Sucrose Translocation and Storage in the Sugar Beet

ROBERT T. GIAQUINTA
Central Research and Development Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898

ABSTRACT

Several physiological processes were studied during sugar beet root development to determine the cellular events that are temporally correlated with sucrose storage. The prestorage stage was characterized by a marked increase in root fresh weight and a low sucrose to glucose ratio. Carbon derived from $^{14}$C-sucrose accumulation was partitioned into protein and structural carbohydrate fractions and their amino acid, organic acid, and hexose precursors. The immature root contained high soluble acid invertase activity ($V_{max}$ 20 micromoles per hour per milligram protein; $K_m$ 2 to 3 millimolar) which disappeared prior to sucrose storage. Sucrose storage was characterized by carbon derived from $^{14}$C-sucrose uptake being partitioned into the sucrose fraction with little evidence of further metabolism. The onset of storage was accompanied by the appearance of sucrose synthetase activity ($V_{max}$ 12 micromoles per hour per milligram protein; $K_m$ 7 millimolar). Neither sucrose phosphate synthetase nor alkaline invertase activities were detected during beet development. Intact sugar beet plants (containing a 100-gram beet) exported 70% of the translocate to the beet, greater than 90% of which was retained as sucrose with little subsequent conversions.

In spite of the agronomic importance of translocation, the cellular processes associated with the partitioning of translocate to the harvestable portions of crops represent one of the most poorly understood areas in translocation. In terms of import region physiology, the mechanism of sucrose storage in sugarcane stalks has perhaps received the most investigation (5–7). The conclusion reached from these studies was that sucrose translocated to the stalk enters the free space where it is hydrolyzed by a cell wall acid invertase (6). The resulting hexoses are actively accumulated into the storage parenchyma and resynthesized to sucrose phosphate by sucrose phosphate synthetase. Sucrose transport into the storage vacuole is presumably mediated by a specific sucrose phosphate phosphatase (7). In the mature cane stalk, alkaline invertase has been proposed to regulate sucrose storage and utilization. In contrast, immature cane contains an intracellular acid invertase in addition to a cell wall acid invertase. During cane development the intracellular acid invertase activity disappears with a concomitant increase in alkaline invertase activity (5).

The sucrose storage mechanism in sugar beet roots differs from that in the sugarcane. The Russian investigators (10–12, 14) have studied sugar beet root metabolism extensively and have proposed that sucrose synthetase, a reversible enzyme catalyzing both sucrose hydrolysis and synthesis, plays a pivotal role during sucrose accumulation. In the sugar beet, sucrose produced in the leaves may enter the apoplast of the root prior to its accumulation into the storage parenchyma (8, 16), but unlike the cane, free space hydrolysis is not a prerequisite for uptake (3). Recently, Stein and Willenbrink (16) have shown a correlation between sucrose accumulation and energy charge during beet development, and have suggested that this is consistent with an energy-dependent concentration step occurring at the storage parenchyma plasmalemma.

In this study, several cellular processes, including sucrose concentration, carbon partitioning, and enzymology, were studied during sugar beet root development to determine their temporal relationship with the process of sucrose storage. Additionally, the metabolic fate of $^{14}$CO$_2$-derived translocate was determined in mature, sugar-storing beets.

MATERIALS AND METHODS

Sugar beet plants (Beta vulgaris, monogerm hybrid, size 3) were grown for 4 months in a controlled environment under the following conditions: 12-h photoperiod, 3,500 ft-c, 23/17 C day-night temperature, and 60% RH. $^{14}$C-Metabolite distributions following $^{14}$C-sucrose uptake and translocation of $^{14}$C-assimilates were determined by ion exchange and paper chromatography as described previously (3, 4). Sucrose and glucose in the neutral fraction were determined by the Wortingham Statzyme assay. Distribution of $^{14}$CO$_2$-assimilates was determined by pulse-labeling the source leaf of five different sugar beet plants, with approximately 30 $\mu$Ci of $^{14}$CO$_2$ for 10 min followed by $^{14}$CO$_2$ exposure for up to 10 h in the light. Each plant was trimmed to a simplified source-path-sink leaf-beet system about 16 h before the experiment. The beet weight of the plants was about 100 g. At 0.5, 1, 2, 5, and 10 h after the $^{14}$CO$_2$ exposure the plant parts were collected, frozen in solid CO$_2$, and lyophilized for later analysis (4). For the enzyme studies, root tissue was homogenized in 85 mM Hepes (pH 8), 20 mM dithioerythritol, 20 mM sodium diethyl dithiocarbamate, 15 mM EDTA (pH 7), and 1.5% Polyclar AT (4). The 20,000g supernatant was brought to 65% saturation with ammonium sulfate at 4 C and after 30 min the 10,000g pellet was resuspended in 5 mM Hepes (pH 7) and passed through a G-25 column (4). Invertase, sucrose synthetase, and sucrose phosphate synthetase were determined as described previously except that the glucose and sucrose formed in the invertase and sucrose synthetase reactions were determined by the Statzyme reagent.

RESULTS AND DISCUSSION

Beet Growth and Sucrose Content. The time course of growth and sucrose accumulation during beet ontogeny was used as a framework to investigate various cellular correlates of the storage process. Figure 1 shows the increase in root fresh weight during beet development. During the first 60 to 70 days after planting, the root fresh weight increased more than 5 $\times$ 10$^3$-fold (0.013 to 70 g), and by 120 days the beets weighed about 250 g under our growth conditions. Concomitant with the increase in root weight, sucrose concentration increased markedly reaching 40% sucrose

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on a dry weight basis (about 10% on a fresh weight basis) by 120 days (Fig. 2). Immature 8-day-old roots had a low sucrose to glucose ratio while the reverse was true for the mature beet (Table I). Sucrose storage commenced between 30 and 60 days (1–20 g beet weight) (Fig. 2), and this time scale was used to study changes in carbon partitioning and enzymology in relation to beet growth and sucrose storage.

Carbon Partitioning. The metabolite distribution of $^{14}$C derived from $^{14}$C-sucrose accumulation into root tissue during beet development is shown in Figure 3. The young root is characterized by an equal distribution of $^{14}$C between the insoluble (protein, structural carbohydrates) and soluble fractions. During the first 30 to 40 days (beet weight < 1 g), the partitioning of carbon changed markedly with greater than 90% of the $^{14}$C remaining in the H₂O soluble fraction. Fractionation of the water-soluble components showed a similar change in partitioning of $^{14}$C from the amino acid and organic acid precursors of the insoluble components into the sugar fraction (neutral). That little $^{14}$C was retained in sucrose in young roots following $^{14}$C-sucrose uptake agrees with the data in Table I showing low sucrose content in immature roots. The decline in the distribution of $^{14}$C into hexoses was accompanied by a reciprocal increase in the $^{14}$C partitioned into the sucrose fraction. These data agree with the observation that young, actively growing roots utilize sucrose primarily for growth and metabolism.

The onset of sucrose storage was accompanied by the majority of $^{14}$C-sucrose entering the sucrose storage compartment. Recently, we reported a similar trend in carbon partitioning during the leaf import-export transition, another process where an assimilate-utilizing region (young leaf) differentiated into an assimilate-accumulating (sucrose) and exporting source leaf (4).

Enzymology. Russian workers have postulated that sucrose synthetase plays an important regulatory role in the sucrose storage process (9–11). Additionally, they have shown the presence of invertease activity in young sugar beet roots (1, 12). We determined the activities of these enzymes along with sucrose phosphate synthetase during beet development with particular emphasis on their temporal relationship to growth, sucrose storage, and carbon partitioning (Figs. 1–3). The immature root contained high invertase activity which dramatically decreased during the first 30 days of growth, the period before sucrose storage began (Fig. 4). Sucrose synthetase activity (Fig. 4), absent during the prestorage stage, appeared by 30 to 35 days and increased parallel with sucrose accumulation (Fig. 2). Sucrose phosphate synthetase activity, which catalyzes sucrose synthesis in both the mature sugarcane stalk (5) and exporting sugar beet leaves (4), was very low at all stages of beet development. The high invertase activity most probably accounted for the low sucrose content and extensive carbon partitioning in the young root. The majority of the invertase activity (> 90%) was soluble rather than cell-wall-bound, indicating an intracellular location. The invertase obtained from
(NH₄)₂SO₄, showed a $V_{\text{max}}$ of 18 \( \mu \text{mol/h \cdot mg protein} \) and a $K_m$ of 2.1 \( \text{mm} \) (Fig. 5). The $K_m$ of 2 to 3 \( \text{mm} \) for sucrose agrees well with the $K_m$ of invertase from sugar beet source and sink leaves (4).

The $V_{\text{max}}$ of invertase, however, was about 4- to 5-fold higher in the young roots than in the leaves. Unlike sugarcane, beet development is not accompanied by the appearance of substantial alkaline invertase activity (Fig. 6). It should be noted that although the activity of invertase was low in the mature beet (Fig. 6), there was a slightly higher activity at the alkaline pH values compared to pH 5, suggesting that a low level of neutral invertase may be present in the mature beet as suggested by Wyse (17).

These data indicate that the change in metabolism of the young beet is correlated with the loss in the ability (invertase activity) to hydrolyze sucrose. Invertase inhibitors have been found in many storage tissue, including the beet (15), thus the decrease in invertase activity may have been caused by the formation of an invertase inhibitor.

The onset of sucrose storage was characterized by the appearance of the protein fraction precipitating at 65% (NH₄)₂SO₄ had a $V_{\text{max}}$ of 110 \( \mu \text{mol/h \cdot mg protein} \) and a $K_m$ of 2.9 \( \text{mm} \) for sucrose. Invertase activity from 8-day-old beet roots, assayed from a desalted, 20,000g homogenate supernatant, not concentrated with
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Fig. 6. pH dependence of invertase activity in immature and mature sugar beet roots. Days refer to time after planting. Activities determined on protein fraction precipitating at 65% (NH₄)₂SO₄.

Fig. 7. Kinetic parameters of sucrose synthetase activity of a mature sugar beet root. Beet weight was approximately 700 g. Activity determined on protein fraction precipitating at 65% (NH₄)₂SO₄.

Fig. 8. Metabolic fate of ¹⁴C₀₂-derived translocate in the mature beet of an intact plant.
the vacuole may be coupled to the co-transport of protons. Further information is needed on the processes associated with sucrose storage in the beet in particular, and with assimilate utilization in the harvestable portions of crop plants in general before the ultimate goal of photosynthate regulation is achieved.

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LITERATURE CITED

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