ELSEVIER

Plant Science 104 (1995) 153–160

PLANT SCIENCE

Effect of mannose on the plasma membrane ATPase from sugar beet (*Beta vulgaris* L.) leaves

Clyde Wilson*[a], Monica A. Madore[b]

[a]*USDA-ARS, U.S. Salinity Laboratory, 4500 Glenwood Drive, Riverside, CA 92501, USA*
[b]*Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA*

Received 2 September 1994; revision received 6 October 1994; accepted 1 November 1994

Abstract

Experiments were conducted to determine if the observed reduced influx of sugars following mannose treatment could be attributed to changes in ATPase activity. Mannose was supplied to *Beta vulgaris* L. (sugar beet) leaves via the transpiration stream for either a 16- or 24-h light period. The plasma membrane was then isolated using the aqueous polymer two-phase partitioning technique. Analysis of ATP hydrolytic activity showed that mannose pretreatment had a progressive inhibitory effect on the H^+-ATPase activity. These results are consistent with previous reports that mannose can dramatically influence membrane transport events in sugar beet leaves. Measurement of Mg ATP-dependent, pH-gradient formation by plasma-membrane vesicles indicated that mannose pretreatment shifted the maximal initial rate of proton pumping to more alkaline regions compared with control membranes. At pH 6.5, mannose pretreatment also inhibited the total quench. We cannot attribute this to increased leakage of protons out of the vesicles as mannose pretreatment had no effect on the passive backflow of protons out of the vesicles. Mannose appears to elicit a general perturbation of membrane transport processes which is not limited to sugars and amino acids. Collectively, these studies indicate that mannose may inhibit sugar transport, at least in part, by affecting the activity of the plasma-membrane ATPase.

Keywords: *Beta vulgaris* L.; Plasma membrane; Sugar transport; H^+-ATPase; Proton pumping; Mannose

Abbreviations: BSA, bovine serum albumin; BTP;1,3-bis[tris(hydroxymethyl)-methylamino]propane; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; Hepes, (*N*-(2[Hydroxyethyl] piperazine-*N'*-[2-ethanesulfonic acid]); EGTA, ethyleneglycol-bis-(β-aminoethyl ether)*N*,*N*,*N'*,*N'*-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; mannose 6-P, mannose 6-phosphate; Mes 2-(*N*-morpholino)ethanesulfonic acid; P_i, inorganic phosphate; pmf, proton-motive force; PMSF, phenylmethylsulfonyl fluoride; SPS, sucrose-phosphate synthase; Tris, (tris[hydroxymethyl]aminomethane).

Mention of company names or products is for the benefit of the reader and does not imply endorsement, guarantee, or preferential treatment by the USDA or its agents.

* Corresponding author.

1. Introduction

An earlier study on the regulation of sucrose transport across the plasmalemma demonstrated that mannose caused a reduction in exogenous [^{14}C]sucrose influx [1]. Further investigations revealed that mannose treatment reduced the influx of not only sucrose, but other sugars and amino acids as well [2]. The inhibition of glucose, fructose, and arginine uptake into mannose-treated tissue indicates that mannose may elicit a

0168-9452/95/$09.50
SSDI 0168-9452(94)04024-B

perturbation of all membrane transport processes. Further analysis of adenine nucleotide levels suggested that the mannose inhibition of influx could not be explained by a reduction in adenine nucleotide levels alone.

Mannose has frequently been used to investigate the regulation of plant metabolism with regard to the role of orthophosphate. It is generally believed that in species lacking phosphomannoisomerase, mannose is able to reduce cytosolic inorganic phosphate (P_i) levels by phosphorylation to mannose 6-P which can only then be slowly metabolized [3–5]. If the transport of P_i between the vacuole and cytosol is slow [5,6], then the addition of mannose results in a sequestration of cytosolic P_i and a reduction of ATP levels needed for phosphorylation reactions [7,8].

With respect to sugar transport across the plasmalemma, it now appears that sugars are transported by a sugar:H^+ cotransport mechanism and a first-order kinetic process. Recent reports using isolated membrane vesicles have identified an active, proton-coupled sucrose transport system in the plasmalemma [9–11]. In these investigations, the proton-motive force (pmf) needed to drive secondary sugar-transport systems was generated by isolating the vesicles in buffered solutions at around pH 8.0 and then diluting them in an acidic transport solution. In the intact plant, it is believed that the plasma-membrane proton-pumping ATPase is responsible for the pmf needed to drive sucrose:H^+ cotransport.

Since the plasma-membrane ATPase is necessary for active sucrose transport, we decided to investigate whether the observed inhibition by mannose could be explained, at least in part, by an effect on the ATPase. While the reaction mechanism of the plasma-membrane ATPase has been investigated quite closely [12], little is known concerning the regulation of the plasma-membrane H^+-ATPase, especially within the tissues of the leaf.

In the present study, we investigated the effect of mannose on the plasma-membrane H^+-ATPase activity from leaves of *Beta vulgaris* L. (sugar beet). By supplying mannose to the leaves via the transpiration stream, we attempted to reduce ATP levels and sequester cytosolic P_i, thereby making it unavailable for regulatory purposes. We then tested the activity of the plasma-membrane H^+-ATPase by first isolating the plasma membrane using the aqueous polymer two-phase partitioning method and comparing ATP hydrolysis and proton-pumping rates with membranes from untreated control leaves. Consistent with its effect on other membrane transport processes, mannose treatment affected both H^+-ATPase specific activity and proton pumping. Collectively, these studies indicate that mannose may inhibit sugar transport, at least in part, by affecting the activity of the plasma-membrane ATPase.

2. Materials and methods

2.1. Plant material

Beta vulgaris L. (var. SSBN1, USH10, Lot 3102 or USH11, Lot 82313) seeds were planted in sand tanks and grown in a greenhouse from mid March to mid April. The mean maximum/minimum temperatures and relative humidities (RH), ±S.D., were 30 ± 3°C and 19 ± 1°C, and 57 ± 8% and /135 ± 4% RH. The nutrient solution had the following composition in mM: $Ca(NO_3)_2$, 2.5; KNO_3, 3.0; $MgSO_4$, 1.5; KH_2PO_4, 0.17; Fe (as sodium ferric diethylenetriamine pentaacetate), 0.05; H_3BO_3, 0.023; $MnSO_4$, 0.005; $ZnSO_4$; $CuSO_4$, 0.0002; and H_2MoO_4, 0.0001. The third pair of leaves (after the cotyledons) of 4-week-old sugar beet plants were used.

2.2. Mannose pretreatment

Two hours after sunrise, the leaf-petiole complex of the third pair of leaves (after the cotyledons) were excised from the base of the crown while the sugar beet plant was submerged under nutrient solution. The leaf-petiole complex was then placed either in distilled water (control) or a 10 mM mannose solution before being placed for 24 h in a controlled-environment chamber with fluorescent and incandescent lighting (350 μmol m^2/h) and a constant temperature of 25°C. The photoperiod was either 16 h or 24 h.

2.3. Kinetic studies

Kinetic studies were performed using the method of Maynard and Lucas [13] as modified by

Wilson et al. [14]. After mannose (or water) pretreatment, the leaf was placed on a rubber pad in a dish containing in solution 20 mM $CaCl_2$. Six leaf discs were used for each sucrose concentration, and these discs were given a 30-min preincubation in a solution containing 20 mM $CaCl_2$, 25 mM Mes (pH 5.0), and enough mannitol to adjust the osmolality to 500 mOsm. Sucrose at the concentration specified was also included. Preincubation was followed by a 30-min uptake period in solution containing radioactive sucrose (D-[U-^{14}C]sucrose, International Chemical and Nuclear Corp., Irvine, CA). The specific activity of the uptake solution was maintained at 1.78×10^7 Bq/mmol. The incubation period was followed by three 7-min washes in medium containing 20 mM $CaCl_2$, 120 mM Mes, and 140 mM sorbitol. Leaf discs were placed in scintillation vials containing 0.1 ml glacial acetic acid and a drop of ethylene glycol monomethyl ether (Fisher Scientific). After a 60-min digestion period, 5 ml of Scintiverse BD scintillation mixture (Fisher Scientific) was added and the vials were counted in a Beckman 3801 scintillation counter. Experiments were performed at 24 ± 1°C under laboratory lighting.

2.4. Membrane isolation

Plasma membrane-enriched vesicles were isolated using the aqueous two-phase technique described by Larsson [15] and modified by Bush [9]; all solutions were kept at either 4°C or on ice. Briefly, 80 g fresh weight of previously described leaf material was rinsed with deionized water, chilled in ice water for 10 min, spun and blotted to remove excess water. The leaves were homogenized in a commercial Waring blendor at low speed for three 20-s bursts in 250 ml of homogenization buffer. This buffer consisted of 250 mM sorbitol, 50 mM Hepes-BTP (pH 8.0), 5 mM EGTA, 5 mM ascorbic acid, and 10 mM KCl. Just before use 0.6% polyvinylpyrrolidone, 5 mM DTT, and 1 mM PMSF (dissolved in 95% ethanol), and 0.5% protease-free BSA (final concentrations) were added. The homogenate was filtered through four layers of cheesecloth and then centrifuged for 15 min at 10 000 × *g* using a Sorvall GSA rotor. The supernatant fluid was subsequently centrifuged at 85 000 × *g* using a Beckman 45 Ti rotor for 40 min. The resulting microsomal pellet was gently resuspended using a ground-glass homogenizer in a medium consisting of 350 mM sorbitol, 5 mM DTT, 10 mM KCl, and 5 μg/ml chymostatin dissolved initially in DMSO. PM-enriched vesicles were obtained from the microsomal vesicles using aqueous two-phase partitioning. Three 36-g phases consisting of 6.2% (w/w) dextran T500, 6.2% (w/w) polyethylene glycol (average molecular mass 3350), 350 mM sorbitol, 5 mM KCl, and 5 mM K_2PO_4 (pH 7.8) were used. The first phase included microsomal vesicles resuspended in enough resuspension buffer to bring the weight of the resuspended microsomal vesicles to 9 g.

The phases were separated by centrifugation at 1000 × *g* for 10 min at 4°C using a Sorvall SS34 rotor. The final upper phase (U_3) was diluted about 6-fold in a wash buffer of 350 mM sorbitol, 25 mM Hepes (pH 7.8 with 1,3-bis[tris(hydroxymethyl)-methylamino]propane), BTP, 10 mM KCl, and 2.5 mM DTT and pelleted at 85 000 × *g* for 40 min. The resulting pellet was resuspended in wash buffer and pelleted again as above. The final pellet was resuspended in 1 ml of wash buffer containing 5 μg/ml chymostatin. A 30-μl aliquot was taken for protein analysis. The vesicles were stored in liquid nitrogen until used (1–6 days). The colorimetric assay of Peterson [16] was used to determine membrane protein concentrations using BSA as the protein standard.

2.5. Measurement of ATPase Activity and ΔpH

ATPase hydrolysis assays were performed at 30°C for 30 min in 25 mM BTP–Mes (pH 7.5) buffer containing 3.75 mM $MgSO_4$, 3.75 mM Tris–ATP, 0.5 mM $(NH_4)_6Mo_7O_{24}$, and 0.005% (w/v) lysolecithin. Salts (50 mM) and 100 μM Na_3VO_4 were added as indicated. A titration experiment was performed to determine the optimal concentration of lysolecithin for sugar beet. The assay temperature was maintained at 30°C. Boiled controls were included to determine background phosphate. Inorganic phosphate was measured by the method of Peterson [17].

The pH-gradient formation experiments were performed using the method of Suhayda et al. [18] with some modification. Proton transport activity was measured in the presence of different salts by

the quenching of the fluorescent probe, quinacrine, using a SLM 8000 C spectrofluorometer at 30°C. Excitation and emission wavelengths were 430 and 500 nm, respectively. The membrane vesicles were assayed in a buffer consisting of 200 μg of plasma-membrane protein in 250 mM sorbitol, 25 mM BTP–Mes (pH 7.5), 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), 2.5 μM quinacrine, 1 mg/ml fatty-acid free BSA, and 50 mM monovalent ions.

Experiments on the rate of dissipation of the ATP-generated pH gradient were performed essentially as described by Garbarino and DuPont [19]. The assay buffer contained 200 μg of membrane protein in 250 mM sorbitol, 25 mM BTP–Mes (pH 7.5), 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), 2.5 μM quinacrine, 1 mg/ml fatty-acid free BSA, and 50 mM KNO_3. The assay temperature was maintained at 30°C. After a steady-state pH gradient was formed by the addition of 3.75 mM MgATP (about 500 s), 8 mM Tris–EDTA was added in order to complex the Mg^{2+} and stop proton pumping. H^+ leakage was monitored by the increase in fluorescence.

3. Results

3.1. Effect of mannose on ATPase activity

Lucas and Wilson [2] demonstrated that mannose-induced phosphate sequestration in sugar-beet leaves resulted in a progressive inhibition of sugar and amino acid uptake and suggested that mannose may elicit a general perturbation of all membrane transport processes. The present experiments were designed to investigate the effect of mannose on the activity of the plasma-membrane H^+-ATPase. After mannose (or water) treatment, the control leaves remained turgid while the mannose-treated leaves were wilted with a water-soaked appearance. It has already been demonstrated that mannose treatment in this manner lowers ATP and total adenylate levels in sugar beet [2]. ATP levels are initially reduced along with total adenylates. This was then followed by a subsequent recovery to control levels after 24 h. We supplied mannose to the leaves for 16 h, a time period which would allow sufficient time for mannose to lower ATP levels and dramatically affect sugar transport [2]. The effectiveness of our treatment was monitored by comparing sucrose uptake into leaf discs from control and mannose-treated leaves. Our influx data (data not shown) displayed the same inhibitory effect previously described by Wilson and Lucas [1] and Lucas and Wilson [2]. We then isolated the plasma membrane using the aqueous polymer two-phase partitioning method and measured ATPase hydrolytic activity. This isolation technique is now routinely used and has been shown by many laboratories to be extremely effective in obtaining highly pure plasma-

Table 1
Effect of mannose pretreatment on ATPase specific activity

Treatment	ATPase specific activity	Percentage of control activity		
		+ van	−KCl	$-Mg^{2+}$
μmol P_i/mg protein/h ± SE				
[a]Control	359 ± 2.3	11	88	0
[a]Mannose-treated	273 ± 1.4	9	86	5
[b]Control	326 ± 2.5	6	85	3
[b]Mannose-treated	105 ± 3.3	10	83	3

Leaves were excised at the base of the petiole and then placed in either 10 mM mannose or distilled water (control) before being returned to the controlled-environment chamber. Plasma-membrane vesicles were isolated using the aqueous polymer two-phase technique (see Section 2). ATPase specific activity was assayed in 1.0 ml (30 min at 30°C) in 25 mM BTP–Mes (pH 7.5) buffer containing 3.75 mM $MgSO_4$, 3.75 mM Tris–ATP, 50 mM KCl, 0.5 mM $(NH_4)_6Mo_7O_{24}$, and 0.005% (w/v) lysolecithin; 100 μM Na_3VO_4 was added as indicated.

[a]Leaf-petiole complex was placed for 24 h in a controlled-environment chamber with a 16-h light/8-h dark cycle.

[b]leaf-petiole complex was placed for 24 h in a controlled-environment chamber with a continuous 24 h light cycle.

membrane vesicles from sugar-beet leaves [9,20–23].

In agreement with the response seen regarding sugar and amino acid uptake, mannose treatment influenced ATP hydrolytic activity in vesicles derived from mannose-treated leaves as compared with control vesicles (Table 1). Since the inhibitory influence of mannose on sugar uptake is progressive with time, the pretreatment period was extended to 24 h. Again, as with our sugar uptake studies, increased pretreatment with mannose resulted in a further decrease in ATPase hydrolytic activity (Table 1).

3.2. Effect of mannose on proton pumping

Since mannose pretreatment appeared to inhibit ATP hydrolysis, we next investigated the effect on proton pumping. Our analyses revealed that the major effect of mannose pretreatment on the initial rate of proton pumping into isolated vesicles

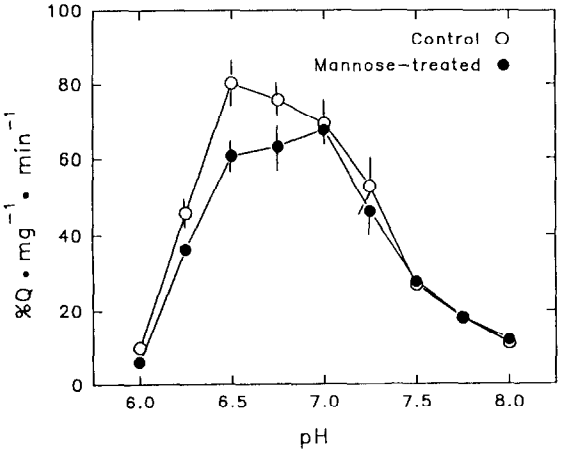

Fig. 1. MgATP-dependent H^+-transport activity of *Beta vulgaris* plasma membrane vesicles as a function of pH. Leaves were excised at the base while the crown of the plant was submerged under water. Petioles were placed in either distilled water (control) or 10 mM mannose before being returned to the controlled environment chamber for 24 h. Membranes from control (O) and mannose-treated (●) leaves were isolated as described in Section 2. Initial rate was determined from at 30°C in a 1.0-ml assay buffer consisting of 200 μg of plasma-membrane protein in 250 mM sorbitol, 25 mM BTP–Mes, 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), 2.5 μM quinacrine, 1 mg/ml fatty-acid free BSA, and 50 mM KNO_3. Means ± S.E. are shown.

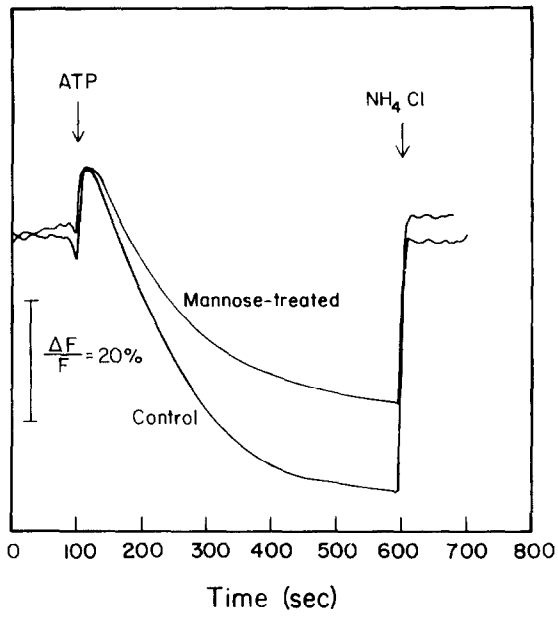

Fig. 2. Fluorescence quench by isolated plasma-membrane vesicles from leaves pretreated with either distilled water (control) or 10 mM mannose for 24 h. Assays were performed at 30°C in a 1.0 ml assay buffer consisting of 200 μg of plasma-membrane protein in 250 mM sorbitol, 25 mM BTP–Mes (pH 6.5), 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), 2.5 μM quinacrine, 1 mg/ml fatty-acid free BSA, and 50 mM KNO_3.

was a shift in the pH optimum to more alkaline regions (Fig. 1). Maximal quenching activity (%Q/mg/min) for vesicles from control leaves was 80.3 at pH 6.5 and for vesicles from mannose pretreated leaves was 70.5 at pH 7.0. In addition, the total extent of quenching at the steady state (where proton pumping and proton leaks are equal) was reduced in vesicles from mannose-pretreated leaves measured at pH 6.5 (Fig. 2).

Apparent proton transport in vesicles can be altered either by changes in H^+-ATPase activity or membrane permeability to protons. It has been previously reported [24] that mannose can markedly affect membrane permeability in spinach beet. Indeed, Lucas and Wilson [2] performed efflux studies on mannose-pretreated leaf discs and discovered that mannose reduced the half-time for [^{14}C]sucrose exchange across the plasmalemma from 40.5 min to 18.8 min. In order to investigate mannose effects on proton efflux, we added EDTA

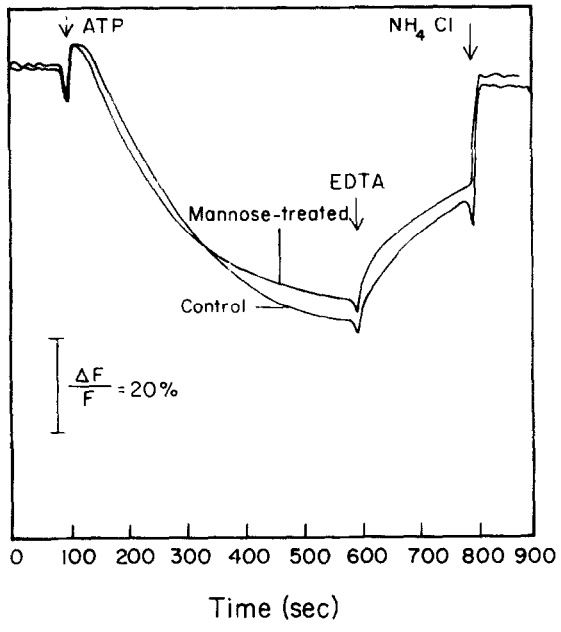

Fig. 3. Recovery of quinacrine fluorescence after the addition of EDTA. Fluorescence quench was followed as described in Fig. 2 at pH 7.0. Additions of 3.75 mM ATP, 8 mM Tris–EDTA, and 3 mM NH_4Cl were as indicated.

to our assay medium 500 s after the addition of ATP in order to complex Mg^{2+} and thereby inhibit proton pumping. The assay was performed at pH 7.0. At this pH there is a difference in initial rate; however, the total quench for control and mannose pretreated vesicles is similar (251 vs. 265 %Q/mg, respectively.) We then monitored the passive backflow of protons (Fig. 3). In contrast to our findings on apparent proton pumping, mannose pretreatment had little influence on the passive backflow of protons from the vesicles.

4. Discussion

Mannose has long been useful as a tool for elucidating regulatory events controlling plant physiological and biochemical processes by sequestering cytosolic P_i [3,25]. Walker and Sivak [26], for example, used mannose to identify the role of P_i in photosynthetic carbon assimilation. Stitt et al. [27] were able to demonstrate that the light effect on sucrose-phosphate synthase (SPS) was indirect and possibly mediated by changes in cytoplasmic P_i by feeding mannose to leaf discs. More recently, mannose was employed as a tool to identify factors regulating the phosphorylation of SPS [8,28]. In the case of sugar beet, Lucas and Wilson [1] discovered that mannose pretreatment caused a dramatic and progressive perturbation of sugar and amino acid influx into the mesophyll tissue leading them to suggest that the mannose effect may be a consequence of a more general membrane phenomenon.

In our experiments, the activity of the plasmamembrane H^+-ATPase in vesicles isolated from source leaves was influenced by mannose pretreatment. Large differences were found between ATP hydrolytic activity in vesicles from control and mannose-treated leaves subjected to 16 h mannose pretreatment and 24 h pretreatment. This similarity was noted regardless of whether lysolecithin, an activator of the enzyme, or Brij 58 was used to disrupt the membrane barrier (data not shown). Thus, it appears that lowering cytosolic P_i and ATP levels may affect the activity of the plasmamembrane H^+-ATPase in subsequently isolated vesicles.

Consistent with our observations on ATP hydrolysis, we observed differences in proton pumping due to mannose pretreatment. The major effect of mannose on the initial rate was to shift the pH optimum towards more alkaline regions. Additionally, the total extent of quenching at steady state was reduced at pH 6.5. This reduction could not be attributed to differences in H^+-leakage from the vesicles. Thus, one manner in which mannose treatment may be altering the H^+-ATPase is by altering its sensitivity to pH

Whereas the effect of mannose on H^+-ATPase activity at pH 7.5 was characterized by a decrease, the effect on proton-pumping was more complex. Proton-pumping at pH 7.5 was similar in vesicles isolated from control or mannose-treated leaves. But, ATP-induced pumping was decreased at pH 6.5 in vesicles derived from mannose-treated leaves (Fig. 2). It should be pointed out that measurements of ATPase activity may not be directly comparable with H^+-transport. On the other hand, if these results do indeed reflect in vivo activity, then mannose treatment may have different effects on

the dual functions of this enzyme, ATP hydrolysis and proton pumping.

The mechanism of mannose inhibition of the plasma-membrane ATPase activity is still unclear. One possibility is that H^+-ATPase activity is reduced due to a post-translational regulatory change in the enzyme brought about by a reduction in cytosolic P_i. Currently, it is hypothesized that some covalent modification of the enzyme such as phosphorylation/dephospohorylation involving multiple sites occurs [29–34]. Such an event would likely be mediated by a protein kinase activated by either basic peptides, changes in cytosolic calcium, or diacylglycerol. There are reports in the literature indicating that an H^+-ATPase from the plasma membrane of red beet storage tissue is phosphorylated [35,36]. Alternatively, mannose treatment may have decreased the relative abundance of ATPase in the plasma membrane. Since the mannose preincubation times were long (16 and 24 h), dramatic changes in the composition of the plasma membrane could have occurred.

Another possibility is that mannose treatment may be eliciting an induced expression of a different ATPase isoform. Harper et al. [37], recently reported that in *Arabidopsis thaliana* the plasma-membrane H^+-ATPase is encoded by a multigene family with 10 isoforms. Further work by Dewitt et al. [38] indicates that various isoforms may be differentially expressed in tissues with unique transport functions. This interpretation is supported by changes in the pH maximum of the ATPase. However, other explanations for the shift in pH maximum, including post-translational modification or changes in the membrane itself, are still possible.

Earlier, it was reported that feeding mannose to the leaves via the transpiration stream has a drastic effect on membrane permeability [2]. Specifically, this treatment was reported to decrease the half-time for sucrose efflux from both the cytosolic and vacuolar compartments. We investigated the effect of mannose on membrane permeability to protons. Our results were unexpected. When we added EDTA to our assay medium 500 s following the addition of ATP, we did not observe any differences in the passive backflow of protons. Apparently, the permeability changes of the plasma membrane due to mannose do not include protons.

These results are in agreement with those obtained on sugar and amino acid influx into sugar-beet leaf discs, and support the previous hypothesis that mannose pretreatment can elicit a perturbation of many membrane transport processes. Given the role of the plasma-membrane ATPase in providing the pmf needed for secondary active transport systems, it appears from our data that the observed mannose inhibition of sugar influx may be attributed to mannose effects on the ATPase. We should point out, however, that our data do not preclude effects on the sugar carriers themselves. If the mannose-pretreatment effects on the H^+-ATPase are due to some post-translational regulatory change in the enzyme, then mannose may be a very useful tool in the study of the regulation of the ATPase.

References

[1] C. Wilson and W.J. Lucas, Influence of internal sugar levels on apoplasmic retrieval of exogenous sucrose in source leaf tissue. Plant Physiol., 84 (1987) 1088–1095.

[2] W.J. Lucas and C. Wilson, Influence of mannose on the apoplasmic retrieval systems of source leaves. Plant Physiol., 85 (1987) 423–429.

[3] G.C. Harris, P.A. Gibbs, G. Ludwig, A. Un, M. Sprengnether and N. Kolodny, Mannose metabolism in corn and its impact on leaf metabolites, photosynthetic gas exchange, and chlorophyll fluorescence. Plant Physiol., 82 (1986) 1081–1089.

[4] M.J. Kime, R.G. Ratcliffe and B.C. Loughman, The application of ^{31}P nuclear magnetic resonance to higher plant tissue. J. Exp. Bot., 33 (1982) 670–681.

[5] M. Miginiac-Maslow and A. Hoarau, Variations in the adenylate levels during phosphate depletion in isolated soybean cells and wheat leaf fragments. Z. Pflanzenphysiol., 107 (1982) 427–436.

[6] F. Rebeille, R. Bligny, J-B. Martin and R. Douce, Relationship between the cytoplasm and the vacuole phosphate pool in *Acer pseudoplatanus* cells. Arch. Biochem. Biophys., 225 (1983) 143–148.

[7] T.E. Humphreys and L.A. Garrard, Glucose uptake by the corn scutellum. Phytochemistry, 3 (1964) 647–656.

[8] C.H. Foyer, The effect of sucrose and mannose on cytoplasmic protein phosphorylation, sucrose phosphate synthetase activity and photosynthesis in leaf protoplasts from spinach. Plant Physiol. Biochem., 28 (1990) 151–160.

[9] D.R. Bush, Proton-coupled sucrose transport in plasmalemma vesicles from sugar beet (*Beta vulgaris* L. cv. Great Western) leaves. Plant Physiol., 89 (1989) 1318–1323.

[10] L.E. Williams, S.J. Nelson and J.L. Hall, Characterization of solute transport in plasma membrane vesicles isolated from cotyledons of *Ricinus communis* L. II. evidence for a proton-coupled mechanism for sucrose and amino acid uptake. Planta, 182 (1990) 540–545.

[11] J.H. Slone, T.J. Buckout and W.J. VanDerWoude, Symport of proton and sucrose in plasma membrane vesicles isolated from spinach leaves. Plant Physiol., 96 (1991) 615–618.

[12] D.P. Briskin, Intermediate reaction states of the red beet plasma membrane ATPase. Arch. Biochem. Biophys., 248 (1986) 106–115.

[13] J.W. Maynard and W.J. Lucas, A reanalysis of the two-component phloem loading system in *Beta vulgaris*. Plant Physiol., 69 (1982) 734–739.

[14] C. Wilson, J.W. Oross and W.J. Lucas, Sucrose uptake into *Allium cepa* leaf tissue: an integrated approach. Planta, 164 (1986) 227–240.

[15] C. Larsson, Plasma membranes, in: H.F.D. Linskens and J.F. Jackson (Eds.), Modern Methods of Plant Analysis, New Series, Vol. 1, Cell Components, Springer-Verlag, Berlin, 1985, pp. 85–104.

[16] G.L. Peterson, A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem., 83 (1977) 346–356.

[17] G.L. Peterson, A simplified method for analysis of inorganic phosphate in the presence of interfering substances. Anal. Biochem., 84 (1978) 164–172.

[18] C.G. Suhayda, J.L. Giannini, D.P. Briskin and M.C. Shannon, Electrostatic changes in *Lycopersicon esculentum* root plasma membrane resulting from salt stress. Plant Physiol. 93 (1990) 471–478.

[19] J. Garbarino and F.M. DuPont, NaCl induces a Na^+/H^+ antiport in tonpolast vesicles from barley roots. Plant Physiol., 86 (1988) 231–236.

[20] C. Larsson, S. Widell and M. Sommarin, Inside-out plant plasma membrane vesicles of high purity obtained by aqueous two-phase partitioning. FEBS Lett., 229 (1988) 289–292.

[21] T.J. Buckout, Sucrose transport in isolated plasma membrane vesicles from sugar beet (*Beta vulgaris* L.). Evidence for an electrogenic sucrose-proton symport. Planta, 178 (1989) 393–399.

[22] R. Lemoine and S. Delrot, Proton-motive-force-driven sucrose uptake in sugar beet plasma membrane vesicles. FEBS Lett., 249 (1989) 129–133.

[23] M.G. Palmgren, M. Sommarin, P. Ulvskov and C. Larsson, Effect of detergents on the H^+-ATPase activity of inside-out and right-side-out plant plasma membrane vesicles. Biochim. Biophys. Acta, 1021 (1990) 133–140.

[24] A. Herold, Induction of wilting by mannose in spinach beet leaves. New Phytol., 81 (1978) 299–305.

[25] A. Herold, D.H. Lewis and D.A. Walker, Sequestration of cytoplasmic orthophosphate by mannose and its differential effect on photosynthetic starch synthesis in C_3 and C_4 species. New Phytol., 76 (1976) 397–407.

[26] D.A. Walker and M.N. Sivak, Can phosphate limit photosynthetic carbon assimilation in vivo? Physiol. Végetale, 23 (1985) 829–841.

[27] M. Stitt, S.C. Huber and P.S. Kerr, Control of photosynthetic sucrose formation, in: M.D. Hatch and N.K. Boardman (Eds.), Biochemistry of Plants, Vol. 10, Academic Press, New York, 1987, pp. 328–409.

[28] H. Weiner, R.W. McMichael Jr. and S.C. Huber, Identification of factors regulating the phosphorylation status of sucrose-phosphate synthase in vivo. Plant Physiol., 99 (1992) 1435–1442.

[29] D.P. Briskin and R.J. Poole, Evidence for a β-aspartyl phosphate residue in the phosphorylated intermediate of the red beet plasma membrane ATPase. Plant Physiol., 72 (1983) 1133–1135.

[30] R. Scalla, A. Amory, J. Rigaud and A. Goffeau, Phosphorylated intermediate of a transport ATPase and activity of protein kinase in membranes from corn roots. Eur. J. Biochem., 132 (1983) 525–530.

[31] F. Vara and R. Serrano, Partial purification and properties of the proton-translocating ATPase of plant plasma membranes. J. Biol. Chem., 257 (1982) 12826–12830.

[32] G.E. Schaller and M.R. Sussman, Phosphorylation of the plasma-membrane H^+-ATPase of oat roots by a calcium-stimulated protein kinase. Planta, 173 (1988) 509–518.

[33] R. Nickel, M Schutte, D. Hecker and G.F. E. Scherer, The phospholipid platlet activating factor stimulates proton extrusion in cultured soybean cells and protein phosphorylation and ATPase activity in plasma membranes. J. Plant Physiol., 139 (1991) 205–211.

[34] G.E. Schaller, A.C. Harmon and M.R. Sussman, Characterization of a calcium-dependent and lipid-dependent protein kinase associated with the plasma membrane of oat. Biochemistry, 31 (1992) 1721–1727.

[35] D.P. Briskin, Phosphorylation and dephosphorylation reactions of the red beet plasma membrane ATPase studied in the transient state. Plant Physiol., 88 (1988) 84–91.

[36] L.E. González de la Vara and G. Medina, Phosphorylation by inorganic phosphate of the plasma membrane H^+-ATPase from red beet (*Beta vulgaris* L.). Plant Physiol., 94 (1990) 1522–1527.

[37] J.F. Harper, L. Manney, N.D. Dewitt, M.H. Yoo and M.R. Sussman, The *Arabidopsis thaliana* plasma membrane H^+-ATPase multigene family: genomic sequence and expression of a third isoform. J. Biol. Chem., 265 (1990) 13601–13608.

[38] N.D. Dewitt, J.F. Harper and M.R. Sussman, Evidence for a plasma membrane proton pump in phloem cells of higher plants. Plant J., 1 (1991) 121–128.