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Communication

Evidence for the Uptake of Sucrose Intact into Sugarcane Internodes

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ABSTRACT

Application of [^{14}C]fructosyl sucrose was used to determine whether sucrose cleavage was necessary for sucrose uptake by sugarcane (*Saccharum* spp.) internode tissue. Although approximately 25% of ^{14}C in the apoplast was present as fructose, indicating some sucrose cleavage, less than 15% of the label was randomized in the sucrose that remained in the tissue after a 30 minute osmoticum rinse. This is insufficient to support cleavage and resynthesis as the sole sucrose transport scheme. The lack of randomization of label between the glucose and fructose moieties of the sucrose molecule was taken as presumptive evidence that sucrose does not have to be cleaved prior to uptake by parenchyma cells in sugarcane internode tissue.

It is generally believed that sucrose must be cleaved to glucose and fructose prior to transport into the storage tissue of sugarcane (*Saccharum* spp.) (10–12). Maretzki and Thom (16, 20) proposed a group transporter functioning at the tonoplast to be responsible for sucrose transport in sugarcane, but recent reports have suggested that the earlier results may have been in error (17, 18). A group translocator for sucrose uptake has also been proposed for beet roots (9, 19) and grape berries (5). However, there is some evidence that the uridine 5′-diphosphoglucose group transporter is not the sole sucrose transport mechanism in sugarbeet roots (13). Also, studies on sugar uptake in the stem tissue of sweet sorghum, a plant closely related to sugarcane, showed sucrose cleavage was not required for transport into the vacuole (14).

Most of the studies that suggested sucrose cleavage as a prerequisite for sugar transport in sugarcane were done using immature tissue or suspension cultures (3, 4, 10, 20), not mature, sucrose-storing internode tissue. De Leon *et al.* (7) showed that transport mechanisms in isolated vacuoles may be impaired by the buffers used during isolation. Critical evaluation of the literature also reveals uptake studies on tissue over long periods of time (2, 3, 10), during which additional invertase activity may have been induced (1), and studies on tissue rinsed in tap water (2, 3, 10), which would introduce the complication of turgor effects known to have an effect on sucrose transport in other tissues (6, 22).

Lack of randomization of label after the application of fructosyl-labeled [^{14}C]sucrose has been used as evidence in support of the presence of a sucrose carrier in other tissues (21). In the following study this method was used to investigate whether sucrose uptake is accompanied by sucrose cleavage in immature (meristematic and elongating) and mature storage tissue of sugarcane.

MATERIALS AND METHODS

All plant material was obtained from a greenhouse in Weslaco, TX. Stems of the sugarcane (*Saccharum* spp.) cultivar CP 70-321 were sampled when the stem had 10 aboveground internodes. Internode 1 from the top was identified as that subtended by the leaf with the topmost exposed collar. Tissue from internodes 1 (meristematic), 3 (elongating), and 5 (sucrose-storing) was sampled using a cork borer 0.8 cm in diameter, and 1.0 to 1.5 mm discs were cut freehand from the cylinders using a razor blade. Discs were rinsed in 250 mM mannitol, 1 mM $CaCl_2$, and 25 mM K-Mes (pH 5.5) for 60 min with several changes of solution prior to uptake experiments. Groups of 15 discs were blotted, weighed, and transferred to culture tubes containing 1.5 mL of 1 mM [^{14}C] fructosyl sucrose (specific activity 69.1 kBq μmol^{-1}), 249 mM mannitol, and 25 mM K-Mes (pH 5.5). During a 3 h uptake period, humidified air was bubbled through the solutions to agitate and aerate the tissue, and solutions were changed at 1 and 2 h to minimize sucrose depletion from the medium. After uptake, discs were rinsed 30 min at 30°C in 250 mM mannitol, 1 mM $CaCl_2$, and 25 mM Mes (pH 5.5) with changes at 2 and 8 min. Tissue was then heated to 60°C in 1.5 mL of 80% ethanol for 30 min, and placed at −20°C overnight. Radioactivity in 100 μL aliquots of each sample was determined by scintillation spectroscopy.

Sugars in the first, 2-min rinse and ethanol extract were separated by HPLC using an Aminex HPX-87C[2] column (Bio-Rad, Richmond, CA), and fractions were collected in 7 mL scintillation vials. Fractions were counted to determine ^{14}C in sucrose, glucose, and fructose. The sucrose fraction was collected, freeze-dried, treated with 10 units of invertase (pH 4.5) at 37°C for 30 min, chromatographed, and the glucose

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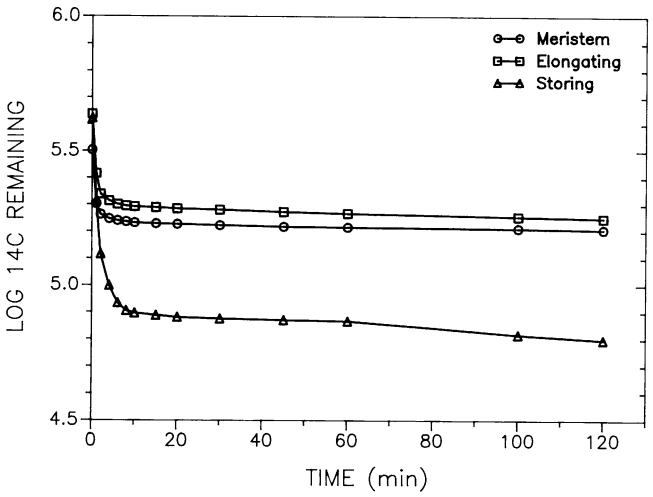

Figure 1. Washout curves of soluble ^{14}C from meristematic (internode 1), elongating (internode 3) and sucrose-storing (internode 5) sugarcane internode tissue. Discs were incubated in 1 mM [U-^{14}C] sucrose, 249 mM mannitol, 1 mM $CaCl_2$, 25 mM K-Mes (pH 5.5) for 3 h, then rinsed with frequent changes in 250 mM mannitol, 1 mM $CaCl_2$, 25 mM K-Mes (pH 5.5) for 2 h, followed by extraction with 80% ethanol.

Table I. *^{14}C as Free Fructose and Sucrose and Ratio of Labeled Glucose to Fructose in Sucrose in the First 2-Minute Rinse and Ethanol Extracts of Sugarcane Storage Tissue*

Tissue discs were cut from internode 1 (top, meristematic), internode 3 (elongating), and internode 5 (sucrose-storing), and incubated for 3 h in 1 mM [^{14}C]fructosyl sucrose, 1 mM $CaCl_2$, 249 mM mannitol, 25 mM Mes (pH 5.5), rinsed for a total of 30 min in 250 mM mannitol, 1 mM $CaCl_2$, 25 mM Mes (pH 5.5), and extracted in 80% ethanol. Values are means of four replicates ± SE.

	Fructose	Sucrose	Sucrose Glc/Fru
	Bq ± SE		
2-Min rinse			
Meristem	1469 ± 499	4317 ± 210	0.0089 ± 0.0083
Elongating	3535 ± 273	6458 ± 344	0.0032 ± 0.0010
Storing	3543 ± 338	9692 ± 528	0.0004 ± 0.0001
Ethanol			
Meristem	671 ± 277	883 ± 55	0.0250 ± 0.0100
Elongating	172 ± 15	908 ± 53	0.0830 ± 0.0500
Storing	204 ± 22	584 ± 19	0.0930 ± 0.0100

and fructose fractions were counted. There were four replications, and data were analyzed by analysis of variance.

For the washout study, discs were incubated 3 h as above with 1 mM [U-^{14}C]sucrose (69.1 kBq μmol^{-1}). During a 2 h rinse, the solutions were changed at frequent intervals, and radioactivity in the rinses and the 80% ethanol-soluble extract was determined. Reducing sugar in each wash was determined by the method of Dygert *et al.* (8), and total sugar was determined by the same method after treatment with invertase. Sucrose was calculated by subtraction.

RESULTS AND DISCUSSION

The results of the washout studies (Fig. 1) are consistent with a three-compartment system (15). The first few washes after the uptake period removed sugar from the apoplast and sugar adhering to cell walls, while sugar remaining after 30 min was in a long-term storage compartment. In the washout study, sucrose was chemically detectable only in the first wash and ethanol extract of the meristematic and elongating tissues. After rinsing for 2 h, the ethanol extract contained 80 to 90% of the reducing sugar, and 95 to 99% of the sucrose extractable from the tissue. Since the phloem is unlikely to contain large amounts of reducing sugars (23), the most likely long-term storage compartment in the tissue is the vacuole.

After incubation with [^{14}C]fructosyl sucrose, the tissue was rinsed for 2 min. The presence of [^{14}C]fructose in this wash indicates that there was cleavage of sucrose in the apoplast (Table I), but more than 65% of the ^{14}C in this compartment was still sucrose with a labeled glucose to fructose ratio of less than 0.009. The glucose to fructose ratio of the applied [^{14}C] sucrose was less than 0.0001. The increase in this ratio indicates a small amount of randomization, implying some cleavage and resynthesis occurring.

After an additional 28 min of rinsing, sugar remaining in the tissue was extracted with ethanol. There was free [^{14}C] fructose in the ethanol extracts (Table I), especially in the meristematic tissues. However, the ratio of labeled glucose to fructose in sucrose in the storage compartment was low in all tissues, indicating less than 15% randomization of label. The group transporter hypothesis of Maretzki and Thom (16, 20) requires complete randomization for uptake into the vacuole, since they have reported that [^{14}C]fructose was not taken up by isolated vacuoles, but required conversion through UDPG for transport. In this study, however, although some randomization of label was observed in sucrose from ethanol extracts, it was not nearly enough to substantiate a requirement for sucrose cleavage and resynthesis during transport.

Maretzki and Thom (16, 20) have presented the strongest evidence that sucrose must be cleaved and resynthesized for transport into sugarcane, although the presence of a group transporter has recently been questioned (17, 18). The data presented here also would seem to be in conflict with their initial data. However, the isolation of vacuoles from suspension cultures and sugarcane tissue may inactivate a sucrose transporter, as well as stimulate the production of saccharides not normally produced in sugarcane (18). It has been shown that transport into vacuoles isolated from barley (*Hordeum vulgare*, L.) mesophyll cells is affected by the buffer used during isolation (7). The evidence presented here shows that the mechanism of sucrose transport in sugarcane is still open to question. Further research on sugar transport in sugarcane using intact tissue would be helpful in clarifying the understanding of this process.

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