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Identification of Actively Filling Sucrose Sinks[1]

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ABSTRACT

Certain actively filling plant sucrose sinks such as a seed, a tuber, or a root can be identified by measuring the uridine diphosphate and pyrophosphate-dependent metabolism of sucrose. Sucrolysis in both active and quiescent sucrose sinks was tested and sucrose synthase was found to be the predominant sucrose breakdown activity. Sucrolysis via invertases was low and secondary in both types of sinks. Sucrose synthase activity dropped markedly, greater than fivefold, in quiescent sinks. The tests are consistent with the hypothesis that the sucrose filling activity, *i.e.* the sink strength, of these plant sinks can be measured by testing the uridine diphosphate and pyrophosphate-dependent breakdown of sucrose. Measuring the initial reactions of sucrolysis shows much promise for use in agriculture crop and tree improvement research as a biochemical test for sink strength.

The acknowledged prominence of sinks in agricultural production work has raised many questions about how sink yield can be increased. In plant biology one speaks about 'sink strength,' thereby implying that some sinks are stronger in receiving and/or metabolizing assimilate than others. How can sink strength within a plant be measured, or what are the determinants of sink strength? Since the proportion of imported assimilate used for respiration in some sinks can be substantial (over 40% in barley roots, 9), conventional methods of measuring sink strength, *e.g.* growth rate, net accumulation of dry matter, or the synthesis rate of carbon reserves, fail to assess the true ability of a sink tissue to receive and to metabolize assimilate (14). Ho (14) suggests that the import rate of assimilate, measured as the sum of the net carbon gain and respiratory carbon loss, by a sink organ should give a more appropriate estimate of sink strength, but he also does not report a biochemical method for measuring sink strength.

It is known that sucrose is the major form of translocated carbon in most plant species; hence, it is the major form of carbon which plant sinks grow on (5, 31, 33). When translocated sucrose arrives at a recipient plant cell it enters the cytoplasm through plasmadesmata or by crossing the plasma membrane either via a sucrose carrier or by diffusion (5). Sucrose is fed into metabolism either by sucrose synthase or by an invertase (5, 15, 31, 32); indeed, we have reassessed plant glycolysis and gluconeogenesis recently (31) and would propose that they should be more correctly termed sucrolysis and sucroneogenesis. Before recent work it was customary to assume that sucrose synthase action resulted in the formation of UDP-glucose and then other nucleotide sugars leading into sugar polymer synthesis, such as plant cell walls. However, a substrate level pool of PPi was measured in plants (2, 7, 27), and we successfully tested the pyrophosphorolysis of UDP-glucose feeding glucose 1-P directly into plant metabolism (32). These steps are an integral part of the recently proposed sucrose synthase pathway (1, 15, 30). Then, of course, from glycolysis carbon can be directed into essentially every metabolic activity of a cell. Therefore, the ability of a tissue or organ to metabolize sucrose must be one determinant of sink strength. Here we have tested the feasibility of biochemically measuring sucrose sink strength by assaying sucrose cleavage activities, *i.e.* sucrolysis via either the invertases or by the sucrose synthase pathway. The reasons we developed this UDP and PPi-dependent assay for sucrose synthase activity were (a) to measure activity in the sucrose breakdown direction whereas many other workers measured the opposite, namely sucrose synthesis (3, 4, 22, 26), and (b) others who measured sucrose breakdown often assayed for UDP-glucose accumulation as a precursor of the synthesis of cell walls or other nucleotide sugars (6, 22). But in our assay we couple through the PPi-dependent UDP-glucopyrophosphorylase, which is very active in plants, to the formation of glucose 1-P which feeds carbon directly into glycolysis and possibly on to starch formation (31, 32).

The hypothesis we test here is that certain actively filling plant sinks can be identified by measuring UDP and PPi-dependent sucrolysis. The experiments demonstrate that sucrose synthase is the predominant sucrose breakdown activity in the cytoplasm of actively filling sinks whereas its activity decreased more than fivefold in mature or quiescent sinks. We can distinguish the sink strength even of similar sinks, *e.g.* individual tubers of similar weight attached to the same potato stolon. Sucrolysis by invertase(s) is secondary in these sinks. Thus, the UDP and PPi-dependent sucrose synthase pathway can be used as a biochemical measurement of sink strength.

MATERIALS AND METHODS

Plant Materials

Potato (*Solanum tuberosum*), lima bean (*Phaseolus lunatus*), and cassava (*Manihot esculenta* Crantz) plants were grown with good cultural practices either in a greenhouse or in field plots. Both sweetgum (*Liquidambar styraciflua* L.) and pecan (*Carya illinoinensis* (Wangenh.) C. Koch) seedlings were in the second growing year in field plots.

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Enzyme Extraction from Plant Organs

For tree roots, enzymes were extracted from the cambial zone which includes xylem mother cells and ray cells. All plant tissues except stored potato tuber, cassava root, and lima bean seed, were freshly harvested. One to 3 g of tissues were powdered and homogenized in liquid N_2 with a mortar and pestle. The extraction solution contained 200 mM Hepes/NaOH (pH 7.5), 3 mM magnesium acetate, 5 mM dithiothreitol (DTT), 2% (v/v) glycerol, and 1% (w/v) soluble and 1% (w/v) insoluble PVP. For the extraction of tree tissues, in addition to the above mentioned solution, we changed or included: 10% glycerol, 10% (w/v) soluble PVP, and 1% (w/v) Dowex-1. The ratio of tissue weight (g) and extraction solution volume (mL) was 1:5. The homogenate was passed through one layer of Miracloth and centrifuged at 34,000*g* for 20 min at 4°C. The supernatant was then fractionated with 30 to 70% $(NH_4)_2SO_4$. The 70% $(NH_4)_2SO_4$ pellet was resuspended in a solution of: 10 mM Hepes/NaOH (pH 7.5), 2 mM DTT, and 2 mM Mg acetate. A Sephadex G-25 column was used for desalting all samples. We carefully checked enzyme recovery in crude soluble extracts *versus* the $(NH_4)_2SO_4$ fractionation and found over 85% recovery with excellent recovery of sucrose synthase.

Enzyme Assays

The assay for sucrose synthase was previously described in detail (32). Two endogenous plant enzymes were involved in this assay, *i.e.* sucrose synthase and UDP-glucopyrophosphorylase. The 1 mL reaction mixture contained 100 mM Hepes/NaOH (pH 7.5), 3 mM Mg acetate, 5 mM DTT, 0.02 mM glucose 1,6-bisphosphate, and 0.5 mM NAD. Sucrose (50 mM), UDP (1 mM), and PPi (1 mM) were added to start the reactions. The glucose 1-P produced was coupled via phosphoglucomutase (2 units) and *Leuconostoc* glucose-6-P dehydrogenase (2 units) to form 6-phosphogluconate and NADH. NADH production was monitored spectrophotometrically at 340 nm with a Beckman DU-7. Since UDP-glucopyrophosphorylase activities were always over 1 unit/mg protein in these plant extracts, the limiting activity measured was sucrose synthase.

The invertases were assayed at pH 5.0 and 7.0 for acid and neutral invertase, respectively. Sucrose, 50 mM, was added to the incubation buffer (70 mM K_2HPO_4/40 mM citrate for acid invertase and 160 mM K_2HPO_4/20 mM citrate for neutral invertase) containing plant extracts. The standard incubation time was 15 min at 25°C and reactions were stopped by boiling. Aliquots of incubation mixture were added to the assay mixture: 100 mM Hepes/NaOH (pH 7.5), 3 mM Mg acetate, 5 mM DTT, 0.02 mM glucose 1,6-bisphosphate, 0.5 mM NAD with hexokinase (2 units), and glucose 6-P dehydrogenase (2 units) as coupling enzymes. ATP, 1 mM, was added to start the reaction and the reaction was completed when there was no more increase at A_{340} nm. All enzyme assays have been described in more detail earlier (1, 27, 32). Extract protein contents were determined using the Bradford procedure with BSA as the standard protein.

RESULTS AND DISCUSSION

A major role for the sucrose synthase-initiated sucrose breakdown pathway is illustrated in Table I with several plant sink tissues. Each of these tissues, *i.e.* lima bean seeds, potato tubers, and deciduous tree seedling or cassava roots, when actively growing and/or filling with nonstructural carbohydrates, is a sucrose sink. There the UDP and PPi-dependent sucrose breakdown is much more active than either the acid or neutral invertase. However, in storage tissues and in quiescent tree roots which are not importing sucrose as in the winter (2), the sucrose synthase pathway activity is lower (Table I). All invertase activities tended to be nearly equal and low in the sink tissues in Table I.

With each of the active or quiescent sink tissues in Table I, we have measured the activities of other glycolytic enzymes, namely, PPi-dependent phosphofructokinase, ATP-dependent phosphofructokinase, fructokinase, glucokinase, UDP-glucopyrophosphorylase, or phosphoglucomutase and found none of these activities followed a pattern like sucrose synthase.

Sucrolysis in Developing Lima Bean Seeds

Thus, we predicted from tests as in Table I that growing filling sucrose sinks should have different amounts or specific activities of sucrose synthase indicative of differences in their sink strengths. Indeed, in a more detailed study with lima bean seeds throughout their development, we found that the total amount of sucrose synthase activity increased with increasing seed fresh weight to near seed maturation when total activity began to decline (Fig. 1). Both specific and total activities of either invertases were less than 2% of the sucrose synthase activities found in lima bean seeds at any stage of embryogenesis. The specific activity of sucrose synthase dropped in these developing seeds (Fig. 1), but this is due

Table I. *Activity of Sucrose Breakdown Enzymes in Plant Sucrose Sink Tissues*

	Sucrose Synthase	Acid Invertase	Neutral Invertase
	milliunits/mg protein		
Active sinks			
Growing potato tuber			
19 g	196	3	6
78 g	188	9	2
Growing lima bean seed			
82 mg	69	1	1
167 mg	76	<1	1
Summer pecan root	123	5	2
Summer sweetgum root	112	13	7
Growing cassava root	110	2	0
Quiescent sinks			
Stored potato tuber			
19 g	2	<1	1
64 g	4	14	1
Dry lima bean seed	1	<1	1
Winter pecan root	18	10	5
Winter sweetgum root	22	8	3
Stored cassava root	22	3	4

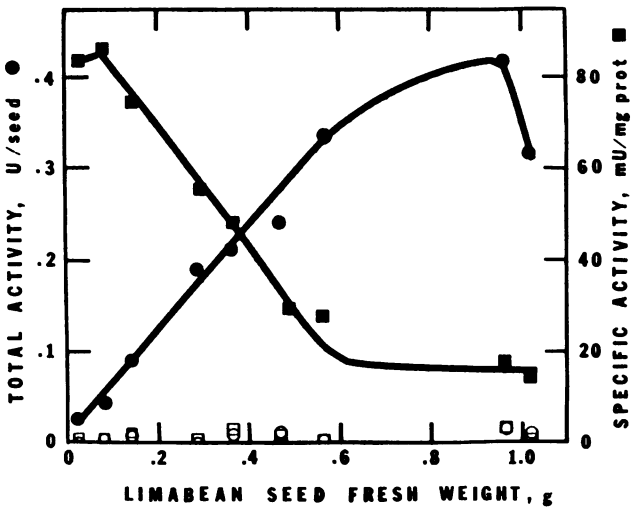

Figure 1. Sucrose cleavage enzyme activities in developing lima bean seeds of various weights. Lima bean seeds were especially selected from field grown freshly harvested pods with all seeds of similar weight. Seeds with a weight of less than 10% deviation from the average were composited. Sucrose synthase total activities (●) are per seed while sucrose synthase specific activities (■) are per mg protein. The open symbols (○) and (□) are for the total activities of acid invertase and neutral invertase, respectively. There were small irregular changes in invertase specific activities throughout seed development.

mostly to the increasing amounts of proteins extracted as these protein-accumulating seeds developed. Therefore, this seed development study strongly supported our hypothesis that the UDP and PPi-dependent sucrolysis mediated the initial cleavage step of sucrose metabolism in an active sink. Similar to these results, Claussen *et al.* (3, 4) reported a close relationship between sucrose synthase activities and sink tissues. They changed a sucrose exporting eggplant leaf into a sucrose sink leaf and observed an increase in the sucrose synthase activity with no change in the invertase activities. Furthermore, incubation of detached eggplant leaves in increasing level of sucrose solution resulted in a corresponding increase of sucrose synthase activity, but the invertase activity did not change (4).

We recognize from work with other sink tissues that sometimes it has been concluded that invertase is a major sucrose breakdown activity. For example, several sucrose importing tissues such as the elongation portion of corn (13) or pea roots (17), the expanding tomato (18) or citrus leaves (26), or grape berries (11) have high invertase activities. However, most of these studies did not report comparable measurements of sucrose synthase activity; in addition, most of these tissues are not filling with nonstructural carbohydrates such as starch and sucrose or proteins as are the sinks we are considering (Table I).

Sucrolysis in the Developing Potato Tuber

We also studied the developing potato tuber which is another strong sucrose sink. In this work, since a tuber is much larger than a seed, we could assay enzyme activities in individual tubers. After several hundred assays we realized (a) that total sucrose synthase actvity plotted *versus* tuber size simply was a scattergram (Fig. 2) with a weak tendency to increase and (b) that on the same plant the sucrose synthase specific activities varied over several-fold (Table II) with little consistent trend relative to tuber size. Indeed, similar sized tubers on the same potato stem, even on the same stolon, had markedly different sucrose synthase activities. But both invertases again had low specific activities (Table II), and their total activities had no relationship to tuber size (data not shown).

Scattergram-type results such as are shown in Figure 2 are not uncommon in the literature. In a study with sugarbeet roots, the relationship between total sucrose synthase activities and root fresh weight is a scattergram with correlation coefficients less than 0.5 (10). However, the more traditionally accepted relationship between enzyme activity and tissue fresh weight was reported with several potato varieties (28, 29). Sowokinos (28, 29) using an extract from a composite sample

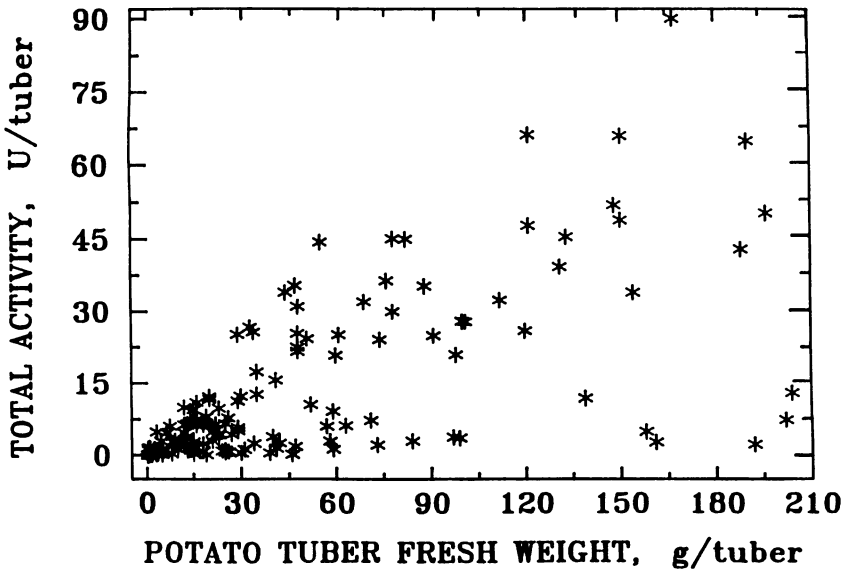

Figure 2. Total sucrose synthase activity of individual potato tubers. Each tuber was assayed immediately after harvesting from greenhouse or field grown potato plants.

Table II. *Variations Among Individual Tubers from the Same Potato Plant in Sucrose Breakdown Enzyme Activities*

Sucrolysis Enzyme	Tuber Fresh Weight, g				
	0.3	1.1	4.9	13.9	150
	munits/mg protein				
Sucrose synthase	12	151	8	81	241
Acid invertase	5	9	6	9	5
Neutral invertase	6	4	3	3	3

of several tubers, presented evidence that specific and total sucrose synthase activities of growing potato tubers increased with fresh weight during early development and decreased appreciably toward fully enlarged tuber; suggestions of sucrose synthase as the indicator of physiological maturity of the potato tuber were made (28). In other words, a mature tuber no longer imports much sucrose, thus sucrose synthase activities decreased accordingly. Besides sucrose synthase, other enzymes have been used to indicate sink strength. For example, ADP-glucopyrophosphorylase activities increased many fold after tuber initiation (12). However, the study did not include individual tuber measurements of ADP-glucopyrophosphorylase nor enzyme measurements with tubers of full size (12). Furthermore, total ADP-glucopyrophosphorylase activities in fast growing tubers were only 1.6-fold of the slow growing tubers when the former weighed at least 3-fold of the latter (12). In a study of tomato fruit development, both ADP-glucopyrophosphorylase and sucrose synthase were suggested to be sink strength indicators while invertase levels were constant (24). However, there was less than a 4-fold decrease in ADP-glucopyrophosphorylase activity concurrent with more than a 10-fold decrease in sucrose synthase activity with maturing fruits 20 to 40 d after anthesis (24). Collectively, previous studies do suggest a good possibility of biochemically measuring sink strength by assaying sucrose synthase activity rather than by assaying other enzymes (10, 12, 24, 28, 29).

However, we still need to explain the scattergram in Figure 2. In production agriculture we recognize that competing sucrose sinks on the same plant grow or fill at different rates. In other words, all sinks on a plant do not fill simultaneously because their sink strength is not equal. An individual stolon on a potato plant can bear many tubers; but the growth rate of each tuber is quite distinct and independent of tuber size at a given sampling time (8, 16, 19–21, 23, 25). For example, Moorby (20) reported essentially no correlation between tuber fresh weights *versus* the ^{14}C content of tubers that were harvested 20 h after exposure of the foliage to $^{14}CO_2$. Even 5 weeks after $^{14}CO_2$ exposure, the tuber ^{14}C contents plotted *versus* tuber fresh weight still was a scattergram quite similar to Figure 2.

Our hypothesis when these studies were initiated was that an individual tuber would have a unique sucrose synthase activity diagnostic of its sucrose sink strength. This hypothesis was tested with tuber from many stems and stolons from different plants and some of those results are among the scattergram of data plotted in Figure 2. Figure 3 illustrates an individual study with a single stolon bearing 8 tubers (tuber 5 was too small to test). On a fresh weight basis, tubers 2, 4, 7, and 8 were similar as were tubers 1, 3, and 6. But when either sucrose synthase total or specific activity was measured, within these two groups tuber 7 was more active than 2, 4, or 8, and tuber 3 was more active than 6 or 1. Invertase activities were low in all of these tubers, similar to those in Table II. Neither fresh weight nor sucrose synthase nor invertase activity had any relationship to the spatial position of an individual tuber on the stolon. Consequently, we conclude that we measured the sink strength of each tuber by assaying its sucrolysis activity. In other words, each tuber acts as a sucrose

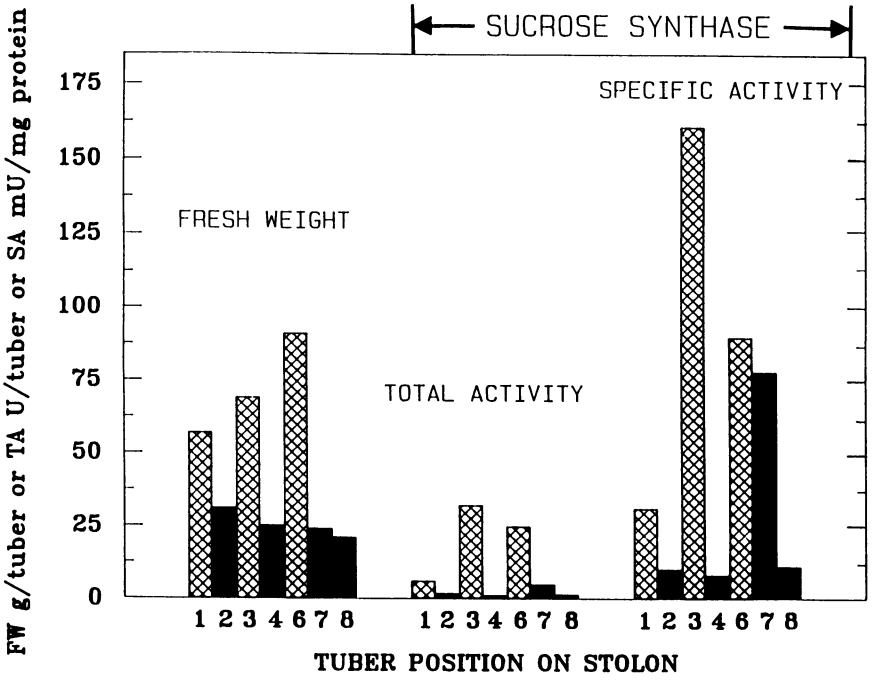

Figure 3. Variations in tuber fresh weight, total sucrose synthase activity, and specific activity for individual potato tubers grown on the same stolon. The tubers were numbered from the stolon tip toward the stem. The tubers were freshly harvested, weighed, extracted, and assayed as rapidly as possible, *e.g.* <2 h total time.

sink but temporally and spatially it is filling and growing uniquely. And we propose that data as in Table II and Figures 2 and 3 are measurements of the sucrose sink strength for each tuber on an individual plant or stolon at the time of sampling. Therefore, one can understand why we obtained the scattergram of sucrose synthase activities plotted in Figure 2.

CONCLUSIONS

We have demonstrated that sucrose synthase is a dominant sucrolysis enzyme in certain plant sinks by showing: high sucrose synthase activities in some actively growing and filling sinks, lower sucrose synthase activities in the same sinks at maturity and when quiescent, and low acid and neutral invertase activities in either of these types of sink tissue. Since sucrose is the major translocation form of carbon in plants (5) and the compound that initiates sucrolysis and glycolysis in plant cells (31), the choice of assaying sucrose cleavage activities in sink tissues gives one a reasonable biochemical measurement of sink strength in these plant organs. Therefore, we conclude that one biochemical determinant of sink strength is the ability of the sink to cleave sucrose. In the sink tissues of this report the sucrose synthase pathway predominates; in other sink tissues invertases may predominate but these will require individual investigations. Assaying for the initial sucrose breakdown reactions as a biochemical test of sink strength shows much promise for use in agriculture crop and tree improvement research.

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