Contributions of Sucrose Synthase and Invertase to the Metabolism of Sucrose in Developing Leaves

ESTIMATION BY ALTERNATE SUBSTRATE UTILIZATION

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ABSTRACT

The relative contributions of invertase and sucrose synthase to initial cleavage of phloem-imported sucrose was calculated for sink leaves of soybean (Glycine max L. Merr cv Wye) and sugar beet (Beta vulgaris L. monohybrid). Invertase from yeast hydrolyzed sucrose 420 times faster than 1-deoxy-1-fluorosucrose (FS) while sucrose cleavage by sucrose synthase from developing soybean leaves proceeded only 3.6 times faster than cleavage of FS. [14C]Sucrose and [14C]FS, used as tracers of sucrose, were transported at identical rates to developing leaves through the phloem. The rate of label incorporation into insoluble products varied with leaf age from 3.4 to 8.0 times faster when [14C]sucrose was supplied than when [14C]FS was supplied. The discrimination in metabolism was related to enzymatic discriminations against FS to calculate the relative contributions of invertase and sucrose synthase to sucrose cleavage. In the youngest soybean leaves measured, 4% of final laminar length (FLL), all cleavage was by sucrose synthase. Invertase contribution to sucrose metabolism was 47% by 7.6% FLL, increased to 54% by 11% FLL, then declined to 42% for the remainder of the import phase. In sugar beet sink leaves at 30% FLL invertase contribution to sucrose metabolism was 58%.

Phloem import and possibly control of phloem unloading are linked to the metabolism of rapidly growing sink organs as demonstrated by inhibitor studies (for review, see Refs. 14, 22). In addition, a pathway for phloem unloading through the symplast in developing leaves (19) and roots (7, 10) suggests that unloading is controlled by removal of the main osmoticum, often sucrose, from the phloem either by compartmentalization or breakdown in nontransport tissues. The initial step in sucrose metabolism is thus an important one in controlling both the movement of sucrose and its utilization. Two enzymes which catalyze the initial step in sucrose metabolism are invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) and sucrose synthase (UDPGlucose:β-fructose 2-α-glucosyltransferase, EC 2.4.1.13) (1, 2). Both invertase and sucrose synthase activities have been found in leaves of a variety of species though activities vary with extraction techniques and species (4, 9; for review, see Ref. 2).

A relationship between invertase activity and growth has been inferred from correlations of growth rate, reducing sugar levels and extractable invertase activity in leaves (4, 11, 16, 18) and stems (17). However, high invertase activities are also found in mature leaves (9, 18). Increases in sucrose synthase activity have also been correlated with growth rate and assimilate distribution in leaves of eggplant (4).

It is difficult to extrapolate in vivo enzyme activities from in vitro measurements because of the inability to recreate the chemical environment, in terms of concentration of substrates, modulators, inhibitors, etc. We have attempted to measure the relative in vivo activities of invertase and sucrose synthase in the breakdown of phloem-supplied sucrose by using an analog of sucrose, FS, which is a poor substrate for invertase but which is cleaved by sucrose synthase (3, 12). The ratio of metabolism of the two sugars was compared in developing leaves of soybean and sugar beet and related to the differential discrimination against FS by sucrose synthase and invertase in vitro.

MATERIALS AND METHODS

Plant Material. Soybean (Glycine max L. Merr cv Wye) and sugar beet (Beta vulgaris L. monohybrid) plants were grown under controlled conditions as previously described (15) with a light period of 14 h. Sugar beet plants were used 3 to 4 weeks after planting and soybean plants 8 to 13 days after planting.

Enzymic Discrimination between Sucrose and FS. Sucrose synthase activity in crude extracts was not stable at room temperature. Sucrose synthase was partially purified from immature first trifoliolate leaves, 1.5 to 2 cm in length (17-22% FLL), from 11 d old soybean plants. Leaves were ground as described previously (12) in 100 mM Hepes-KOH (pH 7.0), 5 mM EDTA, 5 mM DTT, squeezed through Miracloth and centrifuged at 10 KxG for 10 min. The supernatant was centrifuged at 40 KxG for 30 min. The second supernatant, referred to as crude extract, was loaded onto a DEAE-cellulose (Celllex D, Bio-rad) column (37 ml volume) at 5 to 7°C (6). The column was previously equilibrated and eluted with 10 mM Hepes-KOH (pH 7.0), 1 mM EDTA, 1 mM DTT. Flow rate was 17 ml h⁻¹ and 11 ml fractions were collected. Bound protein was eluted with a linear NaCl gradient from 0 to 0.25 m NaCl in equilibration buffer. Aliquots of fractions were assayed for sucrose synthase as described in “Enzyme Assays.” Protein was measured by absorbance at 280 nm. Fractions 10 to 14 were pooled and brought to 50% saturation with ammonium sulfate by stirring for 30 min with an equal volume of saturated ammonium sulfate in 10 mM Hepes (pH 6.0). Protein was pelleted by centrifugation at 40 KxG for

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3 Abbreviations: FS, 1-deoxy-1-fluorosucrose; FLL, final laminar length; UDPG, UDP-glucose.
30 min and resuspended in 0.5 ml 10 mM MES-KOH (pH 6.5), 1 mM DTT, 1 mM EDTA, 1 mM MgCl₂. The crude extract and partially purified extract had sucrose synthase activities of 0.2 and 2.4 mol h⁻¹ mg protein, respectively. Recovery of activity was 30%.

The ratio of \( V_{\text{max}} / K_m \) for sucrose synthase was determined by measuring the \( ^{3}H/^{14}C \) ratio in UDPG after incubating partially purified extract with varying ratios of \([U^{-3}H]\)sucrose to \([U^{-14}C]\)-glucosyl-FS. The 0.1 ml reaction was begun by adding 0.01 ml of partially purified extract to 50 mM sucrose, 20 mM UDP, 14 mM MgCl₂, 1 mM DTT, in 10 mM MES-KOH (pH 6.5). The ratio of \([U^{-3}H]\)sucrose to \([U^{-14}C]\)-glucosyl-FS ranged between 0.12 to 15 with amounts of radioisotopes from 33.7 to 2.8 kBq of \([U^{-14}C]\)-glucosyl-FS and 8.6 to 56.1 kBq of \([U^{-3}H]\) sucrose. The reaction was stopped after 2.5 h by freezing in dry ice followed by freeze-drying. Sugars were redissolved in 30% methanol and UDPG was separated from sucrose and FS by ion exchange chromatography with 0.5 ml of Bio-Rad AG 50W-X 8 resin in formate form. Columns were washed in the following sequence: (a) twice with 11 ml H₂O, (b) 11 ml 50% methanol, (c) 6 ml 8 N formic acid, (d) 0.5 ml 2 N hydrochloric acid, (e) 1 ml 2 N hydrochloric acid. Eluant from the last hydrochloric acid wash contained 80% of the UDPG present and was counted by dual-isotope liquid scintillation counting. Background contamination in UDPG fractions from \([U^{-3}H]\)sucrose and \([U^{-14}C]\)FS of 1.5% and 10% respectively, was subtracted. Initial \( ^{3}H/^{14}C \) ratios were taken as the \( ^{3}H/^{14}C \) ratio in dual-isotope liquid scintillation counting of aliquots of each reaction mixture. These initial ratios were divided by 2 to correct for the \( ^{3}H \) of \([U^{-3}H]\)sucrose in the fructose moiety.

Crude extracts from first trifoliolate leaves of soybean, 1 to 2 cm in length, prepared as described above, did not have sufficient invertase activity to hydrolyze measureable amounts of FS. Hydrolysis of 40 mM sucrose at pH 5.0 occurred at a rate of 5 μmol mg protein⁻¹ h⁻¹, similar to values reported for sugar beet (9) and soybean (21) sink leaves. However, these extracts produced no detectable product when incubated with up to 200 mM FS at pH 5.0 or pH 7.5, demonstrating the resistance of FS to hydrolysis by soybean leaf invertase.

Enzyme Assays. Sucrose synthase was measured in the cleavage direction by production of UDPG from either sucrose or FS. Continuous production of UDPG was assayed in a 1 ml reaction consisting of 100 mM MES-KOH (pH 6.5), 1 mM UDP, 5 mM MgCl₂, 2 mM inorganic pyrophosphate, 1 mM NADP, 3 units of phosphoglucomutase, 1 unit each of UDPG pyrophosphorylase and glucose-6-phosphate dehydrogenase, crude extract and varying concentrations of sucrose or FS. Absorbance at 340 nm was measured continuously. Assay of invertase activity in crude extracts was done as previously described (16).

Transport and Metabolism of Sucrose and FS. All leaves but one sink and one source leaf were removed from sugar beet plants and both cotyledons and one primary leaf were removed from soybean plants the day prior to the experiment. All treatments were started 2 h after the beginning of the photoperiod. Radiolabeled sugars were supplied to source leaves of sugar beet via reverse vein flap feeding (19) and to soybean primary leaves acclimated leaf surface abraded with carbondum. Solution was held on abraded surfaces with Saran Wrap. Solutions were 10 mM sucrose in 25 mM MES-KOH (pH 5.5) with 1 mM CaCl₂.

Initial experiments were done using \([U^{-3}H]\)sucrose and \([U^{-14}C]\)-glucosyl-FS as tracers of sucrose supplied to the same plant, and subsequent experiments were done using \([U^{-3}H]\) sucrose or \([U^{-14}C}\)-glucosyl-FS and \([U^{-14}C]\)-glucosyl-FS supplied to separate plants. For sugar beet plants, 1.8 MBq of each radioisotope was used and for soybean plants, 0.37 MBq of \( ^{14}C \)-sugars and 1.1 MBq of \([H]sucrose was used. Arrival and accumulation of \( ^{14}C \) in a sink leaf was monitored with a Geiger-Mueller tube clamped to the leaf (19). Sugar beet sink leaves were sampled by removing 4 cm diameter discs. Soybean leaves were sampled by removing either an entire leaflet or half of one leaflet of the first trifoliolate leaf. Leaf samples from single isotope experiments were frozen in dry ice and later extracted twice with 80% ethanol at 79°C for 30 min. Both the dried ethanol extracts and the extracted tissue were solubilized with 0.5 ml of NCS tissue solubilizer (Amersham), heated at 50°C for 2 h, cooled, neutralized with 30 μl of acetic acid, and filtered for liquid scintillation counting. Dpm in ethanol extracts and extracted tissue were summed and the percent dpm in insolubles were calculated by dividing dpm in extracted tissue by total dpm.

Leaf samples from dual-isotope experiments were oxidized in a Biological Oxidizer OXZ300 (R. J. Harvey Instrument Corporation, Hillsdale, NJ). Leaf tissue was powdered at dry ice temperature and separate samples were either oxidized for total dpm or extracted in 80% ethanol and extracted tissue oxidized for dpm in insolubles. In several experiments ethanol extracts were further fractionated by ion exchange chromatography (12) and neutral fractions were analyzed for dpm in FS, sucrose, and hexoses by HPLC on a 4.6 mm × 25 cm Du Pont Zorbax Amino column eluted with acetonitrile/water (7:3, v/v).

Sources and Identification of Radiolabeled Sugars. \([U^{-3}H]\) sucrose was purchased from Amersham International and \([U^{-14}C}\)-sucrose and \([U^{-14}C}\]-glucosyl-FS were purchased from New England Nuclear. \([U^{-14}C}\)-glucosyl-FS was synthesized from \([U^{-14}C}\)-glucose from New England Nuclear and 1-deoxy-1-fluorofructose (3) by the following procedure. \([U^{-14}C}\)-d-Glucose (0.5 mM, 0.35 mCi mmol⁻¹) was purified by HPLC as described above to remove nonradioactive contaminants which inhibited subsequent enzymic reactions. Fractions containing \([U^{-14}C}\)-glucose were converted to \([U^{-14}C}\)-glucose-6-phosphate in a reaction mix containing 4.8 mol of ATP, and two units of hexokinase in 0.5 ml of 50 mM HEPES buffer at pH 7.2 which was also 5 mM in MgCl₂. The reaction was allowed to proceed for about 1 h and was checked for completion by TLC of a 0.5 μl aliquot on cellulose using ethanol:1 M ammonium acetate (pH 3.8) (5:2, v/v) as the developing system.
To the [14C]glucose-6-phosphate containing reaction mix was added 4 μmol of UTP and 3 to 4 units each of phosphoglucomutase, UDP-glucose pyrophosphorylase, and inorganic pyrophosphatase. The reaction was checked for completion using the TLC system described above. From 60 to 90% of the applied [14C]co-migrated with UDP-glucose standards. The reaction mix was heated in a boiling water bath, centrifuged and used as the source of [14C]UDP-glucose in the sucrose synthase catalyzed synthesis of FS as described previously (3) except that defatted wheat germ (Sigma Chemical Co.) was used as the source of sucrose synthase.

RESULTS

Enzyme Discrimination Factors. Sucrose and FS were not identical substrates for sucrose synthase or invertase as measured by in vitro kinetic analysis. Approximate Km values for sucrose and FS for sucrose synthase at 1 mM UDP were similar, 40 and 55 mM, respectively, and within the range of reported values for a variety of tissues and species (1, 2). However, the Vmax with FS was 3 to 4 times lower than with sucrose (data not shown).

The discrimination factor for cleavage of the two sugars by sucrose synthase was calculated using low, tracer concentrations of [3H]sucrose and [14C]FS in varying ratios in a large, constant pool of [3H]sucrose. The slope of a plot of the initial ratio of [3H]sucrose to [14C]FS versus Vmax/Km in UDPG is equal to the ratio of the kinetic constant (Vmax/Km) for the two substrates, provided the substrate concentrations are significantly lower than Km. Concentrations of radiolabeled sugars ranged from 3 to 24 μM, well below the Km of 40 to 55 mM. The measured ratio of 3.6 (Fig. 1) agrees well with the ratio of Vmax/Km obtained by calculation of the four kinetic parameters for the two substrates in continuous assays, and is very similar to a factor of 3.1 calculated by the same method using crude extracts of wheat germ as the source of sucrose synthase (data not shown).

Attempts to determine the discrimination factor for hydrolysis of sucrose and FS by invertase using the method described for sucrose synthase were unsuccessful. Reaction conditions necessary for the production of measurable product from FS resulted in complete hydrolysis of sucrose. The reaction rates for hydrolysis of sucrose and FS at equal concentrations were therefore determined separately and compared. From the data of Figure 2, purified invertase from yeast at pH 5.0 and 20 mM substrate, hydrolyzed sucrose 420 times faster than FS when product formation was followed either as appearance of [14C]glucose or by coupled enzyme assay of glucose.

Transport and Metabolism of Sucrose and FS. At tracer concentrations, FS showed the same translocation kinetics into leaves of both soybean (Fig. 3) and sugar beet (data not shown) as sucrose. [14C]FS was not cleaved during transport and accounted for greater than 90% of translocated 14C in sugar beet leaves. FS was metabolized in sink leaves, but at a lower rate than sucrose. In sugar beet sink leaves, 140 min after arrival the percent of total 14C in FS had declined linearly to 60% (20). In the same sink leaf samples, [14C]sucrose from [14C]sucrose supplied to a source leaf was below detection.

The steady state rate of metabolism of phloem-imported sugar was measured by linear regression of the percent of total, translocated radiolabel incorporated into the ethanol insoluble fraction versus time (Fig. 4). Any labeled, ethanol insoluble product must be a product of either invertase or sucrose synthase and the further metabolism of the hexoses derived from those reactions. Although [U-14C]sucrose cleavage yielded label in both hexose moieties and cleavage of [14C]FS yielded label only in the glucose moiety, experiments with either uniformly labeled or glucosyl-labeled sucrose gave the same rates of metabolism and both are.

**Fig. 1.** Ratio of 3H/14C in UDPG produced from incubation of varying ratios of [3H]sucrose to [14C]FS with sucrose synthase purified from crude extracts of soybean sink leaves. Data plotted are three replicates from one extraction. Linear regression gives correlation coefficient = 0.9870 and slope = 3.6.

**Fig. 2.** Hydrolysis of 20 mM sucrose (○) and 20 mM FS (●) by invertase from yeast at pH 5.0. Data points are averages of four experiments in which glucose production was measured by either method described in "Materials and Methods." Linear regression with original data yielded for sucrose, slope = 0.275, correlation coefficient = 0.9976, and for FS, slope = 6.5 × 10⁻³, correlation coefficient = 0.9820. The ratio of the rates of hydrolysis (sucrose/FS) is 4200.

**Fig. 3.** Translocation of [14C]sucrose (X, □) and [14C]FS (A, O) into young soybean leaves. Cpm, measured by Geiger-Mueller counting, were corrected for background and expressed as percent of cpm at 66 min. 14C-Sugars were supplied to the source leaf at time 0.
plotted in Figure 4D.

In soybean, the rate of $^{14}$C incorporation into insolubles from translocated sugar increased with leaf age to a maximum at 11% FLL and then declined (Fig. 4B, Table 1). During the developmental period at which the rate of $[^{14}\text{C}]$sucrose incorporation into insolubles was highest, (Fig. 4, B, C, and D), the rate was initially linear but declined by 40 to 50 min after addition of label to the fed, source leaf. The rate of metabolism for these ages was calculated using only the initial, steady state phase of incorporation. At other ages the rate remained linear for the duration of the experiment. Loss of steady state incorporation was probably due to turnover of the limited supply of $^{14}$C-sugar in the source leaf. Deviation from steady state therefore occurred earlier in sink leaves which were metabolizing more rapidly and earlier with sucrose than with FS.

While the metabolism rate for both sugars increased with age, the increase was not as great with FS as with sucrose. Therefore, the ratio of the rate of metabolism of sucrose to FS also changed with leaf age (Table 1). The low ratio in the youngest leaves increased to a maximum at 11% FLL and declined slightly during the remainder of the import phase.

Percent incorporation of translocated sucrose and FS into insolubles in sugar beet sink leaves at 30% FLL showed the same pattern as soybean sink leaves at 17% FLL (data not shown). The ratio of the rate of metabolism of sucrose to FS was 8.7.

**DISCUSSION**

Sucrose synthase-catalyzed cleavage of phloem-imported sucrose was evident in both species tested and at all developmental stages tested. FS was metabolized 3.4 to 8 times slower than sucrose (Table 1).

If the initiation of metabolism were by sucrose synthase alone, the discrimination in metabolism would be that afforded by cleavage by sucrose synthase, i.e. 3.6 (Fig. 1). The lowest differential in metabolism of sucrose to FS, 3.4 (Table 1), occurred with the youngest leaves and cleavage of sucrose could be accounted for by sucrose synthase. As leaves developed, sucrose was metabolized 6 to 8 times faster than FS (Table 1), indicating that cleavage of sucrose was accomplished by both sucrose synthase and invertase.

Quantitative determination of the relative contributions of sucrose synthase and invertase to the breakdown of phloem-supplied sucrose can be made from measurements of the rates of metabolism of $[^{14}$C]sucrose and $[^{14}$C]FS, and the discrimination factors for the two sugars as substrates for sucrose synthase and invertase. The rate of breakdown of either sucrose or FS is the sum of the rates of cleavage by invertase and sucrose synthase so that:

$$R_1 = I_1 + SS_1$$  
and  
$$R_8 = I_8 + SS_8$$

where $R$ is the total rate of metabolism for either sugar, $I$ is the contribution of invertase to that rate, and $SS$ is the contribution of sucrose synthase.

From the data of Figure 1, the ratio of rates of metabolism of sucrose to FS via sucrose synthase is equal to 3.6 times the ratio of the concentration of $[^{14}$C]sucrose to $[^{14}$C]FS. Although not measured directly as for sucrose synthase, the discrimination by invertase at tracer levels can be estimated at about 4200 from the data of Figure 2. Thus:

$$SS_1 = 3.6 SS_8 \frac{[^{14}$C-sucrose]}{[^{14}$C-FS]}$$  
and  
$$I_1 = 4200 I_8 \frac{[^{14}$C-sucrose]}{[^{14}$C-FS]}$$

The concentration of unmetabolized $[^{14}$C]sucrose and $[^{14}$C]FS was not measured for each experiment and each leaf age. An estimate of the $[^{14}$C]sucrose to $[^{14}$C]FS ratio was calculated as the ratio of percent of radiolabel in insolubles from $[^{14}$C]sucrose versus $[^{14}$C]FS for each sampling time. The percent of $^{14}$C remaining in the soluble fraction was averaged for each time point for each leaf age group. The ratio of amounts of $^{14}$C-solubles from $[^{14}$C] sucrose to $[^{14}$C]FS is one when the $^{14}$C-sugars first arrive, but the ratio decreases with time due to the more rapid metabolism of sucrose compared to FS. The ratios of percent solubles from $[^{14}$C]sucrose to $[^{14}$C]FS ($Y$), calculated for the latest time used in

![Figure 4. Time course of percent incorporation into ethanol insolubles of phloem-translocated sucrose (C) and FS (D) in soybean first trifoliate leaves of varying ages. Radiolabeled sucrose and/or FS were supplied of source leaves as described in "Materials and Methods." Calculated rates and number of plants for each calculation are given in Table 1.](image-url)
Table 1. Percent Contribution of Invertase and Sucrose Synthase Metabolism in Sink Leaves of Soybean with Leaf Development

<table>
<thead>
<tr>
<th>Leaf Age (% FLL)</th>
<th>Rate of Sugar Metabolism*</th>
<th>( R_s / R_{fs} )</th>
<th>Sucrose/FSb</th>
<th>Range of % Contribution to Sucrose Metabolismc (Invertase/Sucrose Synthase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0.25 (3.934)</td>
<td>0.074 (4.915)</td>
<td>3.4</td>
<td>0/100 to 13/87</td>
</tr>
<tr>
<td>7.6</td>
<td>0.64 (6.910)</td>
<td>0.095 (3.791)</td>
<td>6.7</td>
<td>47/53 to 57/43</td>
</tr>
<tr>
<td>11.0</td>
<td>1.2 (2.996)</td>
<td>0.19 (2.954)</td>
<td>7.9</td>
<td>54/45 to 66/34</td>
</tr>
<tr>
<td>17.0</td>
<td>1.6 (6.882)</td>
<td>0.16 (7.864)</td>
<td>6.2</td>
<td>42/58 to 62/38</td>
</tr>
<tr>
<td>40.0</td>
<td>0.37 (0.883)</td>
<td>0.059 (4.757)</td>
<td>6.3</td>
<td>43/57 to 52/48</td>
</tr>
</tbody>
</table>

* \( R_s \) for either sucrose (s) or FS is % total \(^{14}\text{C}\) incorporated into insolubles min^\(-1\), calculated from initial, linear rates in Figure 4. b Ratio of average % total \(^{14}\text{C}\) in insolubles from \(^{14}\text{C}\)sucrose to \(^{14}\text{C}\)FS at final time point used for rate determination. c Calculated using Eqs. 5 and 6 (see “Discussion”). d Number of plants used in rate determination, correlation coefficient of linear regression.

SUCROSE METABOLISM IN DEVELOPING LEAVES

The rate determinations, are shown in Table I. Eqs. 5 and 6 were used to calculate a range of relative contributions of invertase to sucrose synthesis using two values of \( Y \), one and the value at the end of linear increase in percent incorporation into insolubles (Table I).

Solving Eqs. 1 and 2 for the metabolism initiated through invertase and sucrose synthase, substituting from Eqs. 3 and 4, and setting \( Y = \text{[\(^{14}\text{C}\)sucrose]/[\(^{14}\text{C}\)FS]} \) gives the following:

\[
I_s = \frac{R_s - 3.6 R_{fs} Y}{1 - 3.6/4200} \quad (5)
\]

and

\[
SS_s = \frac{R_s - 4200 R_{fs} Y}{1 - 4200/3.6} \quad (6)
\]

A disproportionate overestimate of the percent incorporation into insolubles from \(^{14}\text{C}\)sucrose compared to that from \(^{14}\text{C}\) FS, and hence an overestimate of the contribution of invertase, would occur if significantly more \(^{14}\text{CO}_2\) were respired from \(^{14}\text{C}\) sucrose than from \(^{14}\text{C}\)FS, due to the more rapid metabolism of sucrose. However, if 5% of imported radiolabel were respired and released in 1 h (the time over which the rate was calculated) from leaves which showed the highest differential in metabolism (11% FLL), \( R_s / R_{fs} \) would be decreased from 7.9 to 7.5, which would result in a change in the calculated invertase contribution from 54 to 52%. Further, it is unlikely that even 5% would be respired over 1 h, particularly because these developing leaves were photosynthesizing and any respired \(^{14}\text{CO}_2\) could be refixed. In a sugar beet sink leaf, 25% FLL, no respired \(^{14}\text{CO}_2\) was detected for 14 h during steady state \(^{14}\text{CO}_2\) labeling of a mature leaf, even though the sink leaf accumulated 9 x 10^3 dpm and the detection method for \(^{14}\text{CO}_2\) was sensitive to 1100 dpm (JG Schmalstig, unpublished data).

The estimate of 4200 for the ratio \( I_s / I_{fs} \) is an approximation since insufficient invertase activity was present in nonpurified extracts of soybean or sugar beet leaves to obtain measurable hydrolysis of FS. The position of this coefficient in Eqs. 5 and 6 makes the calculation quite insensitive to variation in the coefficient, provided that the relative discrimination by invertase and sucrose synthase (\( SS_s / SS_{fs} \)) is large. At \( R_s / R_{fs} \) ratios of 6 to 8, as observed in these experiments, variation of \( I_s / I_{fs} \) between 100 and 10,000 change the calculated values of \( I_s \) and \( SS_s \) by less than 4%. From our invertase assays and assays of FS hydrolysis by soybean leaf extracts over a wide pH range (21), we feel that the ratio \( I_s / I_{fs} \) is within the above range.

Clearance by sucrose synthase accounted for the major portion of sucrose metabolized throughout a large part of soybean leaf development (Table I). This in vivo evidence for sucrose synthase activity confirms reports of higher sucrose synthase activity extracted from sink leaves than mature leaves (13). The importance of sucrose synthase to sucrose metabolism in leaves is emphasized by the small contribution of invertase to sucrose metabolism in the youngest leaves tested (4% FLL). Sucrose metabolism predominantly by sucrose synthase in these young leaves may be indicative of high sucrose synthase activity often reported in nonphotosynthetic tissue (1, 2). The contribution of invertase to sucrose metabolism increased abruptly with age such that metabolism initiated by invertase contributed 47 to 57% of total metabolic rate by 7.6% FLL. The increase in invertase contribution relative to sucrose synthase at 7.6% FLL occurred with and apparently accounted for an increased rate of sucrose metabolism. Sucrose metabolism peaked at 11% FLL and was paralleled by a peak in invertase relative to sucrose synthase. As leaves approached maturity, the contribution of invertase declined slightly and the two enzymes contributed fairly equally to total breakdown. Sugar beet leaves also exhibited both enzyme activities, with a sucrose synthase contribution of 42% and an invertase contribution of 58% in leaves at 30% FLL.

The metabolism of sucrose by invertase in sink leaves most likely represents part of a change in developmental state. The maximum contribution of invertase to sucrose metabolism occurred roughly at the end of the exponential phase of cell division and the beginning of the predominance of cell enlargement to leaf growth (5). This relationship between invertase and cell enlargement is similar to that suggested by correlations among soluble acid invertase activity, hexose concentrations, and cell enlargement in leaves (16, 11), and stems (for review, see Ref. 17). 

Sucrose arriving via the phloem is not cleaved before or during uptake in sink leaves of sugar beet (8). Results here demonstrating that FS was transported through sink leaves of sugar beet and soybean at rates identical to sucrose, further support a lack of hydrolysis by cell wall invertase as an obligate step in phloem unloading. Invertase activity on phloem-imported sucrose is most likely intracellular. Schmitt et al. (21) reported alkaline invertase at half the activity of acid invertase in Wye soybean sink leaves but not source leaves, suggesting that intracellular hydrolysis may involve alkaline invertase.

In developing sugar beet leaves, the in vitro contributions of invertase and sucrose synthase to the metabolism of sucrose were nearly equal, although the extractable specific activity of invertase exceeds that of sucrose synthase by about 18-fold (9). The discrepancy between our in vivo measurements and activities in in vitro extracts emphasizes the need for caution in extrapolating physiological function from in vitro assays. The method used here to determine the in vivo rates of throughput of parallel metabolic paths is analogous to the use of isotope discrimination to determine whether carbon fixation has occurred by C3 or C4 pathway of photosynthesis. Application of the method should be possible to any case for which differentially metabolized substrates can be found or developed, and in which these substrates can be supplied by the same route as the normal substrate.
LITERATURE CITED


