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Metabolic Response of Maize Roots to Hyperosmotic Shock[1]

An *in Vivo* ^{31}P Nuclear Magnetic Resonance Study

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ABSTRACT

^{31}P nuclear magnetic resonance spectroscopy was used to study the response of maize (*Zea mays* L.) root tips to hyperosmotic shock. The aim was to identify changes in metabolism that might be relevant to the perception of low soil water potential and the subsequent adaptation of the tissue to these conditions. Osmotic shock was found to result in two different types of response: changes in metabolite levels and changes in intracellular pH. The most notable metabolic changes, which were produced by all the osmotica tested, were increases in phosphocholine and vacuolar phosphate, with a transient increase in cytoplasmic phosphate. It was observed that treatment with ionic and nonionic osmotica produced different effects on the concentrations of bioenergetically important metabolites. It is postulated that these changes are the result of hydrolysis of phosphatidylcholine and other membrane phospholipids, due to differential activation of specific membrane-associated phospholipases by changes in the surface tension of the plasmalemma. These events may be important in the detection of osmotic shock and subsequent acclimatization. A cytoplasmic alkalinization was also observed during hyperosmotic treatment, and this response, which is consistent with the activation of the plasmalemma H^+-ATPase, together with the other metabolic changes, may suggest the existence of a complex and integrated mechanism of osmoregulation.

Water deficit is a common environmental problem encountered by most plants at some stage in their life. In many areas, water availability is a major factor limiting crop production, and consequently, it is important to gain an understanding of how plants respond and acclimatize to water deficit. Low soil water potentials result in a wide variety of physiological changes in plants, such as inhibition of shoot and root growth, enhanced stomatal closure, changes in gene expression and developmental patterns, limitation of photosynthesis, stimulation of compatible solute production, and increased ABA production (12, 21). Although the physiological and whole plant responses to osmotic shock have been studied, the mechanism by which plants perceive water deficit and transduce it into a biochemical response is still poorly understood. Water deficit leads to decreases in turgor pressure, cell volume, and osmotic potential, all of which could conceivably act as signals, and mechanisms by which these signals could be converted into adaptive responses have been suggested.

In the halotolerant alga *Dunaliella* and some higher plants, interest has focused on the possible importance of the plasma membrane H^+-ATPase in the detection of osmotic shock. In support of this hypothesis, it has been shown that *Dunaliella salina* is unable to recover from hypertonic shock in the presence of inhibitors of this ATPase, and in both *D. salina* and *Senecio mikanioides*, osmotic upshock caused an increase in the rate of proton efflux (15, 19). Because the plasmalemma H^+-ATPase provides the driving force for the uptake of sugars and other organic molecules, as well as the transport of ions, it has been suggested that this pump could act both as a detector of osmotic shock (*e.g.* by changes in conformation due to changes in the physical properties of the membrane) and as an effector of the adaptive responses via an increase in its activity, leading to enhanced solute transport and/or changes in the levels of cytosolic ATP or phosphate (14). Inorganic phosphate has been implicated separately as a trigger in glycerol synthesis in *D. salina* exposed to sodium chloride (1). However, further work is needed to confirm any of these mechanisms as responsible for the adaptive processes during exposure to water deficit.

It is also of interest to investigate the bioenergetic responses of plant tissue to decreased water potential, because the survival of the plant under adverse conditions may require increased rates of ion extrusion or compartmentalization, as well as synthesis of compatible osmotic solutes and growth regulators, all of which involve the utilization of cellular energy. The ability of a plant to tolerate a stressful environment may thus be limited by its ability to maintain an adequate flux through its bioenergetic pathways and to channel its energetic resources into appropriate adaptive metabolic processes.

The object of this investigation was to characterize the response of maize (*Zea mays* L. cv LG11) to decreased osmotic potential in terms of metabolite levels and bioenergetic status to assess the ability of the tissue to cope with this condition and, in particular, to identify metabolic changes that could be responsible for stimulating adaptive responses. Cytoplasmic and vacuolar pHs were also measured to investigate whether changes occurred that could provide evidence for

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an alteration in the rate of proton pumping at the plasmalemma or the tonoplast. A variety of osmotica were used to compare the response produced by ionic and nonionic osmotica. ^{31}P NMR spectroscopy was used to characterize the metabolic response because this technique allows a noninvasive and progressive monitoring of the bioenergetic status of the tissue and levels of phosphorylated metabolites. NMR can detect the presence of small, freely mobile molecules that are present in the cell at millimolar concentrations, therefore allowing the visualization of compounds such as inorganic phosphate, nucleotides, sugar phosphates, and other phosphomonoesters (3, 18), and intracellular pH can be calculated from the chemical shift of the Pi resonances (22).

MATERIALS AND METHODS

Sample Preparation for NMR Experiments

Maize seeds (*Zea mays* L. cv LG11) were germinated in the dark at 25°C between sheets of absorbent paper soaked in 0.1 mM $CaSO_4$. After 3 d, 5-mm root tips were excised and maintained in oxygenated standard buffer (1 mM KCl, 0.5 mM $CaSO_4$, 10 mM Mes [pH 6.0]). Approximately 160 root tips were placed in a 10-mm diameter NMR tube, and oxygenated standard buffer was circulated through the sample at a rate of 8 $mL \cdot min^{-1}$ (10). The tissue was allowed to equilibrate for 30 min before the NMR experiment. For control treatment, the tissue was incubated for 6 h on standard buffer. For treatment with osmotica, samples were maintained on standard buffer for 90 min before changing to a solution consisting of standard buffer plus an osmoticum (0.5 M sorbitol, 0.5 M mannitol, 0.43 M PEG-300, or 0.278 M NaCl) for an additional 4 to 5 h incubation. These concentrations of mannitol, sorbitol, PEG, and NaCl all gave an osmotic potential of −1.35 MPa, as calculated using equations described by Money (11).

Preparation of Perchloric Acid Extracts

Two hundred root tips were prepared and incubated in oxygenated Mes buffer with or without an osmoticum to give a comparable treatment to that used in the NMR experiments. The tissue was then extracted by a method adapted from Saez and Lagunas (24) for use with plant tissue. The root tips were frozen in liquid nitrogen and ground to give a fine powder. Two milliliters of 2 M perchloric acid was added, and the mixture was reground and then freeze-thawed three times. Cell debris was removed by centrifugation at 3000*g* for 10 min. EDTA to a concentration of 5 mM was added to the supernatant before adjusting the pH to 5.5 to 6.0 with aqueous K_2CO_3. The solution was centrifuged at 3000*g* for 10 min to remove precipitated $KClO_4$. The supernatant was passed through an Amicon ultracentrifugation membrane (YM5) to remove macromolecules before being passed down a Chelex column to exchange paramagnetic ions; the resulting solution was lyophilized.

Before NMR examination, the lyophilized powder was dissolved in 3 mL of 5 mM EDTA, 100 mM triethanolamine (pH 8.0).

Growth Inhibition by Osmotica

Maize seeds were germinated as described above for 3 d. Groups of eight to 10 seedlings were placed in Petri dishes where they were held between filter papers moistened with one-fifth strength Long Ashton nitrate nutrient medium (9) in which the osmotica had been dissolved. The lengths of the primary roots were measured before treatment and after 24 h incubation in the dark at 24°C.

NMR Methods

^{31}P NMR spectra were recorded on a Bruker CXP300 spectrometer, operating at a phosphorus-31 frequency of 121.49 MHz, using a double-tuned $^{13}C/^{31}P$-probe head. All spectra were acquired with a spectral window of 8064 Hz. Spectra were recorded from tissue *in vivo* using a 90° pulse angle and a 6-s recycle time to give relaxed spectra (except for TEMDP[2] and vacuolar phosphate). These spectra were acquired sequentially during the time course in files of 300 scans (equivalent to 30 min); each free induction decay contained 2K data points. Chemical shifts were measured relative to the signal from a capillary containing a 2% (v/v) aqueous solution of TEMDP, which is at 22.49 ppm relative to the signal from 85% H_3PO_4. The TEMDP resonance was decoupled to improve the line shape.

The tissue volume was measured by circulating standard buffer plus 1 mM MDP through the NMR tube in the presence and absence of root tips; the volume occupied by the sample was calculated from the difference in intensity of the MDP resonance. To calculate the amount of tissue shrinkage caused by osmotic stress, the buffer was changed after 1 h to Mes plus 1 mM MDP plus osmoticum, and the change in MDP intensity was monitored.

The concentrations of metabolites in the tissue were calculated by comparing the resonance intensities with that of a capillary containing 2% TEMDP which had previously been calibrated against 2 mM phosphate, taking into account the percentage volume of the sample (NMR measures concentration averaged over the volume of the NMR tube). The pH values of the intracellular compartments were calculated from the chemical shifts of the Pi resonances after construction of calibration curves as described previously (22).

^{1}H-decoupled ^{31}P NMR spectra of tissue extracts were recorded in files of 1024 scans (each containing 8K data points) using a 90° pulse angle and a 4-s recycle time. The resonances were assigned by comparison with previously published spectra or by spiking with compounds dissolved in 5 mM EDTA, 100 mM triethanolamine (pH 8.0).

RESULTS

Effect of Osmotica on Root Growth

The effects of sorbitol, PEG-300, and NaCl on the growth rate of maize primary roots are shown in Figure 1. It can be

[2] Abbreviations: TEMDP, tetraethyl ester of methylene diphosphonate; DAG, diacylglycerol; G6P, glucose-6-phosphate; m.d., mean difference; MDP, methylenediphosphonate; NTP, phosphate of nucleoside triphosphate; PtdCho, phosphatidylcholine; UDPG, UDP-glucose.

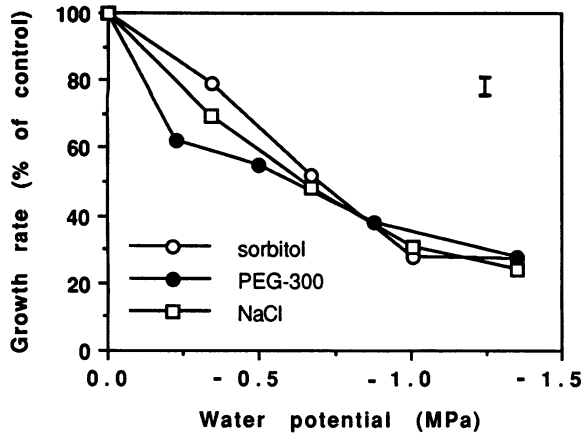

Figure 1. The effect on root growth of intact maize seedlings by sorbitol, PEG-300, and NaCl at various degrees of water deficit. The effect on growth was measured during a period of 24 h, and the growth rate was calculated relative to that of control seedlings grown in nutrient medium in the absence of osmotic agents. The error bar corresponds to the pooled SD (n = 3 for all treatments).

seen that a progressive decrease in growth rate was caused by increasing concentrations of the osmotica, and that at a water potential of −1.35 MPa, all the osmotica produced a similar growth inhibition. The effects of the osmotica on the growth rate were slightly more severe than that which occurs with seedlings grown in vermiculite over a range of water potentials (*e.g.* 70% inhibition by the osmotic agents compared with 60% inhibition in vermiculite at −1.35 MPa [25]), but overall, it appears that these osmotica give a reasonable simulation of soil water deficit. It can be seen that the concentrations of osmotica used in the NMR experiments allowed some root growth to occur.

Effect of Osmotica on Metabolite Concentrations

Figure 2 shows the ^{31}P NMR spectra of excised maize root tips acquired *in vivo* after 3 h of treatment with different osmotica. It can be seen that control tips (incubated in standard buffer only) have higher concentrations of G6P and a lower concentration of phosphocholine and vacuolar Pi than tips that have been treated for the same length of time in mannitol, NaCl, or PEG-300. There do not appear to be substantial changes in the levels of nucleotides or UDPG. The effects produced by treatment with sorbitol were almost identical with those produced by mannitol treatment. The assignments of the resonances and the changes observed *in vivo* were confirmed in ^{31}P NMR spectra of perchloric acid extracts of the tissue.

The intracellular concentrations of phosphorylated metabolites were calculated from the intensities of the NMR resonances. Control root tips showed a number of changes during the time course of treatment: there was an increase in the level of phosphocholine in the tissue (Fig. 3) and slight initial decreases in the levels of G6P and UDPG (data not shown). The levels of γ-NTP (data not shown), cytoplasmic phosphate (Fig. 4), and vacuolar phosphate (Fig. 5) appeared to be stable during the incubation period. Treatment of the roots with

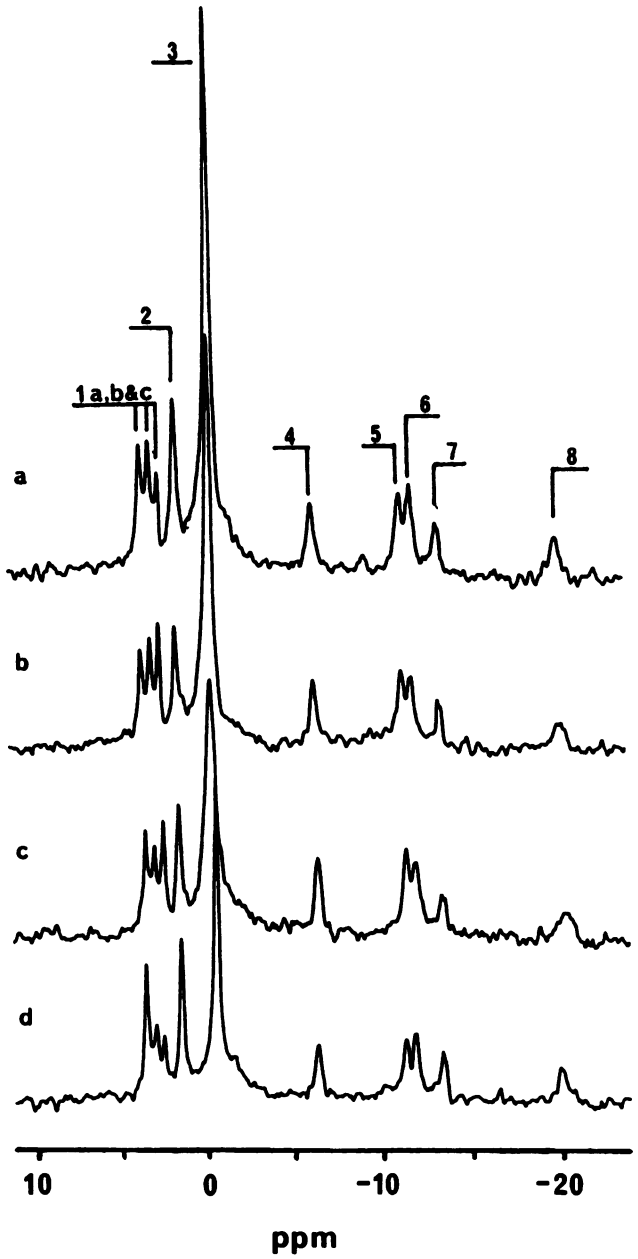

Figure 2. ^{31}P NMR spectra of excised maize root tips treated for 3 h with 0.43 M PEG-300 (a), 0.5 M mannitol (b), 278 mM NaCl (c), or standard buffer (d). The spectra were acquired in 30 min with a 6-s recycle time and are the sum of 300 transients. The resonance assignments are as follows: 1a, G6P; 1b, fructose-6-phosphate and other phosphomonoesters; 1c, phosphocholine; 2, cytoplasmic phosphate; 3, vacuolar phosphate; 4, γ-phosphate of NTP and β-phosphate of nucleoside diphosphate; 5, α-phosphates of NTP and nucleoside diphosphate; 6, UDPG and NAD(P)(H); 7, UDPG; and 8, β-phosphate of NTP. Chemical shifts are quoted relative to 85% H_3PO_4. It should be possible to quantitate changes of >20% for the resonances of interest, given the signal-to-noise quality of these spectra.

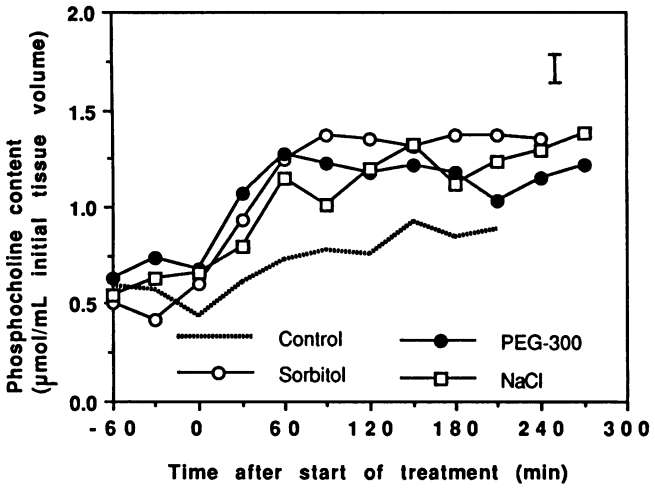

Figure 3. Changes in intracellular phosphocholine content in maize root tissue resulting from hyperosmotic shock. The contents were calculated from the intensities of resonances in the ^{31}P NMR spectra *in vivo* by comparison with a previously calibrated solution of TEMDP contained in a capillary tube and were corrected for the initial percentage volume of the tissue in the NMR tube. The values are the average of several independent time courses for each treatment ($n = 5$ for control, $n = 4$ for sorbitol, $n = 5$ for PEG-300, $n = 3$ for NaCl), and the error bar shows the pooled SD. Analysis of variance of the data at $t = 0$ showed no significant difference between control and sorbitol treatments ($P = 0.05$); the comparisons control-PEG and control-NaCl gave significant differences ($P = 0.05$) with m.d. −0.23 and −0.22, respectively. At $t = 150$ min, there were significant differences for the comparisons control-sorbitol ($P = 0.005$), control-PEG ($P = 0.03$, m.d. = −0.29), and control-NaCl ($P = 0.005$, m.d. = −0.40).

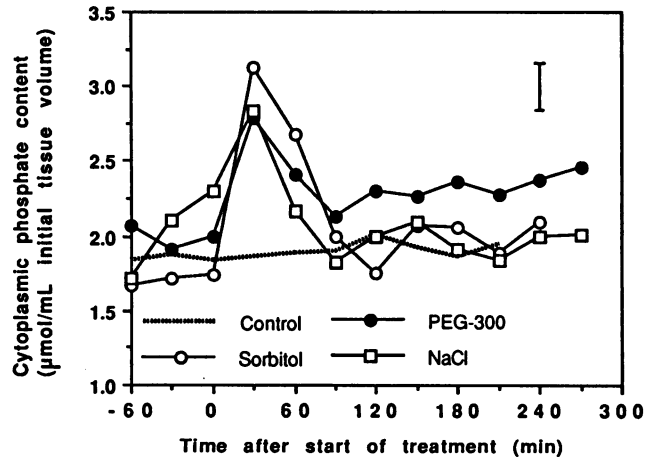

Figure 4. Changes in intracellular cytoplasmic phosphate content resulting from hyperosmotic shock. The number of experiments, error bar, and calculation of the intracellular contents are as described for Figure 3. Analysis of variance of the data at $t = 0$ showed no significant differences for control-sorbitol and control-PEG ($P = 0.05$) and a significant difference for control-NaCl ($P = 0.05$, m.d. = −0.46). At $t = 30$ min, there were significant differences for control-sorbitol ($P = 0.02$), control-PEG ($P = 0.03$), and control-NaCl ($P = 0.03$, m.d. = −0.97).

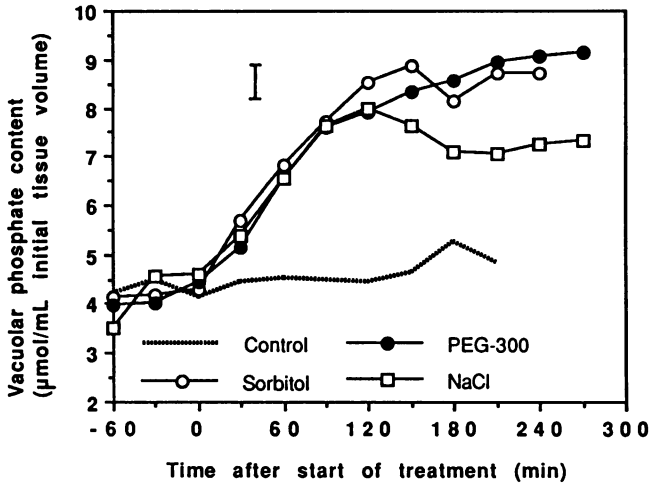

Figure 5. Changes in intracellular vacuolar phosphate content following hyperosmotic shock. The number of experiments, error bar, and calculation of the intracellular contents are as described for Figure 3. Analysis of variance of the data at $t = 0$ showed no significant differences between any groups ($P = 0.05$), whereas at $t = 150$ min, there were significant differences for all the comparisons ($P = 0.001$).

osmotica caused several effects that were distinct from those observed in control tips.

The most obvious changes were in the levels of phosphocholine and intracellular phosphate. The increase in phosphocholine observed in control tissue was considerably enhanced (70–100%) by osmotic stress (Fig. 3), and there was also a 100% increase in the vacuolar phosphate pool over several hours (Fig. 5), as well as a transient increase of about 75% in cytoplasmic phosphate concentration immediately after the start of treatment (Fig. 4). These responses were observed for all the osmotica, with the largest effects being produced by sorbitol and mannitol, which showed very similar responses, and the smallest effect being produced by NaCl. These changes were found to be reversible if the root tips were returned to standard buffer: the recovery was almost complete after 2.5 h for tissue that had been treated in hypertonic solution for approximately 4 h (data not shown).

It was also noted that NaCl produced more noticeable effects on the levels of G6P, UDPG, and γ-NTP than the nonionic osmotica (data not shown). NaCl treatment enhanced the decrease in concentration of G6P, which was observed in control treatment, and caused a larger initial decrease in the level of UDPG. There was also some indication of a slight decrease in the concentration of γ-NTP, which was not observed with sorbitol, mannitol, or PEG. The fact that no noticeable decreases in G6P, UDPG, or γ-NTP occurred with the nonionic osmotica shows that the bioenergetic stress caused by these treatments was not very severe. The partial inhibition of root growth (70%) under these conditions may reflect the redistribution of resources from growth to adaptive metabolism.

Effects of Osmotica on Cell Volume

The effects of ionic and nonionic osmotica on the tissue volume are shown in Figure 6. It can be seen that with both types of osmotica shrinkage of the tissue occurs primarily during the first hour after the initiation of osmotic stress. The effect produced by 0.5 M mannitol, 0.5 M sorbitol, or 0.43 M PEG-300 is approximately 100% greater than that of NaCl; this may be due to the fact that NaCl is taken up into the tissue much faster than the other osmotica. The changes in tissue volume observed will affect the concentrations of intracellular metabolites, but because we have no information concerning the relative volume changes of the cytoplasm and vacuole, it is impossible to quantify these changes exactly.

Effects of Osmotica on Intracellular pHs

The effect of osmotic stress on the pH of the intracellular compartments was also investigated. The changes in cytoplasmic and vacuolar pH observed for tissue incubated in standard buffer and tissue treated with nonionic osmotica are shown in Figure 7. It can be seen that there is a small but significant increase in both the cytoplasmic pH and the vacuolar pH resulting from treatment of the tissue with mannitol, sorbitol, or PEG-300. The increase in cytoplasmic pH became apparent approximately 90 min after treatment commenced, whereas the vacuolar pH increase did not occur until approximately 120 min after the osmotic shock. The effect of NaCl on intracellular pHs are not shown here because very large changes are observed, which are thought to be caused partly by the direct effect of NaCl on the chemical shift of Pi and partly by the activity of a Na^+/H^+ antiporter; these effects will be discussed elsewhere (C.M. Spickett N. Smirnoff, R.G. Ratcliffe, unpublished data). It is unlikely that a direct effect of increased ionic strength due to tissue shrinkage is responsible for the apparent increase in pH observed with the nonionic osmotica, because shrinkage is effectively complete 1 h after the start of treatment, whereas the pH changes do not become evident until after this time.

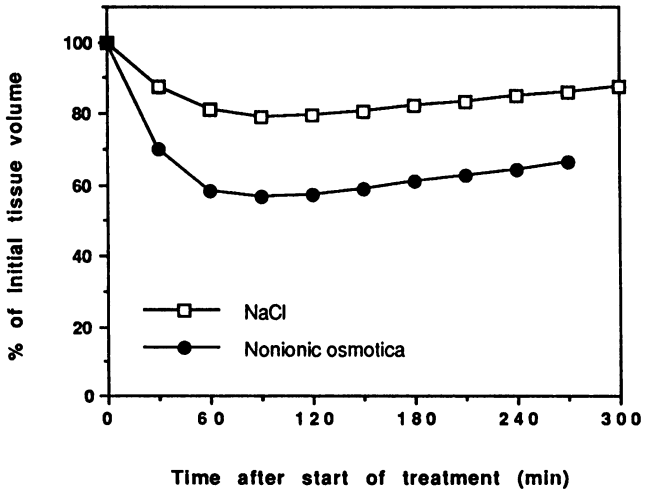

Figure 6. The effect of osmotic treatment on the volume of excised maize root tips. The nonionic osmotica used were sorbitol (0.5 M), mannitol (0.5 M), and PEG-300 (0.43 M). Changes are given relative to the initial percentage volume of the tissue in the NMR tube. The volumes were calculated by circulating buffer containing 1 mM MDP and comparing the intensity of the MDP resonance in the presence and absence of tissue. The results of nonionic osmotic treatment are the average of three independent time courses, one with each osmoticum, and the NaCl results are the average of two independent experiments.

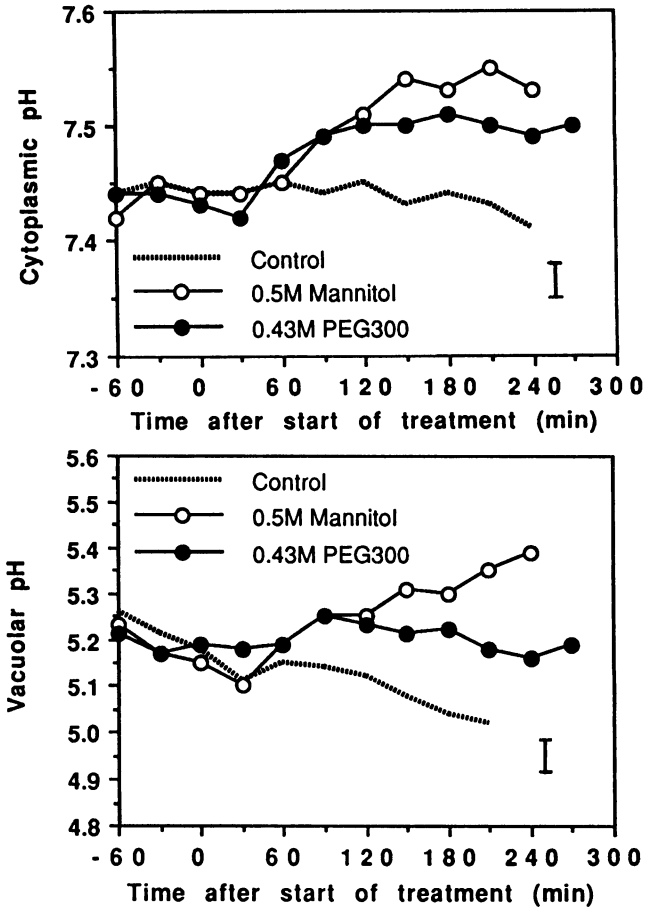

Figure 7. The effect of osmotic treatment on cytoplasmic pH and vacuolar pH of maize root tips *in vivo*. The pHs were estimated from the chemical shift of the cytoplasmic phosphate resonance. The effects produced by treatment with mannitol and sorbitol were very similar. The results are the average of several independent experiments (n = 8 for control and mannitol, n = 5 for PEG-300). Analysis of variance of the data showed no significant differences at t = 0 for both cytoplasmic and vacuolar pH ($P = 0.5$). At t = 180 min, there were significant differences for comparisons of cytoplasmic pH ($P < 0.005$ in both cases) and for comparisons of vacuolar pH (control-sorbitol, $P = 0.001$; control-PEG, $P = 0.02$).

DISCUSSION

The object of this study was to further our knowledge of the early biochemical events that occur when plant tissue is subjected to abrupt changes in external osmotic potential. *In vivo* ^{31}P NMR measurements showed that treatment of maize root tissue with a variety of osmotic agents induced changes

in the concentrations of several important metabolites and in the pH of the cytoplasmic and vacuolar compartments, and it also appeared that there were some differences between the effects of ionic and nonionic osmotica. These biochemical changes will be discussed in the context of the response and acclimatization of the roots to water deficit; in particular, the mechanisms by which environmental changes are detected and adaptive metabolism is triggered.

It should be noted that the metabolite levels are given as intracellular contents, with units of micromoles per milliliter of initial tissue volume, because the relative volumes of the cytoplasm and vacuole are not known, although by comparison with measurements in pea root tips (16) they might be expected to be approximately equal. The real metabolite concentrations are, therefore, likely to be approximately double those quoted. The decrease in tissue volume caused by osmotic treatment has not been taken into account in the calculation of metabolite levels, because in this study we were interested in effects produced by changes in metabolism rather than those due to physical changes (NMR measures concentrations averaged over the volume of the NMR tube; hence, the sample volume at each time point must be taken into account to obtain the real concentration in the sample).

The largest changes observed were the increases in the concentration of phosphocholine and Pi, with a transient increase in cytoplasmic phosphate and a progressive increase in vacuolar phosphate. We suggest that these changes are due to breakdown of membrane components when turgor is lost as a result of cell shrinkage. Pi can be produced by the hydrolysis of any phospholipid, and hydrolysis of PtdCho by phospholipase C will yield phosphocholine, which in turn can be broken down to phosphate and choline. Hydrolysis by phospholipase D will result in the formation of choline and phosphatidic acid, which can be further hydrolyzed to Pi and DAG. The transient increase in cytoplasmic phosphate suggests that phosphate derived from membrane components initially appears in the cytoplasm but is subsequently transported to the vacuole, leading to an accumulation of phosphate in this compartment. The increase in Pi is not balanced by a decrease in the other compounds visible in the NMR spectrum and, therefore, must be derived from NMR invisible phosphate compounds, such as phospholipids and mRNA. Because the increase in phosphocholine is smaller than the increase in phosphate, and there is no substantial increase in choline (data not shown), PtdCho cannot be the only source of Pi.

Similar increases in phosphocholine have been observed previously in stressed plant tissue. Roby *et al.* (23) found increased phosphocholine and glycero-phosphocholine in sucrose-starved cultured sycamore cells, which they attributed to membrane breakdown during stress due to utilization of lipids as an energy source; these effects were reversed when an external carbon source was added. These results suggest that the small increase in phosphocholine levels during control treatment observed in our study may be due to the lack of a carbon supply because the tips were maintained in standard buffer. In the work of Roby *et al.* (23), the effects appeared over a longer time course, but this may simply reflect a higher rate of anabolic metabolism in actively growing roots compared to cultured cells in a stationary phase.

Increased phosphocholine levels have recently been reported in *D. salina* during hyperosmotic shock (4), and it was also suggested that the phosphocholine could be produced from PtdCho by the action of phospholipase C. It is known that osmotic shock affects membrane composition and turnover; in *D. salina*, hyperosmotic shock causes increased turnover of PtdCho and lyso-PtdCho (6), whereas in *Avena sativa*, acclimatization to low water potential results in a decrease in the PtdCho to phosphatidylethanolamine ratio, which would be compatible with the release of phosphocholine from the plasma membrane (13). The appearance of phosphocholine in *D. salina* is likely to have physiological significance because osmotic shock does not decrease the surface area of the plasma membrane (6) and phosphocholine accumulation is not observed in osmotically defective mutants that have very slow glycerol synthesis (4).

The mechanism by which specific phospholipase activity could be induced in response to water deficit is not yet clear, although it has been shown in model phospholipid monolayers that some phospholipases are only active if the surface tension is below a certain level (5; references in ref. 6). Because a decrease in turgor or cell volume would cause a decrease in membrane surface tension, these could directly influence phospholipase activity.

The observed increases in the levels of intracellular phosphate may be interesting in terms of the transduction of information about the extracellular environment. Hyperosmotic shock has been found to cause increases in Pi concentration in *D. salina* and has led to the proposal of a model for the triggering of glycerol synthesis during osmoregulation by Pi (1). Although higher plants are not thought to use glycerol as a compatible solute, phosphate is involved both as a substrate and as an effector in many metabolic processes and, therefore, could potentially act as a metabolic signal in other adaptive responses.

The cytosolic alkalinization observed in this study during treatment with nonionic osmotica is consistent with the hypothesis of plasmalemma H^+-ATPase activation by hyperosmotic shock, although it must be remembered that in biological systems it is incorrect to assume a direct relationship between proton movement and changes in pH because pH is a dependent variable that is influenced by a number of factors, including the net unbalanced charge on the fully dissociated ions in the cytosol (26).

The results obtained are in agreement with previous reports in the literature concerning acidification of the medium by cells exposed to mannitol and other similar osmotica (refs. 19 and 20 and references therein). It has also been shown that inhibition of the plasmalemma H^+-ATPase with vanadate prevents the recovery of *D. salina* from osmotic shock (14). The plasma membrane H^+-ATPase has been proposed as both a detector of loss of turgor and an effector of the adaptive responses in a number of these reports (14, 15, 19, 20). On the other hand, Hartung *et al.* (8) measured intracellular pH in leaf discs of *Valerianella locusta* using compartmental efflux of labeled ABA and reported that treatment with hyperosmotic sorbitol caused a cytoplasmic acidification of 0.2 to 0.3 pH units. This result contrasts with the cytoplasmic alkalinization observed in this study and in the reports discussed above.

Another aspect of plant response to osmotic shock that has emerged in this study is the difference in the changes caused by ionic and nonionic osmotic agents. NaCl appeared to cause smaller increases in the phosphocholine and intracellular phosphate concentrations but larger decreases in the "bioenergetic" metabolites as compared to the nonionic osmotica. The nonionic osmotica did not appear to cause a severe bioenergetic stress, and the partial inhibition of root growth (70%) under these conditions may reflect the redistribution of resources from growth to adaptive metabolism. It appears that NaCl is less efficient as an osmolyte in terms of initiating a membrane breakdown response but that the stress produced is energetically more costly for the tissue. This may be due to the fact that NaCl is taken up into the cell, whereas the other osmotic agents used were not transported at a significant rate during the time course of the experiments.

Influx of salt will reduce the osmotic potential gradient between the external solution and the tissue, and to avoid the accumulation of toxic ions in the cytosol membrane, transport systems (*e.g.* the Na^+/H^+ antiporter) in the plasmalemma and tonoplast must be activated, resulting in the utilization of cellular energy resources. The decrease in levels of G6P and UDPG may reflect a transition to a greater glycolytic flux to accommodate the increased energetic requirement or may result from differential sensitivity of the primary metabolic pathways to the level of NaCl. Differences in response to ionic and nonionic osmotica have been reported previously (20), although in their case a smaller decrease in NTP levels was observed with NaCl as compared to polyol treatment.

If the released phosphocholine is derived from membrane-associated PtdCho, this could have further implications for plant membrane signaling. In mammalian cells, PtdCho breakdown products (DAG and phosphatidic acid) have been implicated in agonist-induced signal transduction (7, 17). Chitlaru and Pick (4) suggested that signal transduction involving PtdCho hydrolysis may be important in osmoregulation in *Dunaliella,* and it is possible that this pathway could exist in the system studied here. However, these processes are usually thought to involve low concentrations of messengers and to occur on a time scale of minutes, although Cabot *et al.* (2) observed phorbol-induced release of choline metabolites during a period of 2 h. Hence, it is not possible to obtain information about these processes from the experiments described here. This agonist-induced signal transduction could result in changes in gene expression and would be distinct from the metabolic signaling that has been investigated and discussed in this study and elsewhere (4).

CONCLUSION

Based on the information obtained in this study and in other studies that have been discussed here, we propose a model for some of the biochemical events that occur during the early stages of adaptation to osmotic shock. Water deficit causes a decrease in turgor and, therefore, in surface tension at the plasmalemma, which could activate membrane-associated PtdCho-specific phospholipase C, resulting in the release of DAG and phosphocholine. Hydrolysis of other phospholipids may also occur, and subsequent hydrolysis of the phospholipids would yield Pi. The released phosphate may be involved in metabolic signaling, leading to an adaptive response; it is not clear whether phosphocholine has any role in the signaling process. The plasmalemma H^+-ATPase may be activated either directly by a conformational change in its structure induced by the decrease in turgor or indirectly by the changes in membrane phospholipid composition resulting from the action of specific phospholipases. Further investigation is needed to confirm that the changes in phosphocholine and Pi concentration observed are due to the turnover of membrane-derived PtdCho and other phospholipids and also to determine more precisely the sequence of events that occurs during detection and signaling of osmotic stress.

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