Carbohydrate Translocation in Sugar Beet Petioles in Relation to Petiolar Respiration and Adenosine 5'-Triphosphate

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ABSTRACT

Earlier studies have shown that the retarding effect of low petiolar temperatures on sucrose transport through sugar beet (Beta vulgaris L.) petioles is markedly time-dependent. Although the initial effect of chilling the petiole to near 0°C is severely inhibitory, translocation rates soon recover (usually within about 2 hours) to values at or near the control rate. In the present studies, selected metabolic parameters were measured simultaneously with translocation. No stoichiometric relationships among petiolar sucrose transport, petiolar respiration (CO₂ production), and calculated petiolar ATP turnover rates were evident. It appears that the major sources of energy input energizing carbohydrate transport in sieve tubes function mainly at either loading or unloading sites and not at the level of individual sieve-tube elements.

Earlier studies have shown that the retarding effect of low petiolar temperatures on sucrose transport through sugar beet petioles is markedly time-dependent (6, 11). Although the initial effect of chilling the petiole to near 0°C (0.1–2.5°C) is severely inhibiting, the degree of inhibition diminishes rapidly with time, and the translocation rate usually recovers to values at or near (and frequently above) the control rate within 1.5 to 2.5 hr. If the "cold-adapted" petiole is rewarmed to room temperatures, and again chilled, the cycle of inhibition-de-inhibition is repeated (11). Thus, sucrose translocation in sugar beet petioles is essentially a homeostatic process.

This homeostasis is interesting, since if sucrose translocation depends on production and use of metabolic energy along the path between source and sink, the reversal of low temperature inhibition with time should be reflected in similar time-dependent changes in various metabolic parameters of the chilled petiole.

The experiments reported here permit comparisons to be made among the following variables at both normal (22–25°C) and low (0.7–2.5°C) petiolar temperatures: (a) the translocation rate; (b) the rate of CO₂ evolution from the petiole; (c) changes in the molar concentration of ATP in the petiole; and (d) the rate of oxygen evolution from the donor-leaf blade. Oxygen evolution measurements were included in these studies to determine whether or not the temporary translocation associated with petiolar cooling may have been associated with a similar temporary reduction in photosynthesis inhibition possibly from transient stomatal closure or other water-stress phenomena induced by the petiolar cooling.

MATERIALS AND METHODS

Sugar beet plants (Beta vulgaris L. monogerm hybrid CV No. SL 129 × 133 MS × SP 6322-0) were pruned to a simplified source-path-sink system (6). Culture procedures were as described by Geiger and Swanson (6), except that for the latter half of the growth period plants were maintained under a bank of six 300-W water-filtered incandescent lamps (14 hr light, 10 hr dark) providing an irradiance of 1100 to 1500 ft-c at leaf level. Under these conditions, petioles elongated to final lengths of 14 to 17 cm, permitting the ready assemblage of plants into test equipment.

The day before each experiment, the hypocotyl of the test plant was girdled with an electrically-heated nichrome wire about 1 cm below the crown to restrict the translocation sink to the young shoot. These plants, therefore, differed significantly from those used in earlier studies in that the ratio of accessible sink tissue to source tissue was considerably smaller because of the exclusion of the root system as a translocation sink.

Figure 1 presents a flow diagram of the analytical system used for measuring translocation to the sink leaf, photosynthesis in the donor-leaf blade, and respiration in the petiole zone subjected to cold treatment. Steady-state labeling of assimilates was obtained by circulating CO₂, at a constant concentration and specific activity through the Plexiglas cuvette (B). The desired concentration of about 600 μl/l was maintained within approximately 1% by means of a motorized syringe pump (J) supplying labeled CO₂ of known specific radioactivity. The analytical circuit through a 40-W capillary inlet in response to error signals from the infrared gas analyzer (K). Oxygen concentrations were followed with a model 777 Beckman polarographic analyzer modified to include a full-scale 1% range with zero suppression from 0 to 100% oxygen (O). Translocation rates were measured in units of Δ rpm min⁻¹ after isotopic equilibrium was established. In three experiments, the counting efficiency (dpm/cpm) was also determined to permit calculation of the actual amount of sucrose (or sucrose equivalents) transported to the sink leaf in unit time (T).

The petiole-respiration cuvette (C) consisted of a double-walled brass chamber, 4 cm long, sealed around the petiole in the 3- to 7-cm zone measured from the blade base. For the first 3 hr of labeling, the petiole was maintained at 25°C; coolant was then circulated through the outer jacket of the petiole.
were then rinsed with N2, weighed, and frozen. Dissolving the tissue in cold 0.1 M Na2HAsO4 buffer at pH 7.4. The final standards obtained by serial dilution were kept on ice to increase stability. Samples and standards were assayed by a luciferin-luciferase system using a Beckman CPM-100 liquid scintillation counter. Firefly lantern extract was reconstituted with 5.0 ml distilled H2O, producing a solution equivalent to 50 mg of the “lanterns” in 0.1 M Na2HAsO4 and 20 mM MgSO4 at pH 7.4. Two-tenths ml of the firefly lantern extract was pipetted into scintillation vials containing 1.7 ml distilled H2O and counted for 12 sec to determine the ATP level in the firefly extract. Following this background determination, a 0.1-ml aliquot of the sample or standard was added to the vial, quickly agitated, and counted for 12 sec after a 60-sec delay. All analyses were carried out in triplicate, and the averaged counts for petiole samples was converted to moles ATP per 10 mg fresh weight of tissue (average fresh weight of the petiole sections was about 125 mg cm⁻²).

RESULTS

Figure 2A summarizes the results of six experiments showing the relatively rapid homeostatic adjustment in petiolar transport rate to low temperatures following initiation of petiolar cooling. The time-response curve conforms well to that reported for earlier studies (5, 11), despite the fact that in the present studies plants with a significantly higher ratio of source to sink tissue were used. The large standard deviation in the data at 100 min reflects the fact that the kinetics of the recovery phase differed substantially from plant to plant. Some evidence has been obtained in the present experiments that translocation rates following recovery are generally higher in hypocotyl-blocked plants than in nonblocked plants relative to

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**Fig. 1.** Schematic of analytical system. A: 8-cm water filter for two 300-w reflector flood lamps; B: Plexiglas cuvette with blade of source leaf enclosed; C: double-walled cuvette enclosing a 4-cm long zone of the petiole, internal atmosphere maintained at desired temperature by circulating coolant through outer jacket; D: infrared gas analyzer; E: 1.2 cm² Geiger-Muller tube connected to ratemeter and potentiometric recorder, sink leaf maintained in fixed geometry by grid affixed to G-M tube; F: vessel holding test plant; G: gas flow meter; H: water trap at 1 C; I: stainless steel bellows pump; J: motor-driven syringe pump, 200 ml capacity, supplying ¹⁴CO₂ of known specific radioactivity through a 40-µm capillary to analytical system in response to error signal from infrared gas analyzer. K: L: gas flowmeter; M: helical fluid U-manometer; O: O₂ polarographic analyzer; and P: mixing chamber.
Fig. 2. Relative rates of translocation, respiration, and photosynthesis. At time 0, temperature of a 4-cm zone of petiole was rapidly lowered to 0.7 to 2.5 C. In A: 100 = 36 nmoles sucrose translocated to sink leaf min⁻¹ dm⁻² source-leaf blade (average of three plants) or Δ97 cpm min⁻¹ (average of six plants); in B: 100 = 4.6 nmoles CO₂ min⁻¹ cm⁻² petiole or 0.037 nmoles CO₂ min⁻¹ mg⁻¹ fresh weight petiole (average of six plants); in C: 100 = 0.168 ml O₂ min⁻¹ dm⁻² source-leaf blade (average of six plants).

Fig. 3. Relative ¹⁴C-translocation rate. 100 = Δ78 cpm min⁻¹ (average of three plants). At time 0, petiole of source leaf was excised 1 cm above cold block and temperature of the treated zone of petiole was lowered to 1 C.

Their respective control rates. This relationship may reflect a higher rate of increase in the sucrose pool of the donor leaf of hypocotyl-blocked plants. In studies by Geiger and Swanson (7), the sucrose pool in the source leaf increased at an average rate of about 7.8 µg min⁻¹ dm⁻² leaf area. Given the more restricted target volume used in the present experiments, it is possible that this parameter increased at a faster rate, leading eventually to a higher loading rate.
To assess what fraction of the increasing translocation rate during the recovery phase may have been due to an increasing mobilization rate of labeled carbohydrates previously accumulated in the petiole and crown tissue, three additional control experiments were carried out. In these, the donor leaf was excised 1 cm above the cold block 3 hr after the start of labeling. As shown in Figure 3, the rate of $^{14}C$ translocation following excision declined 60% in the first few minutes and thereafter at a diminishing rate for the balance of the time period. This is not a valid tracer experiment because of the unknown and diminishing specific radioactivity of the translocate following source leaf excision. The data clearly show, however, that the acceleration in translocation rate during the recovery phase cannot be due in any significant way to remobilization of labeled translocates accumulated along the transport path between cold block and target leaf. Thus, the recovery phase measures the time required for the transport system to compensate for the initial impedance induced by petiolar chilling.

The rate of photosynthesis in the donor leaf blade, measured in units of oxygen production, showed a slow downward drift of about 1.7% per hr during the course of these experiments (Fig. 2C). No evidence for any perturbation in photosynthetic rate coincident with petiolar cooling was obtained. Webb (13, 14) has similarly observed that localized temperature treatments of the petiole did not affect the rates of $^{14}CO_2$ assimilation in that leaf’s blade. Thus the major perturbation induced in translocation rates by cooling cannot be ascribed to a photosynthetic artifact.

As shown in Figure 2B, the respiration rate in the petiole’s chilled zone, measured as rate of $CO_2$ production, decreased logarithmically after cooling started and finally attained a new steady-state rate of approximately 10% of normal after about 2 hr, comparable to the 95% decrease in oxygen uptake rate observed by Weatherley and Watson (12) in isolated strips of Flax viminalis bark at $-2$ C. Despite this marked decrease in respiration, translocation through the respiration-inhibited zone following cold acclimation of the petiole was unimpaired. Data were not obtained in the present study on the rate of $CO_2$ production as a measure of the metabolism of sugars in transit. It does not appear likely, however, in view of the substantial decline in respiration in the petiole tissues as a whole, that this value varied inversely with temperature, as observed by Coulson and Peet for mature stems of Salix viminalis (2). The more probable inference is that the temperature dependence of metabolic breakdown of sugar molecules in transit through petioles of 4- to 6-week-old sugar beet plants used in the present studies corresponded more closely with that observed in young Salix stems (2).

We may speculate that the reversal of low temperature inhibition during cold treatment resulted from a decrease in cytoplasmic viscosity induced by low temperatures. Jones (8–10) presents evidence that low temperatures increase intracellular ATP levels, and that this increase is associated with a rise in cytoplasmic solubility. Accordingly, measurements were made of the molar concentration of ATP in the chilled zone of the petiole as a function of duration of cold treatment. No evidence for any sustained increase in the steady-state concentration of ATP at low temperatures was obtained (Table 1). Conclusions from these data are obscured, however, by the large standard deviations of the means. Although good agreement was usually obtained between duplicate samples from any one petiole, variations between petioles from leaves on different plants (or from different leaves on the same plant) were often substantial. Because of the interpetiole variability, an increase in ATP level exceeding 50% would have been necessary to be significant at the 95% confidence level (the increases reported by Jones varied usually from about 50% to over 100%). To the extent that these data may be considered to indicate no substantial increase in ATP level, and to the extent that an inverse relationship between changes in ATP level and cytoplasmic viscosity holds for sugar beet cells, we may conclude that normalization of translocation rates through cold-treated zones does not result from increased cytoplasmic solubility.

### DISCUSSION

As the above results show, the marked inhibition in the transport capacity of sugar beet petioles by low petiolar temperatures and the subsequent recovery at continued low temperatures of this system with time to full transport capacity, cannot be correlated with any corresponding changes in source leaf photosynthetic rates, in petiolar respiration rates, or in petiolar ATP turnover rates (based on calculated ATP generation rates). Particularly striking is the increasing disparity with time (to a limiting ratio) between translocation rate and respiration rate (Fig. 2A versus 2B). Computed on a relative basis, the translocation rate, after 3-hr cold treatment, had increased on the average about 20% in the same time period that the respiration rate had declined about 90%. Concomitantly, the ATP turnover rate diminished about 10-fold as inferred from the relatively constant steady state level of ATP in the petiole, despite a 10-fold reduction in the petiolar respiration rate following cooling.

Stated in absolute units, the sucrose translocation rate to the sink leaf before petiolar chilling averaged 56 nmole min$^{-1}$ dm$^{-2}$ source-leaf blade and the petiolar respiration rate 4.6 nmole

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**Table I. ATP Levels in nmols/10 mg Petiole (Fresh Weight) as a Function of Duration of Cold Treatment (0.7 to 2.5 C) of the Petiole**

Each figure is the mean of triplicate analyses carried out on duplicate 0.5-cm samples taken from four to nine different petioles (except for the 1080 and 1440 min treatments, in which only one petiole each was sampled). To convert to nmols ATP cm$^{-1}$ petiole, multiply by 12.5.

<table>
<thead>
<tr>
<th>Control</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
<th>1080</th>
<th>1440</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56 ± 0.27</td>
<td>0.70 ± 0.24</td>
<td>0.70 ± 0.14</td>
<td>0.56 ± 0.15</td>
<td>0.65 ± 0.14</td>
<td>0.80 ± 0.31</td>
<td>0.63 ± 0.31</td>
<td>0.54 ± 0.20</td>
<td>0.46 ± 0.06</td>
<td>0.83 ± 0.06</td>
</tr>
</tbody>
</table>

LSD ($P < 0.05$) = 0.27, 0.27, 0.27, 0.29, 0.29, 0.29, 0.29, —, —

1 Except for 1080 and 1440 min data where actual range is given, ±1 standard deviation.
CO₂ min⁻² cm⁻² petiole. After 3 hr of cold treatment, the respective values were 67 nmoles and 0.46 n mole.

As a measure of the energy requirements of the translocation process within the petiole’s conducting system, it is instructive to attempt a calculation of the moles of sucrose transported per mole of ATP generated in the transport cells. The most critical measurement would be the maximum permissible value of this ratio, but this calculation is not possible from present data. A rough estimate, however, of this ratio, based on the assumptions given below, may be calculated from the respective rates of petiole respiration and translocation obtaining after 3 hr of low temperature treatment. At this time, translocation has fully recovered, and the rate of petiole respiration (in the 4-cm chilled zone) has declined to its minimal steady state value.

Assuming full phosphorylative coupling, that no competitive demands exist on the ATP pool other than for sucrose translocation, that the sucrose-transport system is restricted to the sieve-tube lumina, and that the rate of respiration (and correspondingly of ATP synthesis) on a unit cell volume basis averages the same in the sieve-tube elements as in the other cells of petiole tissues, this ratio computes to greater than 900,000 (see appendix for calculations). That is, per molecule of ATP generated in a sieve-tube element, approximately 900,000 sucrose molecules can be transported a distance equal to the length of that element (0.02 cm) at an average velocity of 0.9 cm min⁻¹. If normalized to the ATP generated in both sieve-tube elements and companion cells, this ratio reduces to 630,000. The values of the various parameters used in arriving at this calculation are given in the appendix. Although the several assumptions used in this argument are subject to serious dispute, the substantial magnitude of this ratio appears to justify the conclusion that the major sources of energy input actuating the transport mechanism within the sieve tubes function mainly at loading or unloading sites (or both) and are not directly coupled to energizing longitudinal transport from sieve element to sieve element along the transport path. It appears, therefore, that the metabolic requirements for sieve-tube translocation reflect chiefly the energy requirements for the structural maintenance of this system. It will be of great interest to determine the minimum cytological organization of the sieve-tube elements which permits unimpaired translocation.

APPENDIX

Estimated molar ratio of sucrose translocated to ATP produced.

PARAMETERS

1. Cross-sectional area of donor-leaf petiole = 0.20 cm².
2. Aggregate cross-sectional area of sieve tubes in petiole = 3.4 × 10⁻¹ cm². This value is taken from Geiger et al. (4) and is based on plants of the same cultivar grown under very similar conditions to those used in this study.
3. Percent cross-sectional area of petiole occupied by sieve tubes = 0.17%.
4. Average length of sieve-tube element in petiole = 0.02 cm.
5. Average velocity of transport in sieve tubes = 0.9 cm min⁻¹. This value is taken from Geiger et al. (4). Although this velocity was measured in petioles maintained at room temperature, Geiger and Sovonick (5) have recently shown that the transport velocity in petioles (based on plants of the same cultivar as used in the present study) is the same at 2 C following cold acclimation as at room temperature.

6. Average molar rate of transport in sieve tubes = 89 n moles sucrose min⁻¹. This rate was derived as follows: average molar rate at 1 C to sink leaf after cold acclimation (3 hr at 1 C) = 67 n moles sucrose min⁻¹ dm⁻² source leaf (Fig. 2A); average size of source leaves = 0.664 dm²; estimated ratio of total sink to sink leaf = 2 (this factor is an estimated correction for assessing the total export from the source leaf; it is assumed that the amount of translocate received by other parts of the sink, chiefly the crown tissue, equalled the amount received by the sink leaf, the only part directly measured). Hence, 67 n moles min⁻¹ dm⁻² × 0.664 dm² × 2 = 89 n moles sucrose min⁻¹.

7. Average rate of petiole respiration after 3 hr of cold treatment = 0.46 n mole CO₂ min⁻¹ cm⁻² petiole (Fig. 2B).
8. Average calculated rate of ATP production in petiole after cold acclimation (3 hr at 1 C) = 0.46 n mole CO₂ min⁻¹ cm⁻² petiole × 6 ATP/CO₂ = 2.8 n moles ATP min⁻¹ cm⁻¹.

CALCULATIONS

1. Average residence time of sucrose molecules in transit through sieve-tube elements = 0.02 cm/0.9 cm min⁻¹ = 0.022 min.
2. Average quantity of sucrose in transit through a 0.02-cm length of petiole = 0.022 min × 89 n moles min⁻¹ = 2.0 n moles.
3. Average calculated n moles ATP produced by the sieve-tube elements in a 0.02-cm length of petiole at 1 C during a residence time of 0.022 min for sucrose molecules in transit = 2.8 n moles ATP min⁻¹ cm⁻² × 0.0017 × 0.02 cm × 0.022 min = 2.1 × 10⁻⁶ n moles ATP.
4. Ratio of sucrose molecules in transit through a sieve-tube element to ATP molecules generated in a sieve-tube element during an average residence time of 0.022 min = 2.0 n moles sucrose/2.1 × 10⁻⁶ n moles ATP = 950,000.

LITERATURE CITED