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The impact of mannose and other carbon sources on the elongation and diameter of the primary root of *Arabidopsis thaliana*

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Abstract. To determine to what extent plant growth and morphology are sensitive to perturbed carbon metabolism, we grew *Arabidopsis thaliana* L. (Heynh.) seedlings for 10 d in the presence of various carbon compounds and measured the length and diameter of the primary root. Compounds fell into three groups based on their effect on root length: group one supported about as much elongation as sucrose; group two supported about the same elongation as occurred in the absence of sugar; and group three reduced or even eliminated root growth. No compound changed the diameter of the root notably, although there was a weak, positive correlation between root diameter and elongation. To investigate the inhibition of root elongation by mannose, we transplanted seedlings on to test media and measured primary root growth over the subsequent 2 d. Mannose scarcely changed root diameter, in contrast to 2-deoxyglucose, which caused marked swelling, similar in extent to that caused by tunicamycin. Mannose inhibited elongation rate by 90% within 24 h and required a further 2 d to reduce the elongation rate to zero, with the saturating dose being 30 mM in the presence of 3% sucrose and 0.3 mM in its absence. By contrast, cell production rate was little affected over the first 2 d of treatment. The inhibition of elongation by mannose was not reproduced by two sugar analogs that cannot be phosphorylated on carbon six, was not affected by manipulating phosphate levels in the medium, and was largely prevented by simultaneous treatment with either 30 mM mannoheptulose, 1 mM glucose, or 56 mM fructose. These results suggest that mannose inhibits root elongation via hexokinase-mediated sugar signaling.

Keywords: carbon metabolism, mannose, root diameter, root elongation, sucrose.

Introduction

How is metabolism linked to morphogenesis? At the most basic level, metabolism provides energy for all cellular action, and without this energy the cell will die. But short of this extreme, it remains an open question to what extent metabolic pathways are linked to the pathways that control organ shape. Although metabolism and the processes that direct the shaping of an organ seem separate conceptually, the processes could be intertwined. Metabolism interconverts small molecules and morphogenesis constructs the plant with them. The flux of substrates through various pathways could impact on how structures assemble not only kinetically but also biochemically, by influencing the abundance of reactants. Biochemical changes could change the structure's material properties and hence lead to a difference in cell or organ shape (Harold 1990).

To ascertain how directly morphogenesis in higher plants depends on metabolism, Baskin and Bivens (1995) screened a range of metabolic inhibitors. Over the concentration range where the inhibitors reduced root elongation, very few altered diameter. However, while inhibitors of various pathways were used, inhibitors of carbohydrate metabolism were not included. Yet carbohydrate metabolism is a likely place to find a link to morphogenesis. Many years ago, certain mutants in the filamentous fungus *Neurospora crassa* with altered hyphal shape were found to result from changes in glucose-6-phosphate dehydrogenase (Brody 1973). More recently, Masle (2000) reported that wheat leaves changed shape in response to elevated levels of carbon dioxide, presumably mediated through altered sugar status. In general, sucrose and other forms of transported carbon are being viewed not only as energy sources but also as signaling molecules, analogous to plant hormones (Koch 1996; Jang and Sheen 1997; Smeekens 2000). Therefore, we sought to extend the previous work on metabolism and morphology to carbohydrates by replacing the sucrose in the growth medium with different carbon compounds and evaluating the effect on root elongation and diameter.

In the course of this work, we found that root elongation in *Arabidopsis thaliana* L. (Heynh.) seedlings is highly sensitive to mannose. Recently, the inhibitory effects of mannose have been used to dissect pathways of sugar signaling in plants where mannose appears to trigger a signal transduction cascade begun by hexokinase (Pego

Abbreviations used: DMSO, dimethyl sulfoxide.

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et al. 2000). Most of this recent work on mannose has used photosynthetic shoots, in which mannose and glucose act similarly, for example, both repressing gene expression. However, in the root, mannose acts negatively whereas glucose acts positively, and so the emerging paradigm for sugar signaling worked out on the shoot might not apply to the root. Therefore, we investigated more fully how mannose affects root growth, and in particular whether the mannose effects are mediated via hexokinase.

Materials and methods

Plant materials and growth conditions

Seeds of *Arabidopsis thaliana* L. (Heynh), ecotype Columbia, were stored at 4°C, and at day zero were surface-sterilized in 15% household bleach for 10 min and plated on agar-solidified, modified Hoagland's solution, with the following composition: 1% (w/v) agar, 4 mM KNO_3, 1 mM $Ca(NO_3)_2$, 0.3 mM $MgSO_4$, 2 mM KH_2PO_4, 89 μM Fe-citrate, 46.3 μM H_3BO_3, 9.1 μM $MnCl_2$, 0.77 μM $ZnSO_4$, 0.31 μM $CuSO_4$, and 0.11 μM MoO_3. Carbon sources were added either as powder before autoclaving or from filter-sterilized aqueous stocks after autoclaving, with care to dilute all tested concentrations equally. Tunicamycin was dissolved in dimethyl sulfoxide (DMSO) and added from a 1000-fold concentrated stock, with control media receiving DMSO only. Media made with acidic carbon sources were adjusted to pH 6.0 with KOH. Plates were placed vertically in a growth chamber, with constant temperature (20°C) and light (80 μmol m^{-2} s^{-1}, or for the data shown in Fig. 7, 200 μmol m^{-2} s^{-1}). Light from 40-W warm-white fluorescent bulbs and 25-W incandescent bulbs was filtered through a sheet of yellow acrylic (Plexiglas J2208, Cope Plastic, St Louis, MO, USA). Yellow filters were used to remove ultraviolet and blue wavelengths, which can drive deleterious photo-chemical reactions in media (Hangarter and Stasinopoulos 1991). For media containing simple sugars, 10-cm diameter Petri dishes were used, but for sugar analogs, sugar phosphates, tunicamycin and mannoheptulose, seedlings were transplanted onto media in 6-cm-diameter dishes to conserve reagent. For the experiments, three plates were made for each concentration, and 10 seedlings were grown on each plate.

At plating, the position of the seed was marked with a razor blade at the back of the plastic plate. At 10 d after plating, plates were photocopied at 1.5 times their original size. Root length was measured with a digitizing tablet as the distance along the root from the tip to the mark. Root diameter was measured by placing a coverslip over the roots directly on the agar surface, wetted by a few drops of an aqueous 0.01% (w/v) Triton X-100 solution. Mounted roots were viewed through a compound microscope at low magnification. Diameters were measured with a video digitizer (Image 1/AT, Universal Imaging Co., West Chester, PA, USA). For roots with conspicuous swellings, diameter was measured at its apparent maximum; for control roots, and others without apparent swelling, diameters were measured at the region of root hair initiation. In some experiments, seedlings were grown for 6 or 7 d, and then transplanted onto plates with various additions, as given in the text. Root elongation rate of these plants was then measured over the next 2 d as described by Baskin and Wilson (1997). To measure final cell length of cortical cells, roots were mounted on slides in water, viewed through Nomarski optics to image cortical cell files, and lengths were measured with the video digitizer (Image 1/AT). Twelve cells on each of six roots were measured per treatment. For controls, cells were measured proximal to where root hairs attained their maximal length; for mannose treatments, cells were measured immediately proximal to the meristem, defined by a small but visible increase in the cell size.

Results

Comparison of the effects of different carbon compounds on root elongation

To compare different carbon sources, we incorporated a given compound into the medium and measured the elongation of the primary root over the 10 d following germination. Because the absolute growth increment of roots on control plates varied, from less than 4 cm to more than 5 cm, data are reported as a percentage of the 3% (w/v) sucrose control, which was included in each experiment. Carbon sources fell roughly into three groups (Table 1). The first group, which comprised sucrose, glucose, raffinose, maltose and trehalose, supported more root elongation than occurred when no carbon source was included in the medium. No compound tested was as effective as

Table 1. Comparison of the growth of the roots of *Arabidopsis thaliana* on different carbon sources

Seeds were plated on medium containing the indicated composition and root length was measured 10 d later. Data report mean length ± s.e. of three replicate plates, expressed as a percentage of the value for 3% (w/v) sucrose, plates of which were included in each experiment. ng, no germination; less than 10% of the seeds germinated

	Tested concentration (%)		
	3	1	0.3
Carbon source	Root length (per cent of 3% sucrose)		
Sucrose	100	88.7 ± 9.2	76.2 ± 7.5
None	57.2 ± 3.5		
Group I			
Glucose	69.3 ± 1.0	90.8 ± 0.3	82.4 ± 2.9
Raffinose	87.4 ± 3.6	84.3 ± 3.3	66.8 ± 5.2
Maltose	50.0 ± 1.1	76.8 ± 5.4	77.4 ± 3.2
Trehalose	12.9 ± 0.6	60.2 ± 1.8	75.3 ± 9.6
Group II			
Lactose	55.0 ± 5.1	54.8 ± 3.3	52.5 ± 5.8
Rhamnose	46.9 ± 3.6	66.4 ± 8.2	58.6 ± 13.8
Fructose	19.9 ± 5.1	43.9 ± 6.9	60.4 ± 2.8
Sorbitol	46.3 ± 3.6	59.3 ± 9.0	61.4 ± 2.2
Group III			
Inositol	35.5 ± 2.2	53.3 ± 6.4	51.1 ± 5.6
Mannitol	33.7 ± 1.5	43.7 ± 8.8	46.3 ± 3.7
Glycerol	5.3 ± 0.4	11.6 ± 1.1	13.9 ± 2.5
Arabinose	13.8 ± 1.9	32.9 ± 2.5	30.4 ± 0.2
Ribose	0[A]	7.6 ± 0.9	29.1 ± 1.5
Xylose	0	0	31.3 ± 6.7
Galactose	7.3 ± 0.9	10.5 ± 0.7	15.0 ± 1.8
Glucosamine	0	0	0
Mannose	ng	ng	0
Pyruvate	ng	0	52.0 ± 1.8
Acetate	ng	ng	0
Malate	—[B]	0	0

[A]A value of zero indicates germination but no measurable root elongation.
[B]Plates with 3% malate did not gel.

3% sucrose, but raffinose and glucose were nearly so. This group includes trehalose, even though at 3% it was clearly inhibitory, because at 0.3% it supported essentially the same level of growth as 0.3% sucrose, well above the no-carbon control. The second group, which comprised lactose, rhamnose, fructose and sorbitol, supported about the same growth as no exogenous carbon compound. This suggests that these compounds are not readily taken up or metabolized by the roots. Finally, the third group comprised those sugars that were clearly inhibitory compared with no exogenous carbon. This group was large and contained several sugar alcohols and sugar acids, all of the tested pentoses, as well as glactose and mannose.

Effect of carbon sources on root diameter

To further characterize the effects of carbon sources on growth, we measured root diameter after the 10-d growth interval. In general, roots were slightly thinner on the tested compounds (Fig. 1). Despite this general thinning, there was a trend for diameter to correlate positively with length, as shown by the regression line drawn through the data having a root length that was at least 40% of the control. This suggests that the modest decrease in root diameter observed on most carbon sources was a secondary consequence of inhibited root elongation, as opposed to a specific effect of the compounds on the anisotropy of growth. To support this suggestion, we plotted diameter against length for the control roots, that is, for the 3% sucrose plates included in the experiments, and found that diameter was correlated with length to a similar extent as seen for the experimental data (Fig. 2). Beyond this minor thinning, no compound affected root diameter.

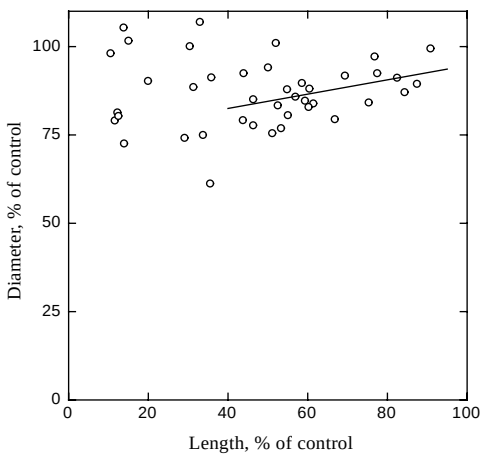

Fig. 1. Relation between root length and diameter for the experiments reported in Table 1 in which detectable root growth occurred. Each datum reports the mean diameter and length of three replicate plates, expressed as a percentage of the 3% sucrose control. The solid line is a linear regression line fitted to all data with greater than 40% root length.

Effect of mannose on root elongation and diameter

Because of the recent interest in mannose as a molecule that interferes with sugar signaling, we studied the effect of this sugar further. To exclude effects on germination (Pego *et al.* 1999), we transplanted 7-d-old seedlings onto plates containing known concentrations of mannose and measured elongation daily for several days. Results for the second day of treatment are shown in Fig. 3. Mannose inhibited root elongation rate maximally by about 90%. Consistent with its reducing elongation rate, mannose reduced root diameter slightly (Table 2). In contrast to mannose, 30 mM galactose inhibited root elongation to only a small extent (Fig. 4*A*). If 3% sucrose (88 mM) was provided with the mannose, the shape of the dose–response curve was similar, but shifted to higher mannose concentrations by nearly two orders of magnitude (Fig. 3). Note that elongation rate did not fall to zero, even at concentrations several orders of magnitude greater than the apparent saturation level of the response. At these levels, the low level of elongation was established some hours after treatment (as also reported for barley roots by Farrar *et al.* 1995), and then declined gradually, requiring 3 d or more to stop completely.

To investigate the effect of mannose on cell division, we measured the length of newly matured cortical cells and calculated cell production rate as the ratio of root elongation rate to cell length (Beemster and Baskin 1998). Mannose reduced mature cell lengths of root cortical cells severely and by about as much as elongation rate was reduced (Table 3). Therefore, the rate of cortical cell production by the meristem was relatively insensitive to mannose. These cell production rates are inexact because the calculation is strictly valid only at steady state, which did not occur here, and because the low elongation rates on mannose were difficult to measure precisely from photocopied images.

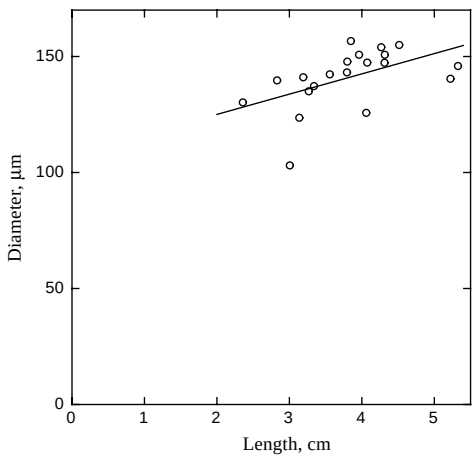

Fig. 2. Relation between root length and diameter for the 3% sucrose controls used in the experiments for Table 1. Note that absolute values are plotted, with the zero of each axis included to delineate the proportionality. The solid line is a linear regression line.

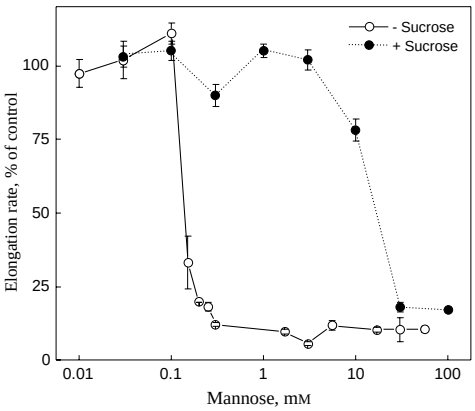

Fig. 3. Effect of mannose on root elongation rate in the presence and absence of 88 mM (3%) sucrose. Seedlings were germinated and grown on control medium for 1 week from plating and transplanted onto medium with the indicated amount of mannose. When sucrose was to be used, it was present in the control medium before transplantation as well as after. Elongation rate is reported over the 2nd day after transplanting, expressed as a percentage of control plants transplanted onto plates with zero mannose. Data are means ± s.e. for three replicate plates.

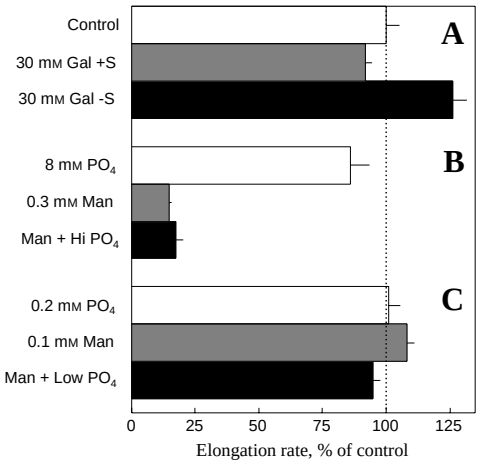

Fig. 4. The effect on root elongation rate of certain additions to the medium. (*A*) Root elongation is not inhibited by 30 mM galactose, in the presence or absence of sucrose. (*B*) Elevated phosphate does not ameliorate the decrease of elongation rate caused by mannose. (*C*) Lowered phosphate does not enhance the effect of a threshold dose of mannose. (*A–C*) Seedlings were germinated and grown on control medium without sucrose for 1 week from plating and transplanted onto medium with the indicated components. Elongation rate is reported over the 2nd day after transplanting, expressed as a percentage of control seedlings transplanted onto plates with no addition. Data are means ± s.e. for three replicate plates.

Nevertheless, the large reduction in cell length and the continued growth on mannose, shows qualitatively that significant production of cells occurred.

Relation between mannose effects and phosphate levels

Mannose has been hypothesized to inhibit growth because of phosphate starvation. The hypothesis originates from the observation that mannose is effectively phosphorylated but slowly dephosphorylated (Goldsworthy and Street 1965; Herold and Lewis 1977), making it possible for high levels of mannose in a cell to sequester phosphate. If so, then mannose toxicity should be ameliorated by high levels of phosphate and exacerbated by low levels. In contrast to this prediction, inhibition at 0.3 mM mannose was not relieved by 4-fold higher levels of phosphate (Fig. 4*B*) nor was threshold inhibition at 0.1 mM mannose enhanced by 10-fold less phosphate (Fig. 4*C*). Inhibition was not enhanced even when the low phosphate treatment was continued for 5 d (not shown). Additionally, the effect of mannose on elongation was not affected by phosphate levels in experiments where seeds were germinated on plates with mannose and various concentrations of phosphate and grown for 10 d. Evidently, phosphate sequestration does not explain the inhibition of root elongation by mannose.

Table 2. Effect of mannose on root diameter

Seedlings were grown on control medium for 1 week, transplanted on to media with various concentrations of mannose, and after 48 h, root diameter was measured. Data are shown for a representative experiment done in the absence of sucrose, as well as for a pool of 12 other experiments, in which mannose reduced elongation rate between 80 and 90% of control

Treatment	Root diameter ± s.d. (μm)
Representative experiment	
Control	137 ± 10
0.1 mM mannose	141 ± 13
0.15 mM mannose	138 ± 15
0.2 mM mannose	128 ± 7
0.25 mM mannose	119 ± 21
0.3 mM mannose	128 ± 7
Pooled experiments	
Control	132 ± 10
Pooled mannose treatments	124 ± 10

Effect of 2-deoxyglucose on root elongation and diameter

Mannose has also been hypothesized to impact cell physiology through the accumulation of the phosphorylated form, mannose-6-phosphate. To assess this hypothesis we first examined the effects of a related sugar, 2-deoxyglucose, which like mannose can be phosphorylated on carbon six. Consistent with the hypothesis, this sugar inhibited root elongation, at even lower concentrations than mannose, and the presence of sucrose shifted the dose–response curve to higher concentrations but did not change its shape (Fig. 5). However, unlike mannose and all of the other carbon sources tested, 2-deoxyglucose increased root diameter substantially. As 2-deoxyglucose is known to inhibit the glycosylation of proteins, we examined another compound with similar

Table 3. The effect of mannose on mature cortical cell length and cell production rate
Seedlings were grown on control medium for 1 week and then transplanted to the medium indicated. Elongation rate of the root was measured at various intervals after transplantation, as indicated, and the length of mature cortical cells was measured during the second day of treatment. Cell production rate is the ratio of elongation rate to mature cell length. Results are shown from two experiments, with slightly different conditions. Data are means ± s.e. for three replicate plates from one experiment. Control data give the elongation rate over the first day of treatment; Mannose treatment data give the elongation rate over the first 3 days of treatment in Experiment 1, and over the first 2 days of treatment in Experiment 2

Treatment	Elongation rate (mm d^{-1})	Cortical cell length (μm)	Cell production rate (cells d^{-1})
Experiment 1			
Control	6.7 ± 0.13	220 ± 5.6	30
0.3 mM mannose (–sucrose)	0.5 ± 0.02	18 ± 0.3	28
30 mM mannose (+sucrose)	0.4 ± 0.03	20 ± 1.6	20
Experiment 2			
Control	5.8 ± 0.35	194 ± 3.5	30
0.3 mM mannose (–sucrose)	0.6 ± 0.06	14 ± 0.7	44
30 mM mannose (+sucrose)	0.5 ± 0.03	15 ± 0.8	34

activity, tunicamycin (Eblein 1987). Similarly to 2-deoxyglucose, tunicamycin inhibited root elongation and stimulated radial expansion (Fig. 6). This suggests that 2-deoxyglucose, although able to be phosphorylated on carbon six, affected root growth through a mechanism different than that used by mannose.

Evidence that mannose inhibits elongation via hexokinase

We examined whether the effects of mannose depended on phosphorylation by the enzyme, hexokinase. In the following three experiments, 6- or 7-d-old seedlings were transplanted on to media of given composition and elongation rate is reported for the second 24-h period of exposure. First, we tested two glucose analogs that cannot be phosphorylated at carbon six. The compounds, 6-deoxyglucose and 3-*O*-methyl glucose, were essentially inert, inhibiting elongation modestly at the highest concentration tested, 10 mM, a concentration which is nearly two orders of magnitude higher than the concentration of mannose needed to inhibit elongation severely (Table 4). Second, we used a competitive inhibitor of hexokinase, a compound called mannoheptulose (Salas *et al.* 1965). At 30 mM, mannoheptulose alone inhibited root elongation slightly, whereas it blocked the inhibition from 0.2 mM mannose nearly completely (Table 5). Third, we compared the ability of mannose toxicity to be ameliorated by exogenous glucose or fructose. Mannose toxicity was overcome completely by either glucose or fructose (Fig. 7); however, the concentration that was approximately 50% effective in overcoming the inhibition by mannose was 0.27 mM glucose and 20 mM fructose. This roughly 100-fold difference is similar to the difference in K_m of hexokinase typically reported for the two sugars (Copeland and Morell 1985; Schnarrenberger 1990; Renz and Stitt 1993). Results from these three experiments consistently implicate hexokinase in mediating mannose toxicity.

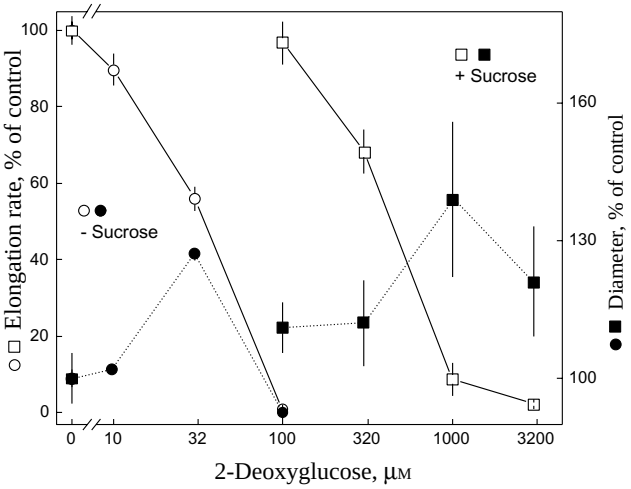

Fig. 5. The effect of 2-deoxyglucose on root elongation rate and diameter in the presence and absence of sucrose. Seedlings were germinated and grown on control medium with or without sucrose for 6 d from plating and transplanted onto medium with the indicated concentrations. Elongation rate is reported over the 2nd day after transplanting and diameter after 48 h. In the presence of sucrose, elongation data are means ± s.e. for three replicate plates and diameter data are means ± s.d. for 20 seedlings on two plates; in the absence of sucrose, data are means ± s.e. for three replicate experiments, each with three plates.

Discussion

The work done here had the objective of determining to what extent root shape could be altered by altering carbon metabolism. We found that a variety of carbon sources inhibited the elongation of the roots of *A. thaliana*, in some cases severely, suggesting that these compounds disrupted metabolism. Treatment with these compounds revealed a weak, positive correlation between elongation rate and diameter, provided that elongation rate was at least one-half

of that occurring on sucrose. A correlation between elongation rate and diameter was reported for *A. thaliana* primary roots (van der Weele *et al.* 2000) and for different classes of roots in other species (Cahn *et al.* 1989; Pagès 1995). The correlation presumably reflects coordination between rates of expansion in length and width. However, with the exception of 2-deoxyglucose, no compound changed the shape of the root markedly, as is caused by interfering with the cytoskeleton or vesicle secretion. This strengthens the conclusion reached by Baskin and Bivens (1995) on the basis of inhibiting other metabolic pathways, that root shape is controlled specifically in *A. thaliana* and is relatively isolated from metabolism. Thus, when root shape is disrupted, it is appropriate to seek a specific explanation.

We hypothesize that 2-deoxyglucose altered root morphology by interfering with protein secretion. This glucose analog, like tunicamycin, has been demonstrated to inhibit protein glycosylation (Schwarz and Datema 1980; Eblein 1987). We report here that 2-deoxyglucose caused roots to swell to a similar extent to that caused by tunicamycin, and similar to that caused by other inhibitors of secretion such as brefeldin and monensin (Baskin and Bivens 1995). Proteins without their customary sugar decorations are apparently interpreted by the secretory system as being misfolded and hence many of them are degraded (Koizumi *et al.* 1999; Leborgne-Castel *et al.* 1999). Enhanced degradation of proteins within the secretory pathway presumably leads to root swelling by disrupting the delivery of one or more critical components to the cell wall.

Interestingly, 2-deoxyglucose has been used for many years to inhibit carbon metabolism because, like mannose, it can be phosphorylated by hexokinase (Salas *et al.* 1965). Indeed, based on the effects in oat coleoptiles of 2-deoxy-

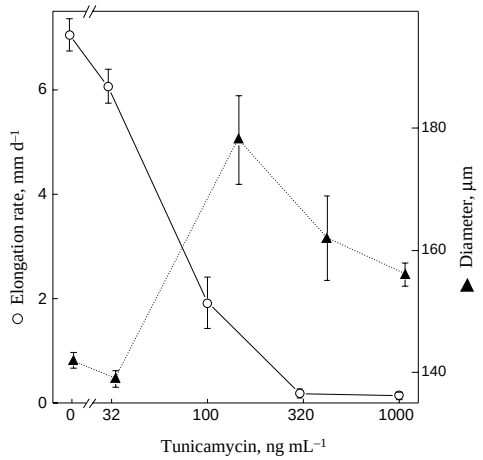

Fig. 6. The effect of tunicamycin on root elongation rate and diameter. Seedlings were germinated and grown on control medium without sucrose for 6 d from plating and transplanted onto medium with the indicated concentrations. Elongation rate is reported over the 2nd day after transplanting and diameter after 48 h. Symbols report the mean ± s.e. of three replicate plates.

Table 4. The effect of selected glucose analogs on root elongation rate and diameter

Seedlings were grown on control medium for 6 d, transplanted on to media with the addition listed, and elongation rate was measured over the second 24-h period of treatment, and diameter after 48 h of treatment. Data are means ± s.e. of three replicate plates, from one experiment

Tested compound	Diameter (μm)	Elongation rate (mm d^{-1})
Control	154 ± 0.3	8.2 ± 0.93
10 m mM 6-deoxyglucose	158 ± 3.7	6.8 ± 0.73
10 mM 3-O-methylglucose	161 ± 1.8	5.8 ± 0.17

Table 5. The effect of mannoheptulose on the inhibition of root elongation by mannose

Seedlings were grown on control medium for 6 d, transplanted on to media with the addition listed, and elongation rate was measured over the second 24-h period of treatment, and diameter after 48 h of treatment. Except where noted, data are means ± s.e. of three replicate plates

Treatment	Elongation rate (mm d^{-1})
Control	6.0 ± 0.09
30 mM mannoheptulose	5.1 ± 0.6[A]
0.2 mM mannose	0.8 ± 0.33
Mannose + mannoheptulose	4.4 ± 0.33

[A]Mean ± s.d. of a single plate.

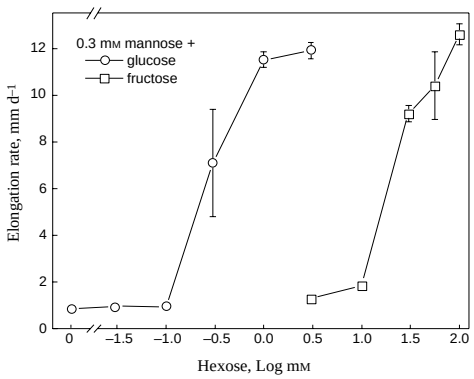

Fig. 7. The effect of exogenous glucose or fructose on the inhibition of root elongation by mannose. Seedlings were germinated and grown on control medium without sucrose for 1 week and transplanted onto plates containing 0.3 mM mannose and the indicated concentration of hexose. Symbols report mean ± s.e. (when greater than the size of the symbol) elongation rate over the 2nd day after transplanting for three replicate experiments, each with three plates. Maximal elongation rates shown are high in this experiment because it was done under doubled light intensity and are equal to that of seedlings in the same experiment grown without mannose.

glucose and mannose being the same, mannose has been proposed to affect elongation by inhibiting protein secretion (Edelmann *et al.* 1995). However, in *A. thaliana* roots, 2-deoxyglucose, like all tested secretion inhibitors, causes swelling, whereas mannose does not, suggesting that in roots mannose does not inhibit protein secretion to a significant extent. Likewise, others have differentiated the effects of 2-deoxyglucose and mannose on roots (Stenlid 1971). Because 2-deoxyglucose inhibits glycoslylation, this analog should be used with care in studies of carbon metabolism. Furthermore, 2-deoxyglucose-6-phosphate is metabolized extensively by plants (Klein and Stitt 1998), apparently producing the metabolites that are responsible for some of the inhibitory activities of the compound (Eblein 1987).

A second objective of the work was to understand the basis for the inhibition of root growth by mannose. While mannose is well known to inhibit root elongation (Herold and Lewis 1977; Farrar *et al.* 1995), to our knowledge we are the first to report that mannose in contrast to 2-deoxyglucose does not cause root swelling, that mannose inhibits elongation more rapidly than cell production, and that mannose inhibits root elongation apparently via hexokinase. Hexokinase substrates mimic mannose activity but non- substrates do not, a hexokinase inhibitor blocks mannose activity, and the difference in K_m of hexokinase toward glucose and fructose explains the difference in the concentration ranges of glucose and fructose that block mannose activity.

An apparent requirement for hexokinase has emerged as a common theme among recent investigations of mannose and sugar signaling (Jang and Sheen 1994; Pego *et al.* 1999; Neta-Sharir *et al.* 2000). Hexokinase phosphorylates mannose to produce mannose-6-phosphate, which frequently cannot be metabolized (Herold and Lewis 1977; Klein and Stitt 1998). Indeed, this build-up has given rise to a selection strategy for plant transformation where incorporation of the gene for phosphomannose-isomerase confers on the transformants the ability to grow in the presence of mannose, thereby allowing mannose to be substituted for the customary antibiotics (Joersbo *et al.* 1998). Accumulation of mannose-6-phosphate gave rise to the original explanation for mannose toxicity, namely phosphate deficit, leading to lowered respiration and ATP levels (Herold and Lewis 1977). This explanation continues to be supported for systems where adding phosphate prevents the deleterious action of mannose (Harris *et al.* 1986; Weiner *et al.* 1992). However, it has become clear that this explanation is not always correct. Examples have been found where mannose reduces neither levels of ATP (Pego *et al.* 1999) nor of cytosolic phosphate (Kime *et al.* 1982) and effects of mannose do not invariably respond to altered levels of phosphate or ATP (Graham *et al.* 1994; Jang and Sheen 1994; Farrar *et al.* 1995; Klein and Stitt 1998).

Another explanation for the inhibitory action of mannose-6-phosphate is now favored: namely, that the phosphorylated mannose participates with hexokinase in sugar signaling, although some have questioned whether hexokinase acts other than metabolically (Halford *et al.* 1999), and no one has discovered the connection between hexokinase and any signal transduction component. The paradigm for sugar signaling is catabolite repression in bacteria, a phenomenon where a high concentration of a sugar represses the expression of enzymes required for its synthesis. In plants, glucose represses genes for carbon fixation as well as other photosynthetic functions and the repression appears to be mediated, at least in part, by hexokinase (Jang and Sheen 1997; Smeekens 2000). That hexokinase can send a signal independently of its metabolic activity is supported by finding that treatment with either glucose or an analog can change mRNA levels faster than concentrations of key metabolites (Klein and Stitt 1998), as well as by experiments with transgenic plants overexpressing hexokinase: the plants are dwarfed and less sensitive to sugar (Dai *et al.* 1999), unless they express instead a yeast hexokinase that has a catalytic domain only, in which case the plants grow normally and symptoms of catabolite repression vanish (Jang *et al.* 1997).

In roots, the inhibition of elongation by mannose is the opposite of what is expected from catabolites. Roots are heterotrophic and sugar stimulates their elongation (for example, Table 1 shows nearly maximal root growth on 165 mM glucose). Mannose on the other hand reduces elongation rate to a very low level in a few hours and to zero after a few days. It is difficult to see how this could be the equivalent of metabolism on high levels of glucose. More straightforward is to argue that in roots mannose remains able to induce catabolite repression, as in leaves, whereas glucose does not. A similar opposition between treatment with glucose and mannose has also been observed in *A. thaliana* for germination (Pego *et al.* 1999). That plants can respond to a sugar phosphate in opposition to the response expected from prevailing metabolism supports the idea of a signaling pathway initiated with hexokinase bound to a phosphorylated hexose. Because of its greater lifetime, mannose-6-phosphate may occupy this site far more persistently than either glucose- or fructose-6-phosphate. Signaling pathways of this kind involving hexokinase are known in animal cells (Van Schaftingen *et al.* 1997) and the challenge is to discover their analogs in plants.

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