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# Sucrose Loading in Isolated Veins of *Pisum sativum*: Regulation by Abscisic Acid, Gibberellic Acid, and Cell Turgor[1]

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## ABSTRACT

**Enzymatically isolated vein networks from mature pea (*Pisum sativum* L. cv Alaska) leaves were employed to investigate the properties of sucrose loading and the effect of phytohormones and cell turgor on this process. The sucrose uptake showed two components: a saturable and a first-order kinetics system. The high affinity system ($K_m$, 3.3 millimolar) was located at the plasmalemma (*p*-chloromercuriphenylsulfonic acid and orthovanadate sensitivity). Further characterization of this system, including pH dependence and effects of energy metabolism inhibitors, supported the $H^+$-sugar symport concept for sucrose loading. Within a physiological range (0.1–100 micromolar) and after 90 min, abscisic acid (ABA) inhibited and gibberellic acid ($GA_3$) promoted 1 millimolar sucrose uptake. These responses were partially (ABA) or totally ($GA_3$) turgor-dependent. In experiments of combined hormonal treatments, ABA counteracted the $GA_3$ positive effects on sucrose uptake. The abolishment of these responses by *p*-chloromercuriphenylsulfonic acid and experiments on proton flux suggest that both factors (cell turgor and hormones) are modulating the $H^+$ATPase plasmalemma activity. The results are discussed in terms of their physiological relevance.**

Assimilate distribution appears to be under sink control and may be hormonally regulated (11, 24). Although many different sites and processes have been described as possible targets of hormonal control, much attention has been paid to the effects of hormones on both phloem loading and unloading of sucrose (17). We developed an experimental system to study the hormone-directed transport in intact pea plants during parthenocarpic fruit set induced by GAs (18). Sucrose transport from source to sink was activated by $GA_1$ and $GA_3$ increasing phloem unloading at the sink (ovary). Moreover, a strong increase in sucrose exported from the source leaf was observed when the GAs reached the leaf adjacent to the ovary, suggesting that sucrose loading at the source could also be activated by GAs.

The current knowledge on the mechanism of phloem loading is based, initially, on studies of sucrose uptake kinetics carried out with leaf discs and other complex plant materials. Attempts have been made to develop new experimental approaches to obtain more homogeneous, phloem-enriched, and still functional tissues. Thus, it is possible in some plants to dissect surgically the vascular components from mesophyll cells, and even to separate phloem from xylem tissue (2, 4). Daie *et al.* (5), using surgically isolated phloem tissue from celery as well as intact plants reported that $GA_3$ and IAA increase sucrose loading and that the hormonal effects were cell turgor-dependent and $H^+$ATPase-mediated (3).

To remove the mesophyll cells of fully expanded leaves, the alternative of choice for most plant systems should be to resort to enzymic maceration. Although sucrose uptake has been characterized using this approach (1, 22, 27), hormonal regulation of this process has not been reported.

The aim of the present work was to use enzymatically isolated functional phloem-enriched tissue from mature leaves of *Pisum sativum* to study the effects of $GA_3$, ABA, and cell turgor on sucrose uptake. Our results show the suitability of this simplified experimental system to investigate factors controlling the process of sucrose uptake.

## MATERIALS AND METHODS

### Plant Material

Plants of *Pisum sativum* L. cv Alaska No. 7 were grown from seeds as described previously (18). Several days before the experiment, plants were transferred from a greenhouse to a controlled cabinet and maintained on a photoperiod of 16 h light (Philips TLD 18W-33 fluorescent tubes, 20 $W \cdot m^{-2}$ at the level of the first flower) and 8 h dark with temperature ranges of 22 to 24°C and 16 to 18°C, respectively.

### Vein Network Isolation

For the isolation of the vein networks, leaves adjacent to the first flower (seventh node) were employed 48 h after

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anthesis. Leaves were cut off and their abaxial epidermis was stripped. The resulting leaves were floated on 5 mL of base solution (BS)[4] consisting of 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM KCl, and 5 mM Mes (pH 6.0), adjusted to the appropriate osmolality with mannitol (Sigma). After 30 min, the BS was replaced by a maceration medium containing BS supplemented with 2% cellulase Onozuka R-10 (Yakult Co Ltd.) and 0.05% pectolyase Y-23 (Seishin Pharmaceuticals), and gently shaken (50 rpm) for 90 min at 28°C. The adaxial epidermis and, with it, all the mesophyll cells, were then removed with a fine forceps. The resulting vein network was rinsed twice in BS.

Image analysis (IBAS 2000, Kontron) of pictures of the isolated vein networks showed that the surface area of the vein system represented 48% of the total surface area of the pea leaf. The dry weight of the isolated veins accounted for 17.1% of the fresh weight of the leaves ($13.7 \pm 0.1$ mg·cm$^{-2}$).

The viability of the vein system was determined with fluorescein diacetate as described by Wildhom (25).

### Light and Electron Microscopy

For morphological studies, tissue samples (vein networks and whole leaves) were prepared as described by Vercher *et al.* (23) for the pea ovaries, except that the fixation and wash buffer was 0.05 M phosphate instead of 0.1 M. Ultrathin sections were observed under a Jeol 100S electron microscope.

### Sugar Uptake

The vein networks were incubated for 1 h in 5 mL of buffer-free BS (pH adjusted to 6.0 with NaOH) BS containing various concentrations of unlabeled sugars and [U-$^{14}$C]sucrose or D-[U-$^{14}$C]sorbitol (Radiochemical Center, Amersham; specific activity, 25.9 MBq·mol$^{-1}$ to 77.7 GBq·mol$^{-1}$). All incubation solutions were isoosmotic (unless otherwise stated, adjusted to 400 mOsm). When used, inhibitors were applied in a preincubation period of 30 min and, except for PCMPS, also during the incubation with sugars. Controls contained the same volume of the inhibitor's solvent. In experiments dealing with hormonal effects on sucrose uptake, growth substances were added to a buffer-free BS, present during both preincubation and incubation periods. After the incubation, excess radioactivity from the surface was completely removed by two successive, 2-min washes with 5 mL of the BS. The tissue was then killed with 80% (v/v) aqueous ethanol at 80°C for 10 min and homogenized. After centrifugation (1600*g*, 10 min) the ethanol-soluble radioactivity was measured by liquid scintillation. For the pH experiments, 5 mM nonpenetrating buffers were used: Mes (pH 4.5 and 5.5), Mops (pH 6.5), Hepes (pH 7.5), and Tricine (pH 8.5). The sugar uptake period was performed with gentle shaking at 30°C under illumination (Osram nitraphot 500-W lamps, 380 W·m$^{-2}$).

### Determination of the Apoplastic Concentration of Sucrose

Apoplastic sucrose of the pea leaf was collected as described by Delrot *et al.* (9). Leaf excision was carried out 3 to 4 h after the beginning of the photoperiod. After quick removal of the lower epidermis, leaves were floated on 5 mL of BS with 250 mM mannitol in the dark at 4°C. At several times, aliquots of 0.3 mL were withdrawn, mixed with 1 μL of 2-mercaptoethanol, and boiled for 10 min. Sucrose concentration was determined spectrophotometrically by enzymatic methods adapted from ref. 13. Leaf apoplastic volume was evaluated according to Morrod (16). Peeled leaves were floated on 50 mL of BS containing 250 mM mannitol, 0.1% inulin (Merck), and [$^3$H]inulin (Radiochemical Center; specific activity, 18.5 kBq·mol$^{-1}$). After different times, tissue was rapidly rinsed in cold medium and extracted with 80% ethanol, and the radioactivity was counted.

### Proton Flux

Proton extrusion activity was continuously monitored using a pH meter (Orion Research Ion Analyzer EA920) connected to an IBM-PC. As standard conditions, about 55 to 60 cm$^2$ of vein networks (preincubated in BS for 2 h) were placed in a vial containing 7 mL of BS continuously mixed with a magnetic stirrer. Measurements were made under room light and temperature.

## RESULTS

The isolated vein networks maintained their original macroscopic structure (Fig. 1A). The use of the fluorescent dye fluorescein diacetate provided additional evidence that the vein networks were largely intact, indicating the viability of this system.

### Ultrastructure of Phloem-Enriched Leaf Tissue of *P. sativum*

Transverse sections of isolated veins were studied by light microscopy to ascertain the integrity of the vascular tissue. The isolated central vein showed a structure similar to the central vein of the intact pea leaf (Fig. 1, B and C), except that the mesophyll cells adjacent to the vascular bundle had their walls disrupted. Xylem and phloem cells did not seem

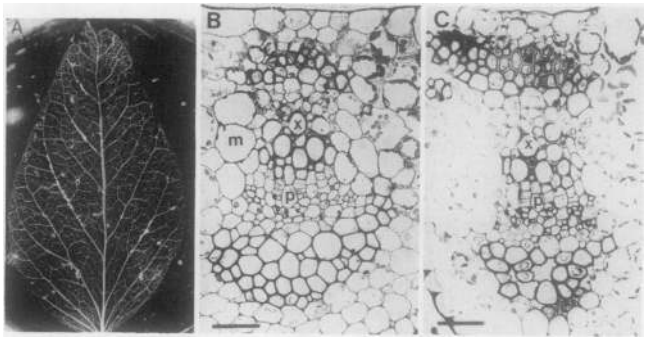


**Figure 1.** Vein networks enzymatically isolated from mature leaves of *P. sativum*. A, Leaf with stripped abaxial epidermis and treated (90 min) with cellulase and pectolyase. B and C, Light micrographs of transverse sections of the central vein of pea leaf. B, intact leaf; C, enzymatically isolated vein. Bar = 50 μm. m, mesophyll; p, phloem; x, xylem.

[4] Abbreviations: BS, base solution; PCMPS, *para*-chloromercuriphenylsulfonic acid; FC, fusicoccin.

affected by the enzymatic treatment, not even the cells of the bundlesheath.

The ultrastructural characteristics of the phloem cells in the isolated veins were essentially the same as the intact leaf.

### Sugar Uptake

The measurement of the sucrose uptake by the isolated vein networks of pea leaves was essentially not affected by the metabolism of this sugar in the tissue. Thus, in the uptake experiments using asymmetrically labeled sucrose ([$^{14}$C]fructosyl sucrose) followed by HPLC analyses of the resulting extracts, we found all the label in the fraction with the retention time corresponding to sucrose. On the other hand, after treatment of extracts with yeast invertase (Boehringer-Mannheim), all the label was found in the fraction with the retention time corresponding to fructose. This total absence of $^{14}$C label in the glucose moiety indicated that sucrose was taken up by the isolated veins without extracellular hydrolysis.

As can be observed (Fig. 2A) the uptake up to 90 mM sucrose by the isolated veins showed a two-component kinetics: a saturable system and an apparently nonsaturable or low affinity system. In the presence of PCMPS, a nonpenetrating thiol binding agent, the kinetics were linear with the same slope as the nonsaturable system (Fig. 2A). The contribution of the saturable component with highest affinity was ascertained by substracting sucrose uptake in the presence of PCMPS from total uptake, using sucrose concentrations below 10 mM (Fig. 2B). The saturable component exhibited an apparent $K_m$ of 3.3 mM (Fig. 2C).

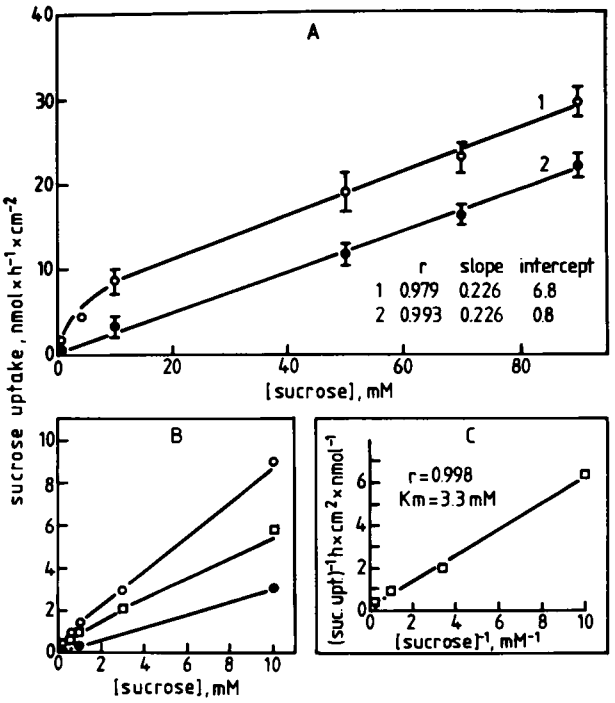


**Figure 2.** Concentration dependence (up to 90 mM) of the sucrose uptake by isolated veins of pea leaves. A, Effect of the presence (●) or the absence (○) of 2 mM PCMPS on sucrose uptake. B, Apparent linear component (●, +PCMPS) is subtracted from the total uptake (○, −PCMPS) for obtaining the saturable component (□). C, Lineweaver-Burk plot for the calculated values in B. Bars are the SE of the mean of three replicates, two leaves per replicate.

Sucrose uptake by isolated vein networks was dependent on cell turgor. Figure 3 shows the variation in sucrose uptake at different concentrations of mannitol, a nonpenetrating osmoticum in this system (data not shown). Highest sucrose uptake occurred at 500 mOsm. In addition, the presence of 5 mM $K^+$ in the medium promoted the sucrose uptake in the range of osmolality tested, the maximum being at 300 mOsm (Fig. 3). According to these results we used 5 mM KCl and 400 mOsm mannitol as standard incubation conditions.

### Secondary Transport of Sucrose

Sucrose uptake in isolated vein networks appears to occur according to the $H^+$-cotransport concept for the secondary transport of sucrose during phloem loading (10). Maximal sucrose uptake in this system was at pH 5.0 to 6.0 (Fig. 4), and the pH dependence was abolished by PCMPS, showing a profile similar to that of the uptake of sorbitol (a diffusion-like process) (Fig. 4). The presence of 10 μM FC (a potent enhancer of the plasmalemma $H^+$ATPase) in the medium resulted in 68% increase in uptake at 1 mM sucrose. The $H^+$-cotransport hypothesis is further substantiated by the observation that several agents that interfere with the generation or maintenance of an electrochemical gradient of $H^+$ inhibited sucrose uptake. Protonophores like 2,4-dinitrophenol (1 mM) and carbonylcyanide *m*-chlorophenylhydrazone (50 μM) dramatically reduced sucrose uptake (88% and 90% inhibition, respectively). The use of selective inhibitors of the proton-pumping activities (20) like orthovanadate (affecting plasmalemma $H^+$ATPase), diminished sucrose uptake rate (32%). However, in the presence of PCMPS that inhibits 64% of total sucrose uptake (Table I), no additional inhibition was produced by orthovanadate. Azide (a selective inhibitor of mi-

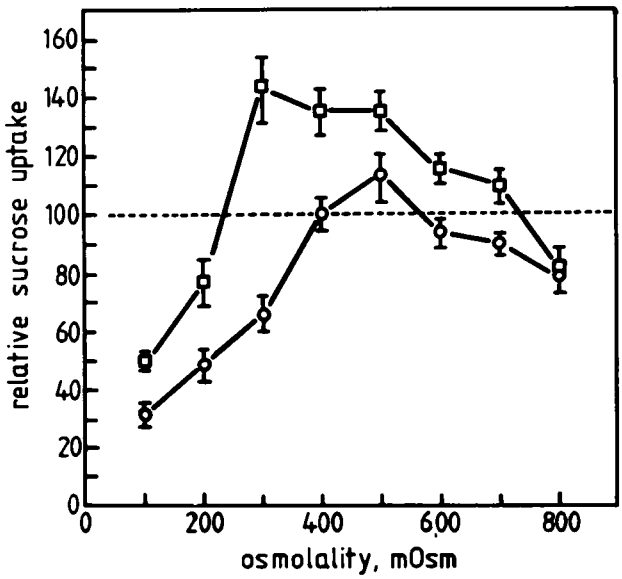


**Figure 3.** Effect of mannitol concentration in the incubation solution on uptake of sucrose (1 mM) by isolated veins of pea leaves. Uptake was in the presence (□) or absence (○) of 5 mM KCl. Bars are the SE of the mean of three replicates, two leaves per replicate.

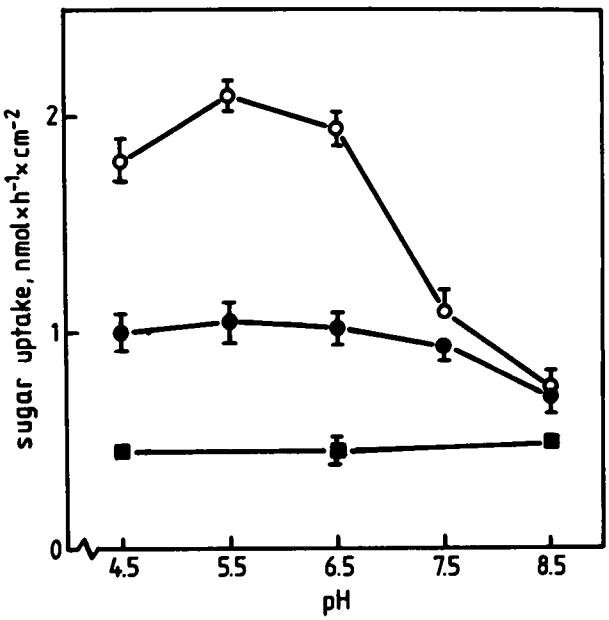


**Figure 4.** Effect of pH on uptake of 1 mM sucrose by isolated veins of pea leaves treated (●) and nontreated (○) with 2 mM PCMPS, and on uptake of 1 mM sorbitol (■). Bars are the SE of the mean of three replicates, two leaves per replicate.

**Table I.** *Effect of FC, ABA, and $GA_3$ on Uptake of Sucrose (1 mM) by Isolated Veins of Pea Treated and Nontreated with 2 mM PCMPS*

Tissue was incubated in buffer-free base medium (400 mOsm). Values are relative to sucrose uptake in controls (2.1 ± 0.7 nmol·$h^{-1}$·$cm^{-2}$, and are the mean of two replicates ± SE, two leaves per replicate.

| Substance Added | Sucrose Uptake (Relative to Control) | |
|---|---|---|
| | −PCMPS | +2 mM PCMPS |
| None (control) | 1.00 | 0.36 ± 0.05 |
| 10 μM FC | 1.67 ± 0.01 | 0.30 ± 0.02 |
| 1 μM ABA | 0.75 ± 0.04 | 0.40 ± 0.02 |
| 10 μM $GA_3$ | 1.40 ± 0.01 | 0.34 ± 0.02 |
| 10 μM FC + 1 μM ABA | 1.25 ± 0.02 | - |
| 10 μM FC + 10 μM ABA | 1.08 ± 0.02 | - |
| 10 μM $GA_3$ + 1 μM ABA | 1.00 ± 0.11 | - |
| 10 μM $GA_3$ + 10 μM ABA | 0.84 ± 0.02 | - |

tochondrial ATP synthase) and dicyclohexylcarbodiimide (a blocking agent of all known $H^+$ATPases) strongly reduced sucrose uptake by 90% and 73%, respectively.

### Hormonal Effects on Sucrose Uptake

After a relatively short period of time (a maximum of 90 min), ABA inhibits (25%) and $GA_3$ promotes (40%) sucrose uptake by the isolated veins of pea (Table I). However, $GA_3$-stimulated sucrose uptake ranged from 26% to 50% in experiments with a different set of plants.

The treatment of the vein networks with PCMPS negates the hormone-induced effects on sucrose uptake (Table I). The ability of isolated veins of pea leaves to acidify the external medium in the presence of ABA or $GA_3$ was determined using 10 μM FC as a positive control (Fig. 5). Abscissic acid (10 μM) caused a 21% inhibition and $GA_3$ (10 μM) a 40% stimulation of $H^+$ extrusion activity compared with the control. Under the same experimental conditions, FC (10 μM) enhances $H^+$ extrusion activity by 74%.

In experiments using mixtures of hormones, ABA diminished markedly both FC and $GA_3$ stimulation of sucrose uptake (10-fold ABA was needed to counteract the positive effect of 10 μM FC compared with 10 μM $GA_3$) (Table I).

The effect of ABA and $GA_3$ on sucrose efflux was measured to examine possible effects of hormones on membrane permeability. Washing the tissue during 2 h in the presence or absence of ABA (10 μM) resulted in equal rates of sucrose efflux up to 40 min but with different retention of label in the tissue after this time (data not shown). Nevertheless, $GA_3$ treatment did not show any effect on sucrose efflux from the isolated veins.

The hormonal effects on sucrose uptake were turgor-dependent (Fig. 6). Whereas $GA_3$ caused an enhancement of sucrose uptake with a clear optimal at 400 mOsm, ABA showed an inhibitory effect along the range of osmolality examined, being larger at intermediate turgor. At intermediate osmolality, where hormones exerted their maximum effects (400 mOsm), and using low osmolality (50 mOsm) as a control, we have observed that ABA showed an increasing inhibition along a broad concentration range (0.1–100 μM), whereas $GA_3$ exhibited an optimal concentration at 10 μM (Fig. 7). Considering the relatively low levels of hormones employed in the experiments, their effects on sucrose uptake may be of physiological significance, although the local concentrations within the cells could differ from the external ones due to the presence of diffusional barriers and/or to accumulation processes.

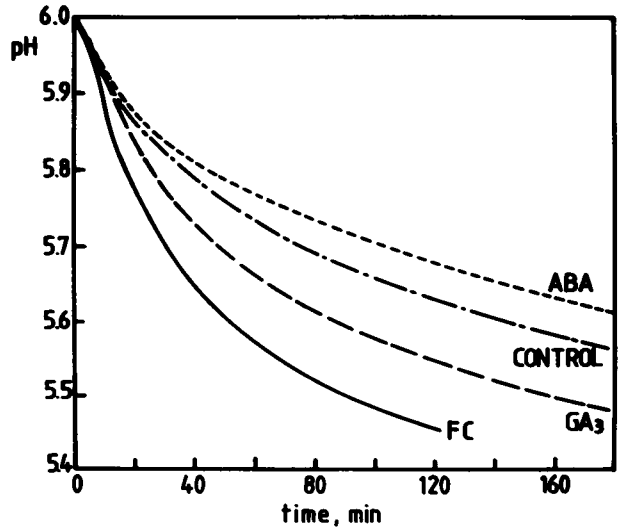


**Figure 5.** Effect of FC, ABA, and $GA_3$ on the proton extruding activity of the isolated vein networks of pea leaves. Tissue was incubated in unbuffered solutions (initial pH, 6.00) containing 400 mOsm mannitol. Each experiment was carried out with the same amount of plant material (58±3 $cm^2$) and the final concentration of added substances (at $t$=0 min) was 10 μM.

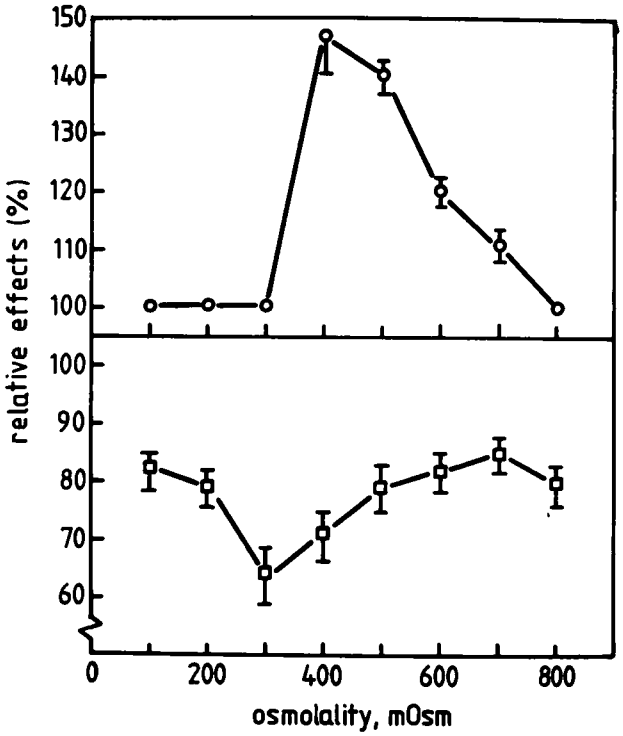


**Figure 6.** Turgor dependence of the ABA and $GA_3$ effects on sucrose uptake by isolated vein networks of pea leaves. Cell turgor was varied with mannitol. Uptake of 1 mM sucrose was in the absence (control = 100%) or presence of 10 μM $GA_3$ (○) or 10 μM ABA (□). Data are the mean of two replicates ± SE, three leaves per replicate.

## DISCUSSION

Isolated vein networks display two components in the kinetics of sucrose uptake. The inhibition of the high affinity saturable system ($K_m$, 3.3 mM) by PCMPS, which binds to the extracellular side of the binding center of the sucrose carrier (15), suggests that it is located at the plasmalemma. Although it has been reported that plasmalemma $H^+$ATPase is sensitive to mercurials (20), the $H^+$ extruding activity of isolated vein networks of pea leaves is not affected by PCMPS (data not shown). Similarly, 1 mM PCMBS exerted a strong inhibition on sucrose uptake, but did not inhibit proton extrusion in *Vicia faba* (8).

Further characterization of the carrier-mediated transport in our system came from studies on its pH dependence and proton efflux, and studies on the effect of energy metabolism inhibitors. Assuming that PCMPS is affecting the carrier but not the $H^+$ATPase of the plasmalemma, an outstanding observation is that, in the presence of PCMPS, orthovanadate did not cause any further inhibition. All evidence taken together indicates that sucrose uptake across the plasmalemma is a secondary transport mechanism based on a $H^+$-sugar symport.

The concentration of sucrose in the apoplast of pea leaves (9.8 mM) was on the order of the one reported by Delrot *et al.* (9) in *V. faba* leaves (1–5 mM). It is worth noting that the $K_m$ of the plasmalemma sucrose carrier is in the range of the sucrose concentration at the apoplast. Therefore, this carrier

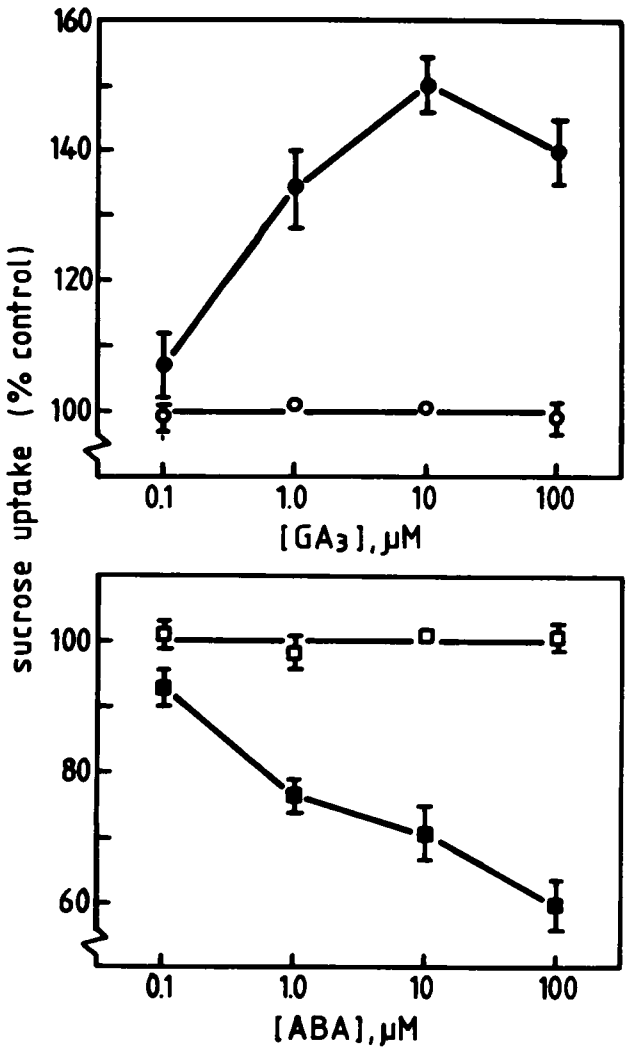


**Figure 7.** Effect of $GA_3$ or ABA on uptake of sucrose (1 mM) by isolated vein networks of pea leaves. Incubation was for 1 h in unbuffered solutions at pH 6 adjusted to 50 mOsm (○, □) or 400 mOsm (●, ■) with mannitol. In each experiment, various concentrations of hormones were added to preincubation (30 min) and incubation solutions (60 min). Data are the mean of two replicates ± SE, three leaves per replicate.

would not be working at maximum activity whereby it could be significant from a regulatory point of view.

On the other hand, taking into account the levels of sucrose in the apoplast of pea leaves, the relative contribution of the linear component of the sucrose uptake *in vivo* should be of little importance. Wilson *et al.* (27), working with stripped and enzymatically digested leaves of *Allium cepa*, and Daie (2), working with surgically obtained vascular bundles of celery, have suggested that a low affinity component represented the contribution of the phloem parenchyma to sugar uptake. Daie (4) has also suggested that the linear component of sucrose uptake by excised celery phloem is the diffusion contribution of xylem parenchyma mainly.

The existence of an active and selective sucrose uptake by the veins, together with the absence of plasmodesmatal connections between mesophyll cells and the vascular system,

points out an apoplastic mechanism (7) for sucrose loading into the phloem of *P. sativum*. In this sense, Turgeon and Wimmers (21), using leaf discs and autoradiographic techniques, have shown recently that an apoplastic pathway is a plausible explanation for the loading of sucrose in pea leaves.

Sucrose uptake rates were higher at low cell turgor than the ones at high cell turgor (see Fig. 5). According to the mass flow theory, high turgor is required to support the hydrostatic pressure gradient between source and sink to facilitate assimilate translocation (12). Thus, below a turgor set point, the sieve element-companion cell complex would respond by increasing the sugar uptake to maintain constant assimilate flow to sink tissues. Some studies (3, 6, 19) support the view that changes in cell turgor are occurring at the plasmalemma of the sieve element-companion cell complex, and suggest that $H^+$ATPase might play a key role in the mechanism of osmotic adaptation. Obviously, this osmoregulatory phenomenon should be transient, if it is occurring *in vivo*.

Sucrose uptake by isolated vein networks was affected by the presence of phytohormones (Table I). The disappearance of the hormonal effects after PCMPS treatment (see Table I) suggests that both ABA and $GA_3$ could affect sucrose loading at the level of the plasma membrane.

Since sugar-$H^+$ cotransport is a plausible explanation of phloem loading in this tissue, at least two possible targets for the hormonal action can be envisaged, namely the sucrose carrier and the $H^+$ATPase, both located at the plasmalemma. A direct effect of these hormones on the sucrose carrier should be excluded because ABA and $GA_3$ did not affect the 1 mM sucrose uptake in buffered solutions (5 mM Mes-OH, pH 6). Therefore, the hormonal action could be focused on the plasmalemma $H^+$ATPase. This supports previous findings of Malek and Baker (14) for ABA acting on sucrose loading in *Ricinus communis*, and by Daie (3) for IAA and $GA_3$ effects on sucrose uptake by isolated phloem segments of celery.

Furthermore, the hormonal response of the vein system was dependent on the osmotic concentration of the solution, indicating an interaction between hormonal effects and cell turgor. We could interpret the observed hormonal effects on sucrose loading in two different physiological situations. Under a *normal water supply*, $GA_3$ may play a role as a positive signal, coming from demanding areas, *i.e.* sinks like developing fruits (18), increasing phloem loading of sucrose at the source leaf. The $GA_3$ effects would be mediated by the plasmalemma $H^+$ATPase, an activity dependent of the cell turgor. In contrast, under a *water deficit* situation, to which pea plants are particularly sensitive (26), the reduction in cell turgor would be a major factor responsible for triggering the change both in the sucrose uptake activity and in the hormonal balance, mainly increasing the ABA level. The apparent hierarchy of the hormonal effects on sucrose loading is outstanding, ABA (negative signal, partially turgor-independent) playing a predominant role over $GA_3$.

This paper shows that, despite the enzymatic treatment, isolated vein networks of pea leaves are suitable to study not only sucrose loading but hormonal regulation of this process. However, further studies are necessary (*i.e.* high-resolution microautoradiography) to show the specific type of cells involved in sucrose loading.

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## LITERATURE CITED

1. **Cataldo DA** (1974) Vein loading: The role of the symplast intracellular transport of carbohydrate between the mesophyll and minor veins of tobacco leaves. Plant Physiol **53:** 912–917
2. **Daie J** (1986) Kinetics of sugar transport in isolated vascular bundles and phloem tissue of celery. J Am Soc Hort Sci **111:** 216–220
3. **Daie J** (1987) Interaction of cell turgor and hormones on sucrose uptake in isolated phloem of celery. Plant Physiol **84:** 1033–1037
4. **Daie J** (1987) Sucrose uptake in isolated phloem of celery is a single saturable transport system. Planta **171:** 474–482
5. **Daie J, Watts M, Aloni B, Wyse RE** (1986) *In vitro* and *in vivo* modification of sugar transport and translocation in celery by phytohormones. Plant Sci **46:** 35–41
6. **Daie J, Wyse RE** (1985) Evidence on the mechanism of enhanced sucrose uptake at low cell turgor in leaf discs of *Phaseolus coccineus*. Physiol Plant **64:** 547–552
7. **Delrot S** (1987) Phloem loading: Apoplastic or symplastic? Plant Physiol Biochem **25:** 667–676
8. **Delrot S, Despeghel JP, Bonnemain JL** (1980) Phloem loading in *Vicia faba* leaves: Effect of *N*-ethylmaleimide and parachloromercuribenzenesulfonic acid on $H^+$extrusion, $K^+$ and sucrose uptake. Planta **149:** 144–148
9. **Delrot S, Faucher M, Bonnemain JL, Bonmort J** (1983) Nycthemeral changes in intracellular and apoplastic sugars in *Vicia faba* leaves. Physiol Vég **21:** 459–467
10. **Giaquinta RT** (1983) Phloem loading of sucrose. Annu Rev Plant Physiol **34:** 347–387
11. **Gifford RM, Evans LT** (1981) Photosynthesis, carbon partitioning and yield. Annu Rev Plant Physiol **32:** 485–509
12. **Hitz WD, Giaquinta RT** (1987) Sucrose transport in plants. BioEssays **6:** 217–221
13. **Jones MGK, Outlaw WH, Lowry OH** (1977) Enzymatic assay of $10^{-7}$ to $10^{-14}$ moles of sucrose in plant tissues. Plant Physiol **60:** 379–383
14. **Malek L, Baker DA** (1978) Effect of fusicoccin on proton cotransport of sugars in the phloem loading of *Ricinus communis* L. Plant Sci Lett **11:** 233–239
15. **M'Batchi B, Delrot S** (1984) Parachloromercuribenzenesulfonic acid. A potential tool for differential labelling of the sucrose transporter. Plant Physiol **75:** 154–160
16. **Morrod RS** (1974) A new method for measuring the permeability of plant cell membranes using epidermis-free leaf discs. J Exp Bot **25:** 521–533
17. **Patrick JW** (1987) Are hormones involved in assimilate transport? *In* GV Hoad, JP Lenton, MB Jackson, RK Atkin, eds, Hormone Action in Plant Development. A Critical Appraisal. Butterworths, London, pp 175–187
18. **Pereto JG, Beltran JP** (1987) Hormone-directed sucrose transport during fruit set induced by gibberellins in *Pisum sativum*. Physiol Plant **69:** 356–360
19. **Reinhold L, Seiden A, Volokita M** (1984) Is modulation of the rate of proton pumping a key event in osmoregulation? Plant Physiol **75:** 846–849
20. **Serrano R** (1984) Plasma membrane ATPase of fungi and plants

as a novel type of proton pump. Curr Top Cell Regul **23:** 87–126

21. **Turgeon R, Wimmers LE** (1988) Different patterns of vein loading of exogenous [$^{14}$C]sucrose in leaves of *Pisum sativum* and *Coleus blumei*. Plant Physiol **87:** 179–182
22. **Van Bel AJE, Koops AJ** (1985) Uptake of [$^{14}$C]sucrose in isolated minor-vein networks of *Commelina benghalensis* L. Planta **164:** 362–369
23. **Vercher Y, Molowny A, Carbonell J** (1987) Gibberellic acid effects on the ultrastructure of endocarp cells of unpollinated ovaries of *Pisum sativum*. Physiol Plant **71:** 302–308
24. **Weaver RJ, Johnson JO** (1985) Relation of hormones to nutrient mobilization and the internal environment of the plant: The supply of mineral nutrients and photosynthate. *In* RP Pharis, DM Reid, eds, Encyclopedia of Plant Physiology (New Series), Vol 11. Springer-Verlag, Berlin, pp 3–36
25. **Wildhom J** (1972) The use of fluorescein diacetate and phenosafranine for determining viability of cultured plant cells. Stain Technol **47:** 189–194
26. **Wilson DR, Jamieson PD, Jermyn WA, Hanson R** (1985) Models of growth and water use of field peas (*Pisum sativum L.*). *In* PD Hebblethwaite, MC Heath, TCK Dawkins, eds, The Pea Crop. Butterworths, London, pp 139–151
27. **Wilson C, Oross JW, Lucas WJ** (1985) Sugar uptake into *Allium cepa* leaf tissue: An integrated approach. Planta **164:** 227–240