solutions were identical to preincubation solutions except they contained various concentrations of labeled sucrose and/or F-sucrose. Sets of discs (0.75 g fresh weight) from mature roots were incubated in the presence of 5 mM [^{14}C]F-sucrose (specific activity 7.6 GBq/mmol). In some experiments, tissue discs were treated with 1 mM PCMBS for 30 min before uptake was initiated (no PCMBS was present in incubation solutions). Immature roots, (75 mg fresh weight) were incubated for 30 min in the presence of various concentrations of labeled F-sucrose or sucrose. All solutions were slowly bubbled with air throughout the experiments in order to prevent anaerobiosis. To remove labeled sugars from the free space and the cut surfaces, tissues were treated with a 3 × 3 min rinse (7 ml each) with their respective preincubation solutions. Each set of tissue was extracted overnight in 2 ml 80% ethanol at 55°C in scintillation vials before radioactivity was determined in a liquid scintillation counter. Uptake experiments were run in triplicate and repeated at least twice.

Translocation. Seedlings were trimmed to one (largest) leaf 24 h prior to any experiment. In the laboratory, translocation was maintained by providing additional light (2 fluorescent tubes giving a light intensity of 45 μmol m^{-2} s^{-1}). Two 1 cm diameter areas of the leaf were gently abraded with carborundum (320 Grit powder) and radioactive sugar solutions were applied in a lanolin well covered with a slide cover glass. Sucrose and F-sucrose were both used at 10 mM (37.0 KBq for 4-week-old plants and 55.5 KBq for 7-week-old plants). Unless otherwise specified, radioactive sugars were applied as [^{3}H](fructosyl)-sucrose (specific activity 373.7 GBq/mmol) and [^{14}C](glucosyl)F-sucrose (specific activity 7.6 GBq/mmol). [^{3}H]Sucrose and [^{14}C]F-sucrose were applied in a 1:1 concentration and dpm ratio. After 6 h, plants were divided into leaf, upper petiole (immediately under the leaf), lower petiole (above the crown), and taproot (the crown region and fibrous roots were discarded). Tissue segments were rinsed in buffer for 3 × 3 min in order to replace and collect the apoplast fraction. To extract sugars, segments were put in 80% boiling ethanol in a Soxhlet apparatus for at least 6 h. Ethanol extracts and washing media were freeze-dried before sugar analysis by HPLC. Each experiment was run in duplicate and was repeated at least twice.

Sugar Analysis. Freeze-dried extracts were dissolved in 1 ml deionized water and were passed through C-18 Prep Sep Columns (Bio Rad) equilibrated with 1 ml methanol. The eluant was freeze dried and redissolved in 60 μl deionized water. Twenty μl aliquots were injected into a Kratos HPLC system (Kratos Analytical, Ramsey, NJ) equipped with a Bio Rad Aminex HP 42C column (Bio Rad) and a refractive index detector (Waters 410; Millipore Corp.) Sucrose and F-sucrose had the same retention time. Sucrose (together with F-sucrose), glucose and fructose fractions were collected in a Pharmacia FRAC 100 fraction collector (Pharmacia, Inc.) directly into liquid scintillation vials. Scintillation solution (Scinti Verse Bio HP, Fisher Scientific) was added to each vial and radioactivity counted in a Beckman 5801 counter, using a dual label dpm program.

To study randomization of label between hexoses, aliquots of asymmetrically labeled sucrose or F-sucrose fractions were subjected to acid hydrolysis with 1 N HCl at 100°C for 1 h. The mixture was then desalted by passing through a Bio Rad anion exchange resin AG-1X-8 (bicarbonate form) and washing with 20 ml of deionized water. CO_2 was evaporated by boiling the eluant and sugars analyzed by HPLC.

Enzyme Assays. Cell-wall-bound and soluble acid invertases (EC, 3.2.1.26) and sucrose synthase (EC 2.4.1.13) activities were measures according to Wyse (22). Enzymes were extracted from 0.5 g of tissue homogenized in a Brinkman homogenizer (Brinkman Instruments) with 5 ml of chilled grinding medium containing at pH 7.0, 50 mM potassium phosphate buffer, 0.1 mM EDTA, 1 mM mercaptoethanol, and 0.1 mM Na_2SO_3. After passage through cheesecloth, the homogenate was centrifuged at 35,000 *g* for 20 min and the pellet washed three times with grinding medium. Supernatant and pellet (2 ml) were dialyzed overnight at 4°C (50 mM sodium acetate buffer [pH 5.0], 0.1 mM EDTA, and 1 mM mercaptoethanol) for acid invertases assay. Another aliquot of the supernatant was dialyzed in a 50 mM potassium phosphate buffer (pH 7.0) for sucrose synthase assay.

Chemical. All chemicals were purchased from Sigma Chemical Co. Radioactive sugars were purchased from New England Nuclear.

RESULTS AND DISCUSSION

Using a 1:1 mixture of [^{14}C]sucrose and [^{3}H]sucrose labeled at different positions showed that the position of label had no bearing on the pattern of ^{3}H/^{14}C distribution in the leaf, petiole, or the root (data not shown). This confirmed that the experimental model was valid and that any change in the ratio of sucrose to F-sucrose within a given tissue would reflect a difference in their uptake, metabolism and/or translocation rates rather than difference in the kind and/or position of the label. Furthermore, total ^{14}C or ^{3}H present in whole seedlings was similar, supporting our assumption that phloem loading of the two sugars was similar.

Enzyme Activity. High cell-wall acid invertase activity was detected in all tissues of the 4-week-old plants (Table I). Note that sucrose synthase activity was detected only in the root tissue. The activity of all enzymes measured was in good agreement with those previously reported at this growth stage (13, 15).

Translocation in 4-Week-Old Seedlings. ^{3}H/^{14}C ratios of sugar fractions extracted from different organs of 4-week-old plants treated with [^{3}H]sucrose and [^{14}C]F-sucrose are shown in Figure 1. Although label was applied at a 1:1 ratio, the ^{3}H/^{14}C ratios along the translocation path were different in various fractions. Total fraction (T) reflected the total label recovered in the soluble extract before HPLC separation. ^{3}H/^{14}C in the total fraction was always less than that in the original solution (M) suggesting that some [^{3}H]sucrose was lost by respiration. More [^{14}C]F-sucrose than [^{3}H]sucrose was present in all tissues so that there was a progressive decrease in ^{3}H/^{14}C in the leaf, upper petiole and lower petiole where it was approximately 12% of the ratio in the original solution. Interestingly, in the root, this ratio was higher than in the petiole but was still much lower than in the medium (about 50%). In the root extract, 85% of the ^{14}C was recovered as [^{14}C]F-sucrose but only 63% of the ^{3}H was recovered as [^{3}H]sucrose. The ratio of ^{3}H to ^{14}C in hexose fractions were greater than 1 in all tissues indicating greater sucrose than F-sucrose hydrolysis.

Distribution of label as % radioactivity in tissue extract is shown in Figure 2A. In the petiole, about 90% of the ^{3}H was recovered in the two hexose fractions indicating almost complete hydrolysis of sucrose in this tissue. As expected, the pattern of ^{14}C distribution was different than that of ^{3}H so that more than 90% of the ^{14}C remained in the sucrose fraction in both petiole and root. In the upper petiole, more than half of the ^{3}H appeared in the glucose fraction. Since [^{3}H]sucrose was labeled in the fructose moiety, the presence of large amounts of label in the glucose fraction would suggest that rapid isomerization has occurred. In the root, 75% of the ^{3}H remained in the sucrose fraction. Considering the high cell-wall invertase activity, and presumed apoplastic unloading, this appeared to be high. However, the observation is explained by [^{3}H]sucrose transport across the plasmalemma as the intact molecule. Alternatively cytoplasmic sucrose synthase (no sucrose phosphate synthase is pres-

Table I. *Acid Invertase and Sucrose Synthase Activity in Various Tissues of Sugar Beet*
Data points are means of 3 replicates ± SE.

Age of Plant	Tissue	Activity		
		Cell wall-invertase	Soluble invertase	Sucrose synthase
weeks		*μmol hexose g^{-1} fresh $wt \cdot h^{-1}$*		
4	Leaf	3.4 ± 0.4		ND[a]
	Upper petiole	2.0 ± 0.1		ND
	Lower petiole	1.8 ± 0.3		ND
	Root	7.5 ± 0.09	6.3 ± 0.5	71.0 ± 10.0
7	Root	0.7 ± 0.05	0.9 ± 0.1	60.0 ± 15.0

[a] Not detected.

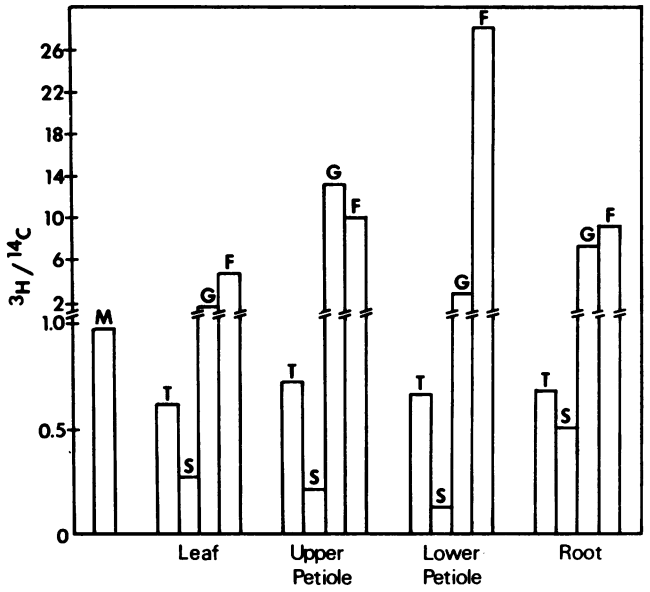

FIG. 1. Ratio of ^3H derived from [^3H](fructosyl)-sucrose to ^{14}C derived from [^{14}C](glucosyl)-F-sucrose in 4-week-old plant sections after 6 h of translocation. $^3H/^{14}C$ was determined in sucrose (S), glucose (G), and fructose (F) fractions after separation on HPLC (see "Materials and Methods"). T, the $^3H/^{14}C$ in the soluble fraction (total); M, $^3H/^{14}C$ of the solution media applied to the leaf. Notice the change in scale above 1.

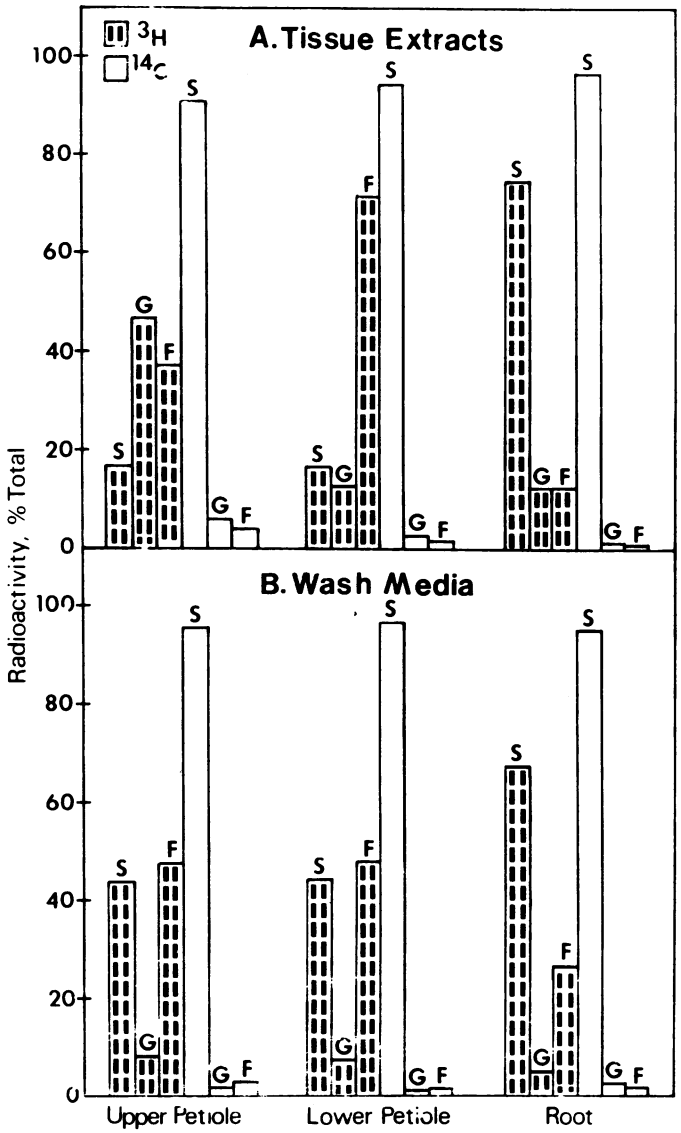

FIG. 2A and B. Percent radioactivity in various sugar fractions in 4-week-old plant tissue extracts and wash media, respectively. Results are expressed as percent of the sum obtained by adding dpm in sucrose (S), glucose (G), and fructose (F) fractions and were obtained from data presented in Figure 1 for petiole and root. ^{14}C is from [^{14}C](glucosyl)-F-sucrose and ^3H from [^3H](fructosyl)-sucrose.

ent in this tissue) may have caused sucrose synthesis from [^3H]hexoses. The possibility remains that at least some of the [^3H]sucrose entered the root symplastically and was not subject to a soluble invertase.

Distribution of label in the wash media representing the apoplastic fraction was also determined (Fig. 2B). In the petiole, the percent of ^3H in the sucrose fraction was higher in the apoplast than in the tissue suggesting an apoplastic unloading in the petiole. Also, in the apoplastic fraction of the petiole, more ^3H was found in the fructose than in the glucose fraction, possibly reflecting the hydrolysis of sucrose in the absence of a cell wall isomerase or preferential retrieval of glucose. In the root, percent of ^3H in the apoplastic sucrose fraction was almost equal to that of root extract. The ability of this tissue to efficiently transport hexose from the apoplast may have resulted in an apparent high radioactivity in the apoplastic sucrose fraction. Almost all of the ^{14}C in the apoplast of the petiole and root (30% of the total; intracellular + apoplastic ^{14}C) remained in the sucrose fraction.

Since high sucrose synthase activity was measured in the roots, we examined a possible randomization of the label in

[^{3}H](glucosyl)F-sucrose (note that in these experiments labeling of sugars was opposite from that of the previous experiments). Randomization would suggest sucrose breakdown by sucrose synthase and possible involvement of the UDPG-dependent group translocator at the tonoplast (21). No randomization of label occurred (>95% retention of ^{3}H in glucose). However, some ^{14}C was recovered in the fructose fraction when [^{14}C](glucosyl)sucrose was used (76% glucose and 24% fructose).

Translocation in 7-Week-Old Seedlings. At this age, the activity of wall-bound acid invertase in the root is only one-tenth of that found in 4-week-old plants (Table I). Therefore, it was expected that ^{3}H and ^{14}C would be distributed similarly in the different sugar fractions (Fig. 3). Results from petiole are not shown since label distribution in this tissue was not changed relative to 4-week-old plants. More than 90% of ^{3}H or ^{14}C remained in the sucrose fraction from both the root extract and apoplast. Due to the insensitivity of F-sucrose to invertase hydrolysis, the labeling pattern for ^{14}C was similar in 4- and 7-week-old plants. Note, however, that 75% and 95% of the ^{3}H remained in the sucrose fractions of 4- and 7-week root extracts, respectively (cf. Fig. 2A and Fig. 3).

In Vitro Sugar Uptake. Using soybean cotyledons, Hitz et al. (11) showed that uptake characteristics of F-sucrose were similar to those of sucrose. Nevertheless, we compared their transport characteristics in sugar beet roots. Transport/accumulation characteristics of the two sugars, determined from flux kinetics analysis described by Saftner et al. (17), indicated that sucrose and its analog shared common transport characteristics across both the plasmalemma and the tonoplast (Table II). More importantly, these efflux data clearly indicate F-sucrose accumulation in the vacuole. In preliminary uptake experiments with fully mature (6 months) sugar beets, slightly higher uptake rates were observed for F-sucrose than for sucrose (Table III). Furthermore, 1 mM PCMBS, a nonpenetrating sulfyhydryl binding inhibitor (6), caused about 50% inhibition of both sucrose and F-sucrose uptake.

To determine whether the two sugars bind to the same carrier, uptake of [^{14}C]F-sucrose was determined in the presence of unlabeled sucrose. In the presence of 5 mM unlabeled sucrose, F-sucrose uptake in both control and PCMBS treated samples decreased (Table III). Moreover, the carrier mediated (active) component of the uptake was inhibited an additional 31% by unlabeled sucrose.

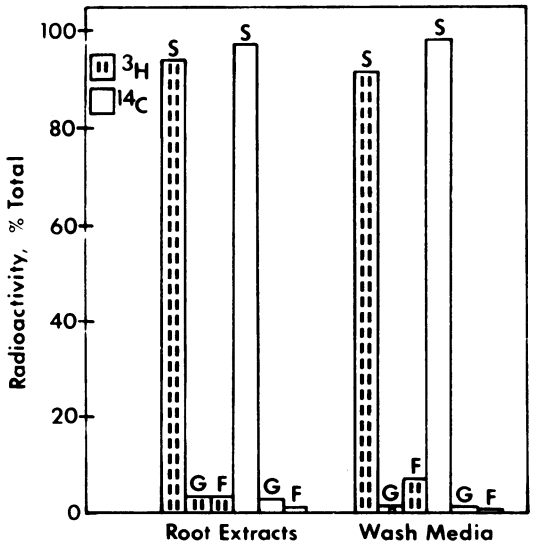

FIG. 3. Percent radioactivity in various sugar fractions in 7-week-old sugar beet root extracts and wash media, respectively. Results are expressed as percent of the sum obtained by adding dpm in sucrose (S), glucose (G), and fructose (F) fractions. ^{14}C is from [^{14}C](glucosyl)-F-sucrose and ^{3}H from [^{3}H](fructosyl)-sucrose.

Table II. *Kinetic Parameters of Sugar Flux Across the Plasmalemma and Tonoplast of Mature Sugar Beet Taproot*

Discs were loaded for 90 min in buffered (pH 6.5) solutions containing 300 mM mannitol and 10 mM labeled sugar.

Parameter	Sucrose	F-sucrose
Total uptake (nmol g^{-1} h^{-1})	248	272
Cytoplasm		
$k(h^{-1})$	7.3×10^{-2}	6.7×10^{-2}
$t_{1/2}$ (min)	9.4	10.3
Vacuole		
$k(h^{-1})$	9.5×10^{-4}	7.9×10^{-4}
$t_{1/2}$ (min)	730	900

In the concentration range of 0.2 to 10 mM, uptake of F-sucrose was biphasic in immature (4-week-old) root tissue (Fig. 4), similar to sucrose uptake kinetics observed with mature root discs (17). Moreover, rates and kinetics of sucrose uptake compared well with that of F-sucrose in this tissue. The presence of 10 mM unlabeled sucrose inhibited F-sucrose uptake resulting in almost complete abolishment of the saturable component. These data clearly indicate that a sucrose-specific carrier was present in immature roots.

The results indicate that F-sucrose in phloem translocated in sugar beets as it is in soybeans (11). Both sucrose and F-sucrose accumulated in the root and to a lesser extent in the petiole. Presence of relatively large quantities of radiolabeled sugars in the petiole is consistent with the role of this sink in buffering the flow of sucrose to the root during periods of high or low assimilate export (5).

As in mature roots (6, 7, 23), sucrose unloading in immature taproot is suggested to be apoplastic. This contention is based on the collective interpretation of the following observations: (a) A significant portion (30%) of the total radioactivity applied to leaves and translocated to the root (*in vivo*) was recovered in the apoplast compartment. Although the presence of radioactivity in the apoplast may have been due to sugar leakage from the cells rather than direct arrival into the apoplastic compartment, it is unlikely that the entire 30% was the result of leakage alone. This assumption is supported by the observation that a major product in the apoplast was expected to be [^{3}H]fructose resulting from the hydrolysis of [^{3}H](fructosyl)-sucrose. Indeed, [^{3}H]fructose accounted for as much as 27% of ^{3}H label compared to 5% for glucose in the root wash media. In case of hexose leakage, radioactivity would be distributed equally between fructose and glucose (intracellular isomerization prior to efflux to the apoplast); (b) F-sucrose was taken up from the external solution (apoplast) by immature root segments. This observation in and of itself does not, however, prove the apoplastic unloading pathway *in vivo*.

If sucrose unloading is apoplastic but a sucrose carrier is lacking, then hexose uptake would be obligatory and a negligible amount of F-sucrose would be expected to accumulate in the roots. For example, in corn roots (9), a tissue lacking a sucrose carrier, F-sucrose uptake rate was four times less than that of sucrose. Since more ^{14}C than ^{3}H accumulated in the roots (*in vivo*) and because F-sucrose uptake by 4-week-old root sections contained a saturable component, subject to inhibition by sucrose, presence of a sucrose carrier is indicated.

F-sucrose is metabolized by sucrose synthase to UDP-glucose and 1′-fluorofructose. Pavlinova and Prasolova (15) and Giaquinta (8) proposed that sucrose synthase was the major enzyme responsible for sucrose metabolism in sugar beet, replacing acid invertase approximately 40 d after emergence. Despite high su-

Table III. *F-Sucrose Uptake in Mature (6 Months Old) Sugar Beet Root Tissue*

Tissue discs were incubated for 1 h in solutions containing 5 mM labeled F-sucrose (pH 5.5). One set of tissue was trated for 30 min with 1 mM PCMBS before uptake was initiated (passive uptake). Carrier-mediated (active) uptake is the difference between control and PCMBS treated. Data points are means of 3 replicates ± SE.

	Control	Uptake		PCMBS Inhibition
		Active	Passive	
	nmol g⁻¹ fresh wt·h⁻¹			%
^{14}C-1′-Fluorosucrose	154 ± 8	71 ± 3	83 ± 5	46
^{14}C-1′-Fluorosucrose + 5 mM sucrose	131 ± 3	49 ± 2 (31%)[a]	82 ± 2	38

[a] Inhibition of active F-sucrose uptake by 5 mM sucrose.

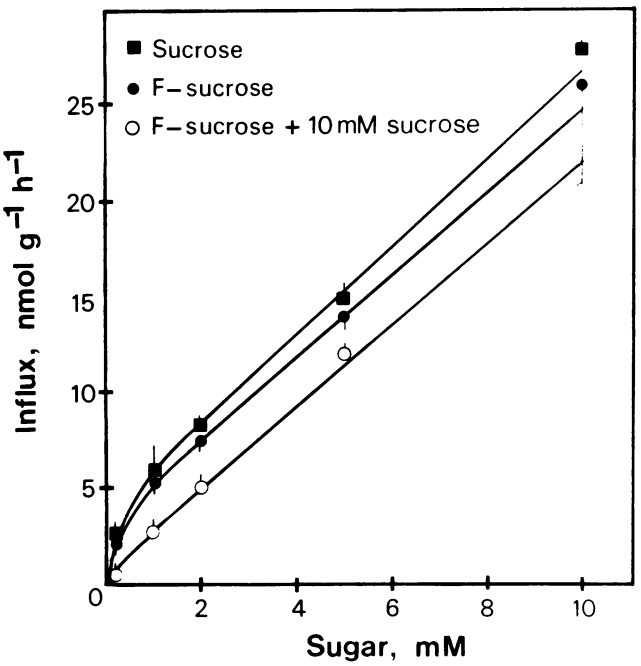

FIG. 4. Concentration-dependent uptake kinetics of sucrose and F-sucrose in the presence or absence of 10 mM unlabeled sucrose in 4-week-old taproots of sugar beet. After 1 h preincubation, 75 mg tissue was incubated for 30 min at pH 5.5. Data points are means of 2 replicates ± range.

crose synthase activity in roots, the pattern of ^{14}C labeling among the sugar fractions was very similar to the one observed in the petiole where no sucrose synthase activity was detected (Fig. 2A). Also, we detected no change in ^{14}C appearance in various fractions from 4- or 7-week-old roots where sucrose synthase activity is high (20). Therefore, it appeared that there was no correlation between F-sucrose metabolism and sucrose synthase activity. However, Schmalstig *et al.* (18) observed F-sucrose metabolism by sucrose synthase in sugar beet sink leaves in which unloading occurs symplastically. A tentative explanation would be that, in the root, F-sucrose was not accessible to the cytoplasmic sucrose synthase. This bypass of the cytoplasm has been suggested by Wyse (23) from the observation of plasma membrane invaginations bringing the plasmalemma and the tonoplast in physical contact (12). We do not have anatomic evidence to substantiate this hypothesis in immature roots but pronounced vacuolization of the sugar beet parenchyma cells has been reported at this stage (1). Furthermore, the lack of correlation between sucrose synthase activity and F-sucrose metabolism may be explained by the assumption that the sucrose synthase assay did not reflect *in vivo* activity. This enzyme is under strict regulation in the cytoplasm (3) and it is possible that this regulation was lost during extraction, leading to an overestimation of its activity. Obviously, further studies are needed to elucidate the role and regulation of this enzyme in sucrose metabolism *in vivo*.

Mechanisms of carrier-mediated sugar transport across the plasmalemma and the tonoplast of immature sugar beet roots are presented in Figure 5. In mature sugar beet roots, the mechanism of sucrose accumulation in the vacuole is reported to be an active H^+/sucrose antiport system located at the tonoplast (16). However, isolated vacuoles from red beet roots were shown to be able to synthesize and simultaneously transport sucrose and sucrose phosphate using a UDP-glucose dependent group translocator (21). Nevertheless, these vacuoles were able to take up sucrose (UDP-glucose-independent) at a similar rate. While we did not directly investigate the presence of such a group translocator in our material, it is obvious that F-sucrose was accumulated inside the parenchyma cells (Table II). In the group

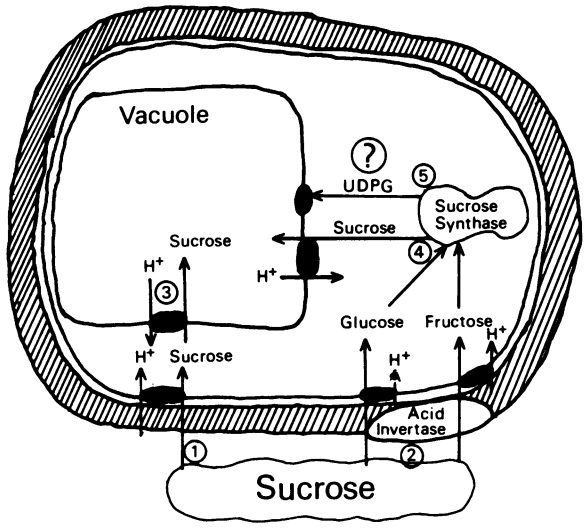

FIG. 5. Schematic presentation of carrier-mediated apoplastic sugar transport into the storage cells of immature sugar beet roots and possible pathways for transport and accumulation in the vacuole. Sucrose may also cross the plasmalemma (as intact molecule or hexose) diffusionally. Numbers are pathways associated with carrier-mediated transport. 1, Sucrose/H^+ co-transport; 2, sucrose hydrolysis and subsequent hexose/H^+/cotransport; 3, sucrose/H^+ antiport across tonoplast; 4, resynthesis of sucrose from hexose in the cytoplasm; 5, UDPG dependent group translocation across the tonoplast. (●), Membrane associated sugar specific carriers.

translocator mechanism, UDP-glucose is the primary donor for both glucose and fructose moieties of sucrose (21). In our material, if part of the UDP-glucose, derived from F-sucrose (asymmetrically labeled in the glucose moiety) had been used by a group translocator, then we should have recovered some label in the fructose fraction of the root extract. However, we did not detect any loss of asymmetry in F-sucrose. Therefore, the group translocator if at all present, does not contribute to sucrose accumulation in sugar beet taproot and the proton-sucrose antiport system may play the major role. Comparative studies are needed to determine the relative importance of each system *in vivo*.

The presence of a sucrose carrier in this tissue would mean that sucrose hydrolysis is not an absolute requirement for sugar accumulation. The question is then raised as to the relative importance of the sucrose carrier and the cell-wall invertase. Cell-wall invertase is believed to support high sink activity in rapidly growing tissues by maintaining a steep sucrose concentration gradient between source and sink regions (14). Eschrich (4) proposed that inversion of sucrose to hexose in the apoplast prevents sucrose reloading into the phloem of sink tissue. Furthermore, cell-wall invertase may facilitate regulation of metabolic (hexose) *versus* storage (sucrose) pools (20).

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