Interaction of Cell Turgor and Hormones on Sucrose Uptake in Isolated Phloem of Celery

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ABSTRACT
Phloem tissue isolated from celery (Apium graveolens L.) was used to investigate the regulation of sucrose uptake by turgor (manipulated by 50–400 milliosmolar solutions of polyethylene glycol) and hormones indoleacetic acid (IAA) and gibberellin acid (GA3). Sucrose uptake was enhanced under low cellular turgor (increase in the Vturg). Furthermore, enhancement of sucrose uptake was due to a net increase in influx rates since sucrose efflux was not affected by cell turgor. Manipulations of cell turgor had no effect on 3-O-methyl glucose uptake. When 20 millimolar buffer was present in uptake solutions, low turgor-induced effects were observed only at low pH range (4.5–5.5). However, the effect was extended to higher external pH (up to 7.5) when buffer was omitted from uptake solutions. A novel interaction between cellular turgor and hormone treatments was observed, in that GA3 (10 micromolar) and IAA (0.1–100 micromolar) enhanced sucrose uptake only at moderate turgor levels. The hormones elicited little or no response on sucrose uptake under conditions of low or high cell turgor. Low cell turgor, IAA, GA3, and fusicoccin caused acidification by isolated phloem segments in a buffer-free solution. It is suggested that enhanced sucrose uptake in response to low turgor and/or hormones was mediated through the plasmalemma H+-ATPase and most likely occurred at the site of loading.

Phloem loading rates are also believed to be hormonally regulated. However, this assumption is based on evidence from complex tissues (6, 11). Recently, Daie (2–4) developed and described a system consisting of a phloem tissue isolated from celery allowing a more direct examination of the regulation of sucrose loading. Using this relatively simple model system, Daie et al. (5) presented evidence indicating that GA3 and IAA had a direct effect on sucrose loading rates not only in isolated phloem segments but in intact celery plants.

Turgor and hormones (1) at the site of loading appear to be important regulators of translocation rates. However, their exact site of action and possible interactions are not well understood. This communication reports on the effects and interactions of cell turgor and exogenous hormones on sugar transport and their relevance to sucrose loading. Our results, together with those of Patrick and colleagues (12, 13) agree with the tenets of the turgor-regulated translocation hypothesis.

MATERIALS AND METHODS

Plant Material. Celery (Apium graveolens L. cv Utah 5270) was grown from seed in a greenhouse in 10 L pots filled with a 3:1 (v/v) peat:perlite mix. The day length was extended to 14 h using high pressure sodium lamps (PL Light Systems, Ontario, Canada). Photon flux density at the canopy level was approximately 600 μmol m⁻²s⁻¹. The temperature in the glasshouse was set at 25/18°C day/night. Plants were fertilized with a 20-20-20 (N-P-K) Peters mix and Peters micronutrients once a week and were harvested when they were 4 to 6 months old. Celery has high calcium requirements; therefore, in addition to the regular fertilization schedule, plants were watered daily with a 0.25 m Ca(NO₃)₂ solution.

Tissue Preparation. Tissue preparation was as described by Daie (2). Briefly, petioles of mature but photosynthetically active leaves were harvested 2–3 h prior to any experiments. Isolated phloem strands were preincubated for 1 h in an aerated base medium adjusted to the appropriate osmolality with polyethylene glycol (PEG, 3350 purchased from Sigma Chemical Co.). In addition to the osmoticum, the base medium contained 1 mM CaCl₂, 2 mM KCl, 5 mM MgCl₂, 1 mM CaSO₄, and in the case of buffered solutions 20 mM nonpenetrating buffers: Mes (pH 4.5 and 5.5), MOPS (pH 6.5), Hepes (pH 7.5), and Tris (pH 8.5). The pH of all buffer solutions was adjusted with bis-tris propane (BTP). After preincubation, phloem strands were cut into 15 mm long segments, pooled, and transferred to the incubation solution.

Measurement of Osmotic Concentration. Osmotic concentration of the sap from freshly isolated phloem strands and PEG solutions were determined in a vapor pressure Osmometer (Wescor, Logan, UT). To obtain sap, tissue was placed in a disposable syringe, frozen in liquid N₂, thawed, and the sap centrifuged through a filter paper into a microfuge tube. Osmotic concentration of tissue sap averaged 370 ± 40 mOsm.

1 Contribution No. D-15192-3-86 from the New Jersey Agricultural Experiment Station.
2 Abbreviations: SE/CC, sieve element/companion cell complex; MOPS, morpholinopropane sulfonic acid; Mes, morpholinoethanesulfonate; mOsm, milliosmolal; 3-OMG, 3-O-methyl glucose; Fc, fusicoccin; PCMB, p-chloromercuribenzenesulfonic acid; CCCP, carbonylcyanide-m-chlorophenylhydrazone; FW, fresh weight.

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Sugar Uptake. Five segments (30 mg FW) were incubated for 1 h (uptake rates were linear for 90 min) in 1 ml incubation solution which was identical to the preincubation medium except it contained various concentrations of labeled sugars at 9.2 KBq ml⁻¹. All incubation solutions were isoosmotic. Unless otherwise stated, inhibitors and hormones were added only to the incubation solutions (same volume of the inhibitor's solvent was added to controls). After incubation, excess radioactivity from cut surfaces and cell wall free space was removed by washing the tissue three times, each time for 3 min using 3 ml of the preincubation solution. Tissue was then extracted overnight at 50°C in 1 ml 80% (v/v) ethanol after which 4 ml scintillation cocktail was added and radioactivity was measured in a 5801 Beckman scintillation counter.

All experiments were run in triplicate under ambient laboratory temperature and light conditions and were repeated at least twice. Data presented in figures was calculated on FW basis measured prior to exposure to treatment solutions with various osmolalities (to avoid change in FW due to gain or loss in turgor).

Sugar Efflux. Tissue was preincubated as previously described, then incubated for 1 h in base solution containing 300 mOsm PEG and 5 mM ¹⁴C-sucrose. After two quick rinses (5 s each) tissue was washed with buffer solution containing 50 or 300 mOsm PEG for 4 h. Wash solutions were changed at various times to monitor efflux. Each wash consisted of 3 ml which was collected and counted. To determine radioactivity in tissue at each time, total radioactivity measured in wash solutions (up to that time point) was added to residual label left in the tissue at the end of the wash procedure.

Media Acidification. One g of freshly isolated phloem strands was exposed to preincubation media containing 50 or 300 mOsm PEG for 1 h. The tissue was then transferred to corresponding fresh solution (4 ml) and the pH of the external solution was monitored over a 4 h period with a combination microelectrode (Microelectrodes, NH) and recorder system. To quantitate acidification, solutions were back titrated to their original pH with HCl at the end of the experiment.

RESULTS AND DISCUSSION

Turgor Effects. Our previous results (7) indicated that sucrose uptake rate was influenced only in the presence of nonpenetrating osmotica such as mannitol. Although mannitol is relatively nonpenetrating in most tissues, it is highly permeable in celery because it is translocated in the phloem (3). In celery, mannitol, and ethylene glycol, another penetrating osmoticum did not, while PEG did, alter sucrose uptake rates. It was evident that only the turgor component and not the osmotic component of the tissue influenced sucrose transport in isolated phloem.

PEG-induced changes in cell turgor were indicated by plasmolysis (incipient) with 500 mOsm PEG. At this concentration more parenchyma than SE/CC were plasmolyzed. Also, PEG treatment resulted in lower tissue FW suggesting loss of water. In subsequent experiments, 50 mOsm PEG solutions were used to generate high and 300 mOsm to induce low cell turgor. While it is recognized that the actual turgor in the cell was not directly determined, an estimate of turgor change in the tissue was possible. Under steady state conditions, tissue water potential is equal to the solution osmotic potential. Knowing that the osmotic potential of cells was 370 mOsm (osmometer reading), then cell turgor potential was calculated to be 0.80 and 0.18 MPa in tissue exposed to 50 or 300 mOsm PEG, respectively (this translates to 0.62 MPa or 6.2 bars decrease in cell turgor). The highest concentration of PEG used (300–400 mOsm) may have caused slight, if any, incipient plasmolysis.

To determine the cell turgor for maximal sucrose transport, uptake of 5 mM sucrose (Κₑ = 5 mM; see Ref. 4) was monitored in the presence of 50 to 800 mOsm PEG. Highest sucrose uptake rate occurred in the presence of 300 mOsm PEG (Fig. 1). While sucrose uptake was inhibited both at low and high solution osmolarity, the inhibition was greater at high as compared to low PEG concentrations. When 2 mM PCMBS (sulfhydryl binding inhibitor) was added to the solutions (passive uptake), no turgor effect was observed indicating that only the active component (carrier-mediated) of sucrose uptake responded to cell turgor.

Isolated phloem is highly specific for sucrose and mannitol uptake (2). To determine the specificity of the turgor response, uptake of sucrose and mannitol were compared to that of 3-OMG (analog of glucose, recognized by the glucose carrier but not metabolized) at low and high turgor. Both mannitol and sucrose uptake rates were higher at low as compared to those at high turgor (Fig. 2). However, 3-OMG uptake was not affected by the external solution concentration (data not shown).

Regardless of the osmotic concentration of the uptake solutions, presence of inhibitors (2 mM PCMBS or 5 μM CCCP) caused a dramatic decrease in sucrose uptake rates as well as changing the uptake kinetics (Fig. 3). However, high turgor-induced inhibition of sucrose uptake was relatively moderate in that it only lowered the Vₘₐₓ. Similar turgor responses were obtained with mannitol (data not shown).

Lower sucrose uptake rates under high turgor may have been due to an enhanced rate of sucrose efflux or decreased rate of influx. Therefore, the effect of cell turgor on sucrose efflux was determined (Fig. 4). Washing the tissue for 4 h in solutions containing 50 or 300 mOsm PEG resulted in identical rates of efflux from the slow releasing compartment and equal retention of label in the tissue. This indicated that high turgor did not enhance sucrose efflux through the carrier-mediated system, and that passive membrane permeability was unaltered during the experiment. Enhanced sucrose influx at low turgor was consistent with results from Senecio leaf discs (14) but was in contrast to results from sink tissues, e.g. sugar beet root discs (16) and bean...
seed coats (13). The difference may be explained by the opposing functions of the two tissues (loading and unloading). At the site of loading, according to the mass flow theory, high turgor is required to maintain the hydrostatic pressure gradient between source and sink to facilitate assimilate translocation, whereas in sink tissue excessive accumulation of solutes resulting in high turgor would stop assimilate flow. Beyond a turgor set point (13) the sink cells would respond by lowering the internal sugar concentration through higher rates of efflux to the free space.

From data presented in Figures 2 and 3, it was apparent that only the $V_{\text{max}}$ and not the $K_m$ of sucrose uptake (and mannitol) was affected by cell turgor, suggesting that the activity of the H⁺-ATPase may have been lowered under high turgor; an assumption consistent with previous reports (10, 14, 16). This H⁺-ATPase is known to generate the transmembrane pH and electrochemical potential gradients. Since sucrose uptake is coupled to primary transport of protons, inhibition of H⁺-ATPase would decrease proton motive force and hence sucrose uptake rates.

If under high turgor sucrose uptake is inhibited solely due to dissipation of the pH gradients, then increasing the H⁺ concentration of the external solution is expected to compensate for the suboptimal H⁺-ATPase activity. Sucrose uptake at high or low turgor was determined in buffered (20 mm) solutions. Regardless of turgor, the pH optima for sucrose uptake was in the acidic pH range (Fig. 5). At low turgor sucrose uptake was higher than that at high turgor only at low external pH range (4.5–5.5). No turgor effect was observed at high (6.5–8.5) pH. Since at low pH range excess protons were available, the inhibition of sucrose uptake at high turgor appeared to result from factor(s) other than H⁺ availability, e.g. decrease in the PMF (10, 16). Furthermore, conformational changes (17) in the carrier may have made the H⁺ binding site inaccessible to its substrate. When identical experiments were conducted in unbuffered solutions (pH adjusted with HCl or NaOH), at low turgor enhancement of sucrose uptake was extended to higher pH (Fig. 6) indicating that low turgor partially compensated for suboptimal external pH. The data in Figure 5 clearly indicated that protonation of the carrier was a prerequisite for the turgor effect. Indeed, in buffer-free solutions (low buffering capacity), it appeared that at low turgor, the system may have been able to partially provide the required protons for carrier protonation. Collectively, data in Figures 5 and 6 suggest that turgor-induced ATPase-mediated transport of sucrose depends on both the transmembrane pH gradient and the PMF. The observed turgor and pH interactions and responses were specific for phloem sugars, because 3-OMG uptake was not affected by pH or turgor manipulations (Table I).

**Media Acidification.** To more directly determine the effect of turgor on H⁺-ATPase activity, acidification of external media by the tissue was determined. Cellular turgor had a significant effect on the ability of isolated phloem to acidify the external medium. During a 4 h incubation, a decrease of 0.3 pH unit (0.3 μmol H⁺ equivalents) occurred in solutions containing 300 as compared to those with 50 mOsm PEG (Table II), pointing to a turgor-regulated ATPase activity (10, 14, 16).

**Hormone Effects.** IAA, GA, and FC are presumed to enhance sucrose uptake through their enhancement of the H⁺-ATPase (11 and references therein). However, such effects would have been masked in our studies because excess protons were available in the uptake media (20 mM Mes, pH 5.5). To examine this possibility, experiments were conducted in buffer-free media (pH adjusted to 7.00 with NaOH) containing 300 mOsm PEG. Under these conditions, 2 μM FC caused 30 to 50% enhancement of
both sucrose and mannitol uptakes (Table III). However, GA and IAA effects were insignificant and variable.

In another set of experiments, the osmotic concentration of the buffer-free solutions was varied. At high and low turgor GA and IAA caused a 0 to 25% enhancement of sucrose uptake (Fig. 7). However, at an intermediate turgor (200 mOsm) both GA and IAA caused a doubling of sucrose uptake rates. IAA was effective at a wide concentration range (0.1–100 μM) but GA exhibited a clear optimal concentration at 10 μM. FC effect (Table III) appeared to be less specific and did not depend on cell turgor. Kinetic analysis of IAA and GA on sucrose uptake (Lineweaver-Burk plot) indicated that only the Vmax of sucrose transport was changed (Fig. 8). Increase only in Vmax is most likely the result of enhanced ATPase activity, a plausible explanation for the hormone role in sugar uptake and especially the observed interaction of hormone and turgor-regulated sugar transport. Similar results were obtained for mannitol (data not shown).

To examine the hormone effect on the H+-ATPase activity,

Table I. Effect of Cell Turgor and External pH on Uptake of 5 mM 3-
OMG in Isolated Phloem Segments of Celery

<table>
<thead>
<tr>
<th>pH</th>
<th>Buffer (20 mM)</th>
<th>Influx (μmol g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>300</td>
</tr>
<tr>
<td>7.5</td>
<td>Heps</td>
<td>1.0 ± 0.2, 0.9 ± 0.1</td>
</tr>
<tr>
<td>6.5</td>
<td>MOPS</td>
<td>1.2 ± 0.2, 1.0 ± 0.3</td>
</tr>
<tr>
<td>5.5</td>
<td>Mes</td>
<td>0.9 ± 0.2, 1.0 ± 0.2</td>
</tr>
</tbody>
</table>

Table II. Effect of Cell Turgor on the Ability of Isolated Phloem
Segments of Celery to Acidify the Buffer-free External Solution (Base
Media)

Solution pH was adjusted to 6.5 with 0.1 M NaOH. Data points are
means of two observations ± SE. The change in pH (300 mOsm)
represented 0.3 μmol H⁺ equivalents.

<table>
<thead>
<tr>
<th>Elapsed time (min)</th>
<th>Osmotic Concentration mOsm PEG (media pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>0</td>
<td>6.51 ± 0.05</td>
</tr>
<tr>
<td>120</td>
<td>6.48 ± 0.08</td>
</tr>
<tr>
<td>180</td>
<td>6.46 ± 0.02</td>
</tr>
<tr>
<td>240</td>
<td>6.46 ± 0.03</td>
</tr>
</tbody>
</table>

Table III. Effect of FC on Sucrose and Mannitol Uptake in Isolated
Phloem Segments of Celery

Data points are means of three replicates ± SE. Tissue was incubated for 1 h in buffer-free base media (300 mOsm); pH of the solutions was adjusted to 7.0 with NaOH. FC was present in both preincubation and incubation solutions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Influx (μmol g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucrose (mm)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td>0.98 ± 0.05</td>
</tr>
<tr>
<td>FC (2 μM)</td>
<td>1.50 ± 0.02</td>
</tr>
</tbody>
</table>
10 μM GA or IAA were added to buffer-free solutions containing phloem segments. Since FC is a known promoter of the H+-ATPase, 5 μM FC was used in separate containers to serve as a comparison point. During a 4 h experiment, both GA and IAA caused significant acidification of the media (Table IV).

The data indicated a three-way interaction between proton availability (solution pH), cell turgor, and hormone levels. In addition to data presented here, it has been suggested that the two latter factors modulate the activity of the H+-ATPase (9, 11, 14, 16). Therefore, it is concluded that the observed interaction between cell turgor and hormone treatment was the result of conditions which lead to maximum H+-ATPase activity and hence sucrose transport. The conclusion gains more credence because sucrose uptake in this tissue was shown to be a H+-cotransport (2). Furthermore, the assumption provides an explanation for results in Figure 7; under high turgor the H+ pump is inhibited, so that hormone effects are minimal. At low turgor the H+ pump is already at its maximal activity so that hormones may not elicit any further response. Under moderate turgor levels the pump is neither inhibited nor maximally activated; therefore, the hormones may synergistically maximize its activity.

Although the H+-ATPase-mediated interaction of hormones and turgor appears to be a plausible explanation, the observed responses may be more complex because at higher turgor, sucrose uptake was inhibited even in buffered (excess proton) solutions. Therefore, it is likely that while hormones and low turgor have a direct effect on the H+-ATPase activity, turgor changes may induce conformational changes in the carrier protein itself (17). In a tissue committed to assimilate export (phloem), fast responses to turgor changes (this study) appear to be ATPase mediated. However, exposure to prolonged mild water stress and alterations in hormonal levels and/or compartmentations may cause changes in gene expression and protein synthesis, leading to modifications in the number of sugar carriers in the membrane. Therefore, reductions in transport $V_{\text{max}}$ may not be solely the result of membrane depolarization, but reduced number of carriers incorporated into the membrane.

Two limitations in our system may be recognized: presence of phloem parenchyma cells in the tissue and the fact that excised segments may not represent in situ translocation. Although the data do not, unambiguously, resolve the exact site of turgor/hormone response, we propose that the observations were, most likely, occurring at the site of loading. It was shown (2) that the contribution of parenchyma cells to sucrose and mannitol uptake was through a diffusional process. Since in this study only the carrier-mediated component responded to treatments, it may be assumed that parenchyma cells were not involved in the response. Moreover, our work with intact celery (5) had indicated the relevance of our observations in vitro to in situ translocation. The fact that the tissue is nontranslocating, does not necessarily mean that the ATPase-regulated response is impaired. Further in vivo work should clarify our contention.

### LITERATURE CITED