Phosphate Uptake by Excised Maize Root Tips Studied by *in Vivo* ^{31}P Nuclear Magnetic Resonance Spectroscopy

ABSTRACT

The extent of phosphate uptake measured by the relative changes in cytoplasmic Pi, vacuolar Pi, ATP, glucose-6-phosphate, and UDPG was determined using *in vivo* ^{31}P nuclear magnetic resonance spectroscopy. Maize (*Zea mays*) root tips were perfused with a solution containing 0.5 or 1.0 millimolar phosphate at pH ~6.5 under different conditions. In the aerated state, phosphate uptake resulted in a significant increase (>80%) in vacuolar Pi, but cytoplasmic Pi only transiently increased by 10%. Under N_2, the cytoplasmic Pi increased ~150% which could be attributed to a large extent to the breakdown of ATP, sugar phosphates and UDPG. Vacuolar Pi increased but only to the extent of ~10% of that seen under aerobic conditions. 2-deoxyglucose pretreatment was utilized to decrease the level of cytoplasmic Pi. When pretreated with the 2-deoxyglucose, the excised maize roots absorbed phosphate from the perfusate with a significant increase in the cytoplasmic Pi. The increase could only be traced to external phosphate since the concentrations of other phosphorus containing species remained constant during the uptake period. With 2-deoxyglucose pretreatment, phosphate uptake under anaerobic conditions was substantially inhibited with only the vacuolar phosphate showing a slight increase. When roots were treated with carbonyl cyanide *m*-chlorophenyl hydrazone, no detectable Pi uptake was found. These results were used to propose a H^+-ATPase related transport mechanism for phosphate uptake and compartmentation in corn root cells.

^{31}P *in vivo* NMR spectroscopy has been utilized as a powerful tool for elucidating metabolic changes taking place in intact plant tissue (4, 21, 23). The technique has been especially effective in determining pH in both the cytoplasm and vacuole of plant cells since the inorganic phosphate chemical shift is pH dependent (1, 24–26, 28). In addition, it allows a simultaneous determination of metabolically induced changes in the cellular level of a number of phosphorus containing cell constituents such as G-6-P,[1] ATP, and UDPG and has been applied to a variety of systems including wheat leaf tissue (30), excised maize root tips (13, 14, 19, 20, 22, 27), intact barley roots (3), pea roots (6), and plant cell suspensions (2, 17, 18). The involvement of compartmented phosphate in the metabolisms of galactose (10) and mannose (11) in plant tissues was also recently studied by this spectroscopic method.

Total phosphate uptake by plant roots, as measured by ^{32}P uptake, revealed a close relationship with the concentration of ATP in corn root tissue (9). Certain aspects of phosphate uptake and intracellular distribution were recently studied by *in vivo* ^{31}P NMR (6, 22, 27). However, the specific factors regulating uptake and compartmentation of phosphate remain to be determined. In this work, we studied the influence of energy status on these processes. Our results indicated that different mechanisms are involved in governing the transport of phosphate into the cytoplasm and vacuole of corn root cells. A regulatory mechanism for controlling the passage of phosphate across the plasma membrane and tonoplast membrane is proposed.

MATERIALS AND METHODS

Approximately 700 to 1000 corn root tips (*Zea mays* L. var FRB-73) 5 to 7 mm in length were excised from seedlings grown at 28 °C in a growth chamber on paper saturated with a 0.1 mM $CaSO_4$ solutions for 48 to 72 h as described previously (29). Immediately after cutting, the root tips were placed in a cold neutral solution of 0.1 mM $CaSO_4$. The tips were then transferred to a 10 mm NMR tube equipped with inlet and outlet perfusion tubes similar to those described by Lee and Ratcliffe (5) and as modified in our laboratories (12, 14). O_2 was continuously bubbled into the perfusion reservoir (1 L) containing 500 to 1000 mL of perfusate. In anaerobic experiments, N_2 was substituted for the O_2 as saturating gas. To change the perfusion medium, a second reservoir was interconnected to a three-way stopcock assembly. Upon switching to a new perfusate, 80 mL of the returning perfusate were discarded to flush the system (the volume approximating that of the NMR tube and connecting tubing). The ^{31}P NMR spectra were obtained with a JEOL[2] 400 MHz, GX-400, spectrometer operating at 161.7 MHz and equipped with a narrow bore 54 mm probe. The spectra were obtained at 22 ± 1 °C over a 16,000 Hz frequency range with 2,048 data points zero filled to 16,384. About 10K transients (29 min) were acquired with a repetition rate of 0.162 s utilizing

† Deceased, February 1989.

[1] Abbreviation: G-6-P, glucose-6-phosphate; HMPA, hexamethylphosphoramide; 2-D-G, 2-deoxyglucose; 2-D-G-6-P, 2-deoxyglucose-6-phosphate; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone.

[2] Reference to a brand name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

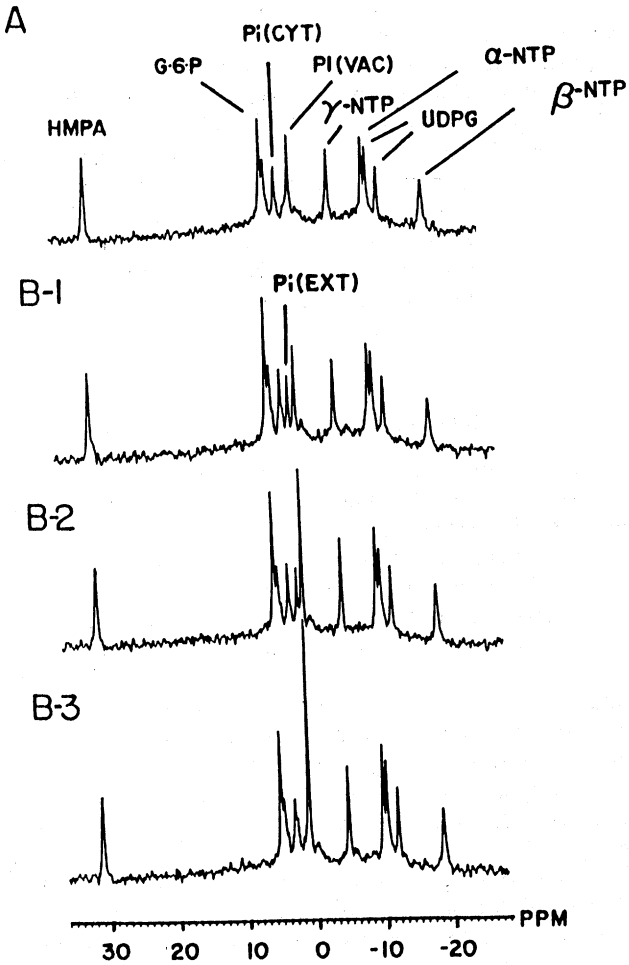

Figure 1. (A) ^{31}P NMR spectrum of excised maize root tips taken under fast acquisition accumulation, 10,000 transients. Perfusate contains 0.1 mM $CaSO_4$, 50 mM glucose. Assignments are as described previously (11). (B-1) Same as (A) but after 0.6 h perfusion with 0.5 mM potassium phosphate added to perfusate, pH 6.5, 22 °C; (B-2) After 2 h perfusion; (B-3) 16 h after washout. NTP represents nucleotide triphosphates with ATP as the predominant species.

approximately a 30 °C pulse (12 μs) without broad band decoupling. HMPA[3] (0.120 M) in a sealed capillary placed at the center of the NMR tube was used as an external reference. Chemical shifts are reported relative to that of 85% phosphoric acid which is 30.73 ppm upfield from HMPA.

Relative changes in cellular levels of mobile phosphorus species were estimated by peak area normalized to that of HMPA. It was found that the relaxation times (T_1) of observed peaks remained unchanged under experimental conditions. Thus, the normalized values (average of two independent experiments) were used to estimate the amounts of each species during the experiments. Reproducibility of relative peak intensities from one set of accumulations to another was 5 to 10%

[3] Caution should be exercised in handling this chemical because it is a known carcinogen.

RESULTS

Phosphate Uptake from an Aerobic Environment

Root tips were first perfused with a solution containing only 0.1 mM $CaSO_4$ and 50 mM glucose for about 1 h and one to two sets of baseline spectra (10K accumulations or 29 min each) were recorded. Then, buffer containing 0.5 to 1.0 mM K-phosphate at the stated pH and added salt as appropriate in addition to $CaSO_4$ and glucose was switched into the system for about 90 to 150 min and four to six sets of spectra (29 min each) were taken. Finally, the initial perfusate was returned to the system and a number of spectra recorded as equilibrium was established. When the roots were first perfused with the aerobic glucose medium, ^{31}P NMR spectra such as A in Figure 1 was obtained. It was generally noted that during this treatment the level of sugar phosphates (including G-6-P) increased slower than that of nucleotide phosphates. When the NMR signals reached a steady state, phosphate-containing perfusion medium was then introduced and the corresponding spectrum recorded (B-1 of Fig. 1). The ^{31}P resonance from the external K-phosphate buffer, pH 6.5, located between vacuolar and cytoplasmic Pi peaks, remained nearly unchanged during the uptake experiment. The most significant change over the course of the experiment was the large increase in the vacuolar Pi peak. Other phosphorus containing compounds showed little changes (Fig. 1, B-2). Since the external phosphate concentration was considerably lower than the initial concentrations of Pi of both cytoplasma and vacuole (at least 1–2 mM, by assuming that the cell compartment volume is the same as the NMR sample volume) of the roots (14), the increase must have resulted from an energy-facilitated process.

The details of the time courses of various resonances are shown in Figure 2. It should be mentioned that after switching

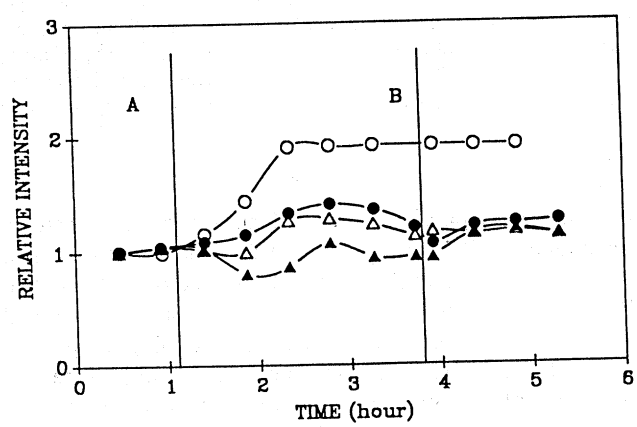

Figure 2. Time course of the relative ^{31}P NMR spectral intensities of selected mobile phosphate containing species within excised maize root tips, before, during and after perfusion with aerated 0.5 mM potassium phosphate solution, pH 6.5, containing 0.1 mM $CaSO_4$ and 50 mM glucose. (○) G-6-P, (△) cytoplasmic Pi, (●) vacuolar Pi, and (▲) ATP. (A and B) Start and end of phosphate buffer perfusion, respectively. Perfusate before and after phosphate treatment contained only 0.1 mM $CaSO_4$ and 50 mM glucose.

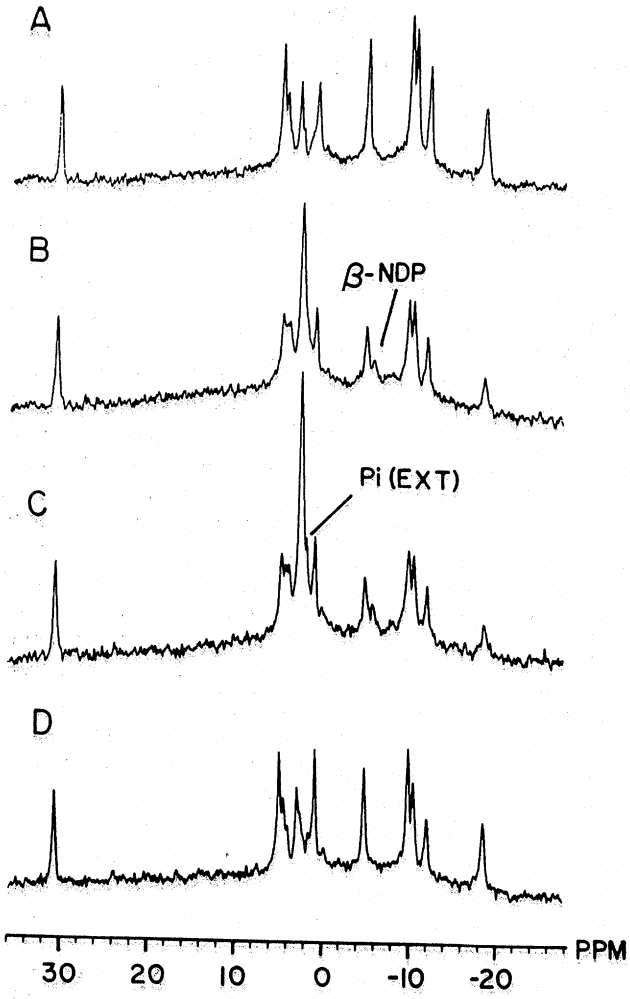

Figure 3. (A) ^{31}P NMR spectrum of excised root tips, 10,000 transients, under fast acquisition times, aerated perfusate containing 0.1 mM $CaSO_4$ and 50 mM glucose; (B) same as (A) but with N_2 gas bubbled through the perfusate; (C) 22 °C, same as (B) but with 0.5 mM sodium phosphate added (pH 6.5) 2 h after phosphate perfusion; (D) same as (A) 3 h after washout of phosphate buffer, under aeration. NDP represents nucleotide diphosphates with ADP as the major species.

back to normal, phosphate-free perfusion media, the spectrum maintained as B-2 (minus external Pi peak) for at least 2 h. An extended washout for 16 h led the spectrum changed to B-3. The further increase in the vacuolar Pi could be attributed to the breakdown of other phosphorus-containing compounds during the extended washout. When the K-phosphate was replaced with sodium salt, no significant increase of Pi accumulation in the vacuole was observed (data not shown). This observation is not compatible with that previously reported (22). The origin of this discrepancy is unknown at the present time. However, the different variety of corn seeds used mav be part of the reason.

Under aerobic conditions the phosphate concentration in the cytoplasm appeared to be tightly controlled with only relatively small changes taking place in the presence of external phosphate; excess phosphate taken up was shunted to the vacuole which appeared to retain it even after the external phosphate was removed. Under the conditions mentioned in Figure 1 (O_2 + glucose), the ATP synthesis via mitochondrial oxidative phosphorylation should be fully operational. Since a significant accumulation of Pi was observed in vacuole, the rate of transport across the tonoplast membrane should be at least as great as that across the plasma membrane. If indeed the uptake of Pi is closely related to the operation of H^+-ATPase, then the primary driving force for the uptake across these two membranes must be quite different. This is because the tonoplast H^+-ATPase is assumed to pump protons in an opposite direction from that of the plasma membrane *in vivo*.

Phosphate Uptake from an Anaerobic Environment

The effect of anaerobiosis on phosphate uptake was next investigated. After a baseline spectrum was recorded as in the initial set of experiments, perfusate containing 0.1 mM $CaSO_4$ and 50 mM glucose saturated with N_2 gas was switched into the root tip sample tube. After three sets of spectra from 29-min spectral accumulations were recorded, the perfusate was changed to one containing 0.5 mM phosphate (pH 6.5) in addition to $CaSO_4$ and glucose, also saturated with N_2 gas. Finally, the root tips were perfused with the normal phosphate-free solution, first saturated with N_2 and then with O_2 during final washout. Representative spectra from the potassium phosphate run were displayed in Figure 3 and the time courses of selected mobile phosphorus compounds are shown in Figure 4.

As seen from Figures 3 and 4, the ^{31}P resonances from both ATP and G-6-P decreased in intensity under anaerobic conditions, even in the presence of external phosphate the min-

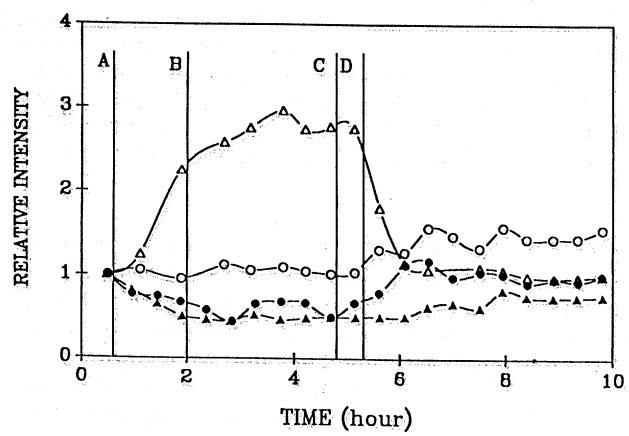

Figure 4. Time course of the relative ^{31}P NMR spectral intensities of species within excised maize root tips before, during and after perfusion with 0.5 mM sodium phosphate solution with N_2, pH 6.5 containing 0.1 mM $CaSO_4$ and 50 mM glucose. (○) G-6-P, (△) cytoplasmic Pi, (●) vacuolar Pi, and (▲) ATP. (B and C) Beginning and end of phosphate buffer perfusion with N_2, respectively. Perfusate before and after phosphate treatment contained only 0.1 mM $CaSO_4$ and 50 mM glucose. Left of (A) O_2 perfusion; at (A) start of N_2 perfusion; at 22 °C, at C washout with N_2 perfusion and at D, washout with O_2 perfusion.

ima being established in about 2 h. When oxygen was reintroduced to the system during washout, these peaks increased in intensity, recovering near fully to the initial levels.

Pi in the cytoplasm increased considerably under anaerobic conditions due primarily to the generation of Pi from the breakdown of ATP and G-6-P. As seen in Figure 3B, ADP was indeed being generated during the anaerobic phase. The cytoplasmic pH as determined by the chemical shift of the Pi resonance (14), initially at 7.6, decreased to 7.1 during the anaerobic phase. This change is in agreement with our earlier studies related to Mn^{2+} transport into corn root tips (14). When roots were washed with a phosphate-free solution under anaerobic conditions, no significant change of the spectrum was observed (Fig. 3C). However, the reintroduction of oxygen brought the spectrum back to resemble that obtained before anaerobiosis (Fig. 3D). After washout, cytoplasmic Pi returned to its initial level while the pH returned to 7.5. The level of Pi in the vacuole however showed a slight increase (~10%) after the aerobic washout.

Under anaerobic conditions, oxidative phosphorylation is not operative and plant cells obtain their ATP supply mainly through the less efficient fermentation pathway (20). In addition to a decrease in the ATP level, the cytoplasmic pH also decreased from 7.6 to 7.1. Since only limited, if any, overall uptake in vacuolar Pi was observed, the transport of Pi from external solution to cytoplasmic region is probably slower than that obtained under aerobic conditions. The presence of a relatively high cytoplasmic level of Pi which mainly derived from the breakdown of phosphorus-containing compounds, did not result in an increase in vacuolar Pi indicated a slowdown of transport of this anion into the vacuole.

Pretreatment with 2-D-G

The results of Figures 2 and 4 suggested that the uptake of phosphate could be related to the level of ATP in the cell cytoplasm. The lowering of the ATP level could also be achieved by fixing part of the cytoplasmic Pi by 2-D-G. This would have the effect of interrupting the normal metabolic pathway of glucose. When 2-D-G is phosphorylated, the product cannot be metabolized via the glycolytic pathway so that the ATP required for this reaction is consumed and cannot be regenerated by glucose metabolism. Thus, ATP levels should fall and 2-D-G-6-P should appear. At the same time, the cytoplasmic Pi level would decrease. Therefore, when the roots were treated with phosphate buffer, any increase in cytoplasmic Pi could be followed more easily.

After a baseline spectrum was established, the root tips were treated with 5 mM 2-D-G in an aerated solution containing only 0.1 mM $CaSO_4$. Spectra were accumulated over shorter periods of time (19.5 min, 5K accumulations) until the cytoplasmic Pi level fell to ~50% of its original value (1.25–1.5 h). Phosphate buffer (pH 6.5) was then introduced under the normal aerobic conditions but without glucose being present. The spectra obtained were similar to those previously reported (13). The time courses of the phosphate resonances are given in Figure 5.

With 2-D-G pretreatment, several events occurred as expected: cytoplasmic Pi and ATP levels decreased with an

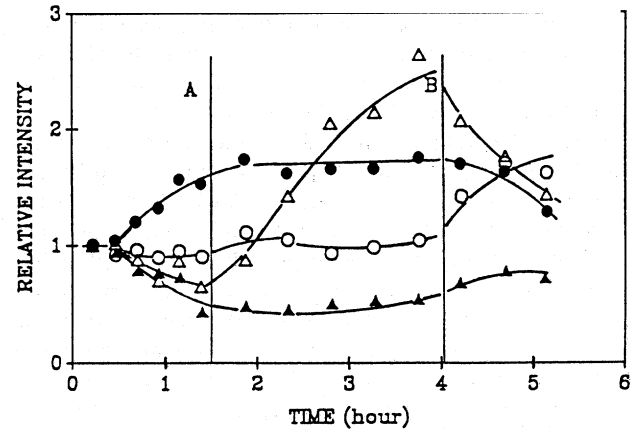

Figure 5. Time course of the relative ^{31}P NMR spectral intensities of species within excised maize root tips, during and after pretreatment with 2-D-G and treatment with phosphate buffer. (○) G-6-P and 2-D-G-6-P, (△) cytoplasmic Pi, (●) vacuolar Pi, and (▲) ATP. Left of (A) 2-D-G pretreatment, 5 mM, with 0.1 mM $CaSO_4$ added; (A) end of 2-D-G pretreatment, start of phosphate treatment, 10 mM sodium phosphate (pH 6.5), and (B) start of phosphate washout, 0.1 mM $CaSO_4$ only, all with O_2 perfusion.

increase in the peak at 5.0 ppm due to the formation of 2-D-G-6-P (which overlays the G-6-P peak). The UDPG resonances also decreased in intensity. When phosphate buffer was introduced, there was a dramatic increase in the cytoplasmic Pi while the other mobile phosphate peaks stayed roughly the same. Finally, on washout (O_2 + glucose), cytoplasmic Pi decreased to approximately its initial level with a concomitant increase in the vacuolar Pi. ATP increased to about 80% of its original value while the intensity of the 2-D-G-6-P peak slowly decreased.

In this set of experiments, the increase in the cytoplasmic Pi could be definitively accounted for by phosphate uptake from the external media. Both the ATP and the glucose phosphate moieties (possible sources of inorganic phosphate) remained constant throughout the period of phosphate accumulation. On washout, it appeared that excess cytoplasmic Pi was shunted off to the vacuole, a phenomena we saw earlier on.

With 2-D-G pretreatment, the cytoplasmic level of ATP and inorganic phosphate both decreased. However, the pH of this compartment remained nearly unchanged. Presumably, the mechanism responsible for the proton gradient present in aerobic cells, was still fully operational. A large influx of inorganic phosphate into the cytoplasm without a concomitant rapid increase in vacuole Pi, indicates that net transport of this anion across the tonoplast was considerably lower under these conditions.

Anaerobic Conditions with 2-D-G Pretreatment

After establishing 2-deoxyglucose pretreatment as a way of more clearly demonstrating the extent of phosphate uptake into cell cytoplasm, we examined the effect of an anaerobic environment on this system. Changes in the mobile phosphate

containing species were measured following anaerobic phosphate uptake and compared with those obtained with anaerobic treatment without phosphate, both following 2-D-G pretreatment. The results are shown in Figure 6.

As seen in Figure 6A, the cytoplasmic phosphate concentration increased significantly upon anaerobic treatment with phosphate buffer, leveling out after about 1.5 h. ATP continued to fall while the sugar phosphate level first increased then rapidly leveled off. The vacuolar phosphate decreased slightly in the absence of external Pi under N_2 (Fig. 6B). In the presence of external Pi, a slight increase in vacuole Pi was noted (Fig. 6A). Thus, the 2-D-G pretreatment followed by anaerobic conditions, brought the cell to a status of having a low level of ATP, a decrease in cytoplasmic pH, an accumulation of Pi in the cytoplasm resulting from the decomposition of phosphorus containing metabolites, and a leakage of Pi from the vacuole (Fig. 6). There was no significant uptake of either external or cytoplasmic Pi into cytoplasm or vacuole, respectively under these conditions.

Effects of Protonophore on Pi Uptake

The results described so far indicated that the uptake and the redistribution of phosphate in corn root cells may be regulated by the level of ATP and the pH value in the cytoplasm. Protonophores, *e.g.* CCCP, are known to stimulate the consumption of high energy substrates by inducing a rapid equilibrium of proton concentration across energy transducing membranes in cells. As shown in Figure 7, the addition of CCCP to aerobic perfusing medium substantially decreased the levels of all NMR observable, phosphorus-containing, high energy compounds and abolished the pH difference between cytoplasm and vacuoles. Consequently, only a broad inorganic phosphate peak was observed. The internal pH appeared to shift to about 6.8 and no visible pH compartmentation was detected. When external phosphate, up to 10 mM, was included in the perfusion medium for up to 2 h, no significant change of intracellular phosphate level was detected. Upon washout, the phosphate signal remained at high level suggesting no or minimal simple diffusion of phosphate from root cells to external solution. Thus, it appears that phosphate uptake requires a CCCP-sensitive energy source and the plasma membrane is not freely permeable to phosphate.

DISCUSSION

One of the significant aspects of phosphate management within the plant root cell to be delineated by ^{31}P NMR is that the level of cytoplasmic Pi appears to be very tightly controlled. For example, both in excised pea roots (6) as well as in suspensions of sycamore cells (17, 19) and *Catharanthus roseus* cells (2), cytoplasmic Pi is maintained at the expense of the vacuolar Pi, excess Pi in the cytoplasm being taken up by the vacuole and the vacuolar Pi pool supplying phosphate to the cytoplasm as required.

In our initial experiments with phosphate uptake under aerobic conditions, this is exactly what was found: while the initial phosphate taken up appeared first in the cytoplasm, it

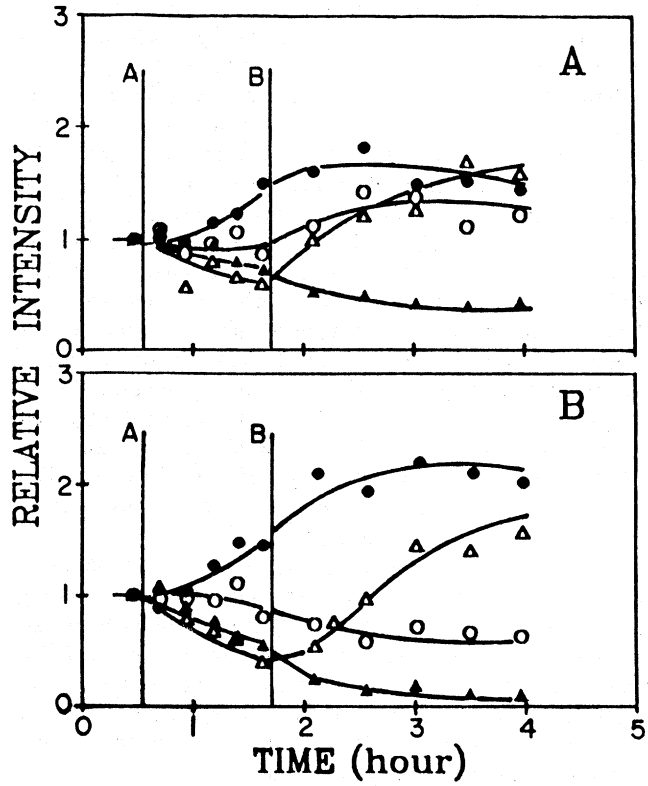

Figure 6. (A) Time course of the relative ^{31}P NMR spectral intensities of species within excised maize roots on pretreatment with 2-D-G, followed by anaerobic phosphate treatment. (○) G-6-P, (△) cytoplasmic Pi, (●) vacuolar Pi, and (▲) ATP. Left of (A) baseline values, perfusate contains only 0.1 mM $CaSO_4$; (A) start of 2-D-G pretreatment, 5 mM with 0.1 mM $CaSO_4$ and oxygen perfusion; (B) end of 2-D-G pretreatment, start of phosphate anaerobic treatment, 10 mM sodium phosphate (pH 6.5) with nitrogen perfusion. (B) same as (A) but perfusate following 2-D-G pretreatment contains no phosphate.

was quickly transported to the vacuole with Pi returning to its initial level and any excess ending up in the vacuole.

Under anaerobic conditions without external phosphate, the energy rich compounds in the cell began to be depleted: ATP, G-6-P, and UDPG levels decreased with a concomitant rapid increase in cytoplasmic Pi as described in (14, 20). When exposed to phosphate, the root tips appeared to take up some external phosphate. The overall net increase seen in the vacuole after aerobic washout was minimal. Therefore, it appears that anaerobic conditions slow down phosphate transport to both cytoplasm and vacuoles in the excised root system.

With the 2-D-G pretreatment, the uptake of phosphate into the cytoplasm under aerobic conditions was very evident from the dramatic increase in cytoplasmic Pi. It was clear that the Pi was not being generated from the mobile phosphate containing moieties since they were either at very low levels (ATP, UDPG) or remained constant (G-6-P, 2-D-G-6-P, UDP-2-D-G). It is interesting that the phosphate influx occurred when the cytoplasmic Pi is at a very low level despite the fact that the level of ATP is also low.

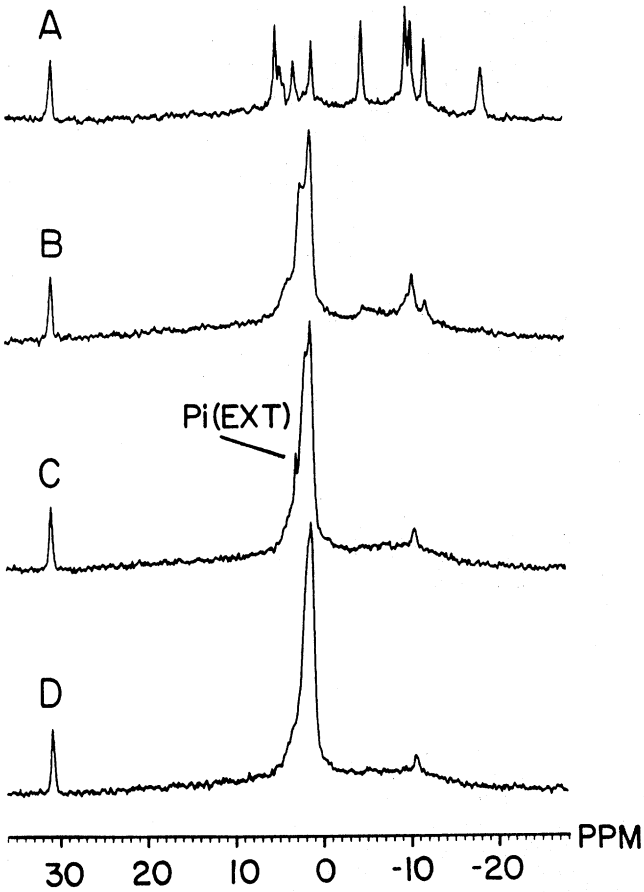

Figure 7. Effects of CCCP on phosphate uptake. After establishing the baseline spectrum (A) under aerobic conditions, 0.2 mM CCCP was added to the perfusion solution (50 mM glucose, 0.1 mM $CaCl_2$, and 2% DMSO, pH adjusted to 7.5). The spectrum changed to (B) 1 h after CCCP addition. External phosphate (0.5 mM, pH 7.5) was then introduced and the spectrum was taken after another hour (C). Fresh perfusion solution was then used to washout added phosphate. Two hours after the washout, the spectrum appeared as (D).

A close examination of the results obtained under various conditions, yielded the following the relationships. Fast uptake through both plasma membrane and tonoplast membrane were noted only when both the ATP level and cytoplasmic pH were high (aerobic conditions) (Figs. 1 and 2). Conversely, the transport across both membranes reached a minimum when both the ATP level and pH of the cytoplasm were low (anaerobic conditions) (Figs. 3, 4, 6, and 7). A low level of ATP but a high cytoplasmic pH allowed the uptake of external Pi to the cytoplasm but hindered the transport of Pi into the vacuole (2-D-G pretreatment followed by aerobic conditions) (Fig. 5). Furthermore, it was also noted that a decrease in the level of cytoplasmic Pi was not an essential requirement for the uptake of external phosphate. Thus, it appears that a lowering of ATP level in the cytoplasma is sufficient to slowdown the net uptake of Pi into the vacuole. However, to significantly decrease Pi transport from external solution to cytoplasm apparently requires a lowered cytoplasmic pH in addition to a low ATP level.

Based on the data mentioned in this report it is not possible to formulate the exact mechanism of Pi uptake in corn roots. However, using the chemiosmotic concept as a guide (15, 16), a working hypothesis to explain our findings, could be developed. We assume that the transmembrane movement of Pi is regulated by the actions of membrane-bound H^+-ATPases of the plasmalemma and tonoplast. Since the direction of proton pumping associated with the plasma membrane ATPase is opposite to that of the tonoplast ATPase, the cytoplasmic compartment has the lowest electric potential and the highest pH comparing to either the external solution or vacuole under the experimental conditions. Accordingly, the observed regulation of phosphate uptake and compartmentation could be achieved as described below.

It is generally agreed that monobasic phosphate ($H_2PO_4^-$) is the preferred anion for moving across hydrophobic membranes (7, 8). Thus, under the aerobic conditions, the outward proton pumping of the plasma membrane H^+-ATPase would favor the formation of $H_2PO_4^-$ and the transport could then be accomplished perhaps through an anion exchanger (Pi − X^-) or a cation symporter (M^+ − Pi). While the transport of monobasic Pi across the tonoplast membrane into the vacuole might be facilitated by the more positive membrane potential in the vacuole, the basic pH of the cytoplasm should favor the presence of dibasic Pi in this compartment. Thus, the observed net Pi uptake in the vacuole could be achieved if the molecular arrangement of the tonoplast membrane (*e.g.* a Pi channel) has a high selectivity toward the dibasic phosphate at the outer surface of vacuole.

The results of 2-D-G pretreatment (Figs. 5 and 6) indicated that a lowering in ATP level had a more pronounced effect on the uptake through the tonoplast membrane. This would be consistent with a suggestion that the activation of the channel for vacuole uptake of Pi requires ATP, *e.g.* phosphorylation of certain membrane signal proteins by a kinase. Thus, a buildup of Pi in the cytoplasm was observed (Fig. 5). Under anaerobic conditions, in addition to a decrease in the ATP level, the pH of cell cytoplasm also decreases. This acidification should increase the concentration of monobasic phosphate (decreasing the concentration of dibasic phosphate) in the cytoplasm and would result in an overall reduction of net phosphate uptake by maize roots.

LITERATURE CITED

1. **Barton JK, Den Hollander JA, Lee TM, Maclaughlin A, Shulman RG** (1980) Measurement of the internal pH of yeast spores by ^{31}P nuclear magnetic resonance. Proc Natl Acad Sci USA **77**: 2470–2473
2. **Brodelius P, Vogel HJ** (1985) A Phosphorus-31 nuclear magnetic resonance study of phosphate uptake and storage in cultured *Catharanthus roseus* and *Daucus carota* plant cells. J Biol Chem **260**: 3556–3560
3. **Jackson PC, Pfeffer PE, Gerasimowicz WV** (1986) Use of ^{31}P NMR to assess effects of DNP on ATP levels *in vivo* in barley roots. Plant Physiol **81**: 1130–1133
4. **Kime MJ, Ratcliffe RG, Williams RJP, Loughman BC** (1982) The applications of ^{31}P nuclear magnetic resonance to higher plant tissue. J Exp Bot **33**: 656–669
5. **Lee RB, Ratcliffe RG** (1983) Development of an aeration system for use in plant tissue NMR experiments. J Exp Bot **34**: 1213–1221

6. **Lee RB, Ratcliffe RG** (1983) Phosphorous nutrition and the intracellar distribution of inorganic phosphate in pea root tips: A quantitative study using ^{31}P-NMR. J Exp Bot **34**: 1222–1244
7. **Lin W** (1979) Potassium and Phosphate Uptake in corn roots. Plant Physiol **63**: 952–955
8. **Lin W** (1980) Corn root protoplasts: isolation and general characterization of ion transport. Plant Physiol **66**: 550–554
9. **Lin W, Hanson JB** (1974) Phosphate absorption rates and adenosine 5′-Triphosphate concentrations in corn root tissue. Plant Physiol **54**: 250–256
10. **Loughman BC, Ratcliffe RG, Schwabe JWR** (1989) Galactose metabolism in Zea Mays root tissues observed by ^{31}P-NMR spectroscopy. Plant Sci **59**: 11–23
11. **Loughman BC, Ratcliffe RG, Southon TE** (1989) Observations on the cytoplasmic and vacuolar orthophospate pools in leaf tissues using in vivo ^{31}P-NMR spectroscopy. FEBS Lett **242**: 279–284
12. **Pfeffer PE, Tu S-I, Gerasimowicz WV, Boswell RT** (1987) Effects of aluminum on the release and-or immobilization of soluble phosphate in corn root tissue. Planta **172**: 200–208
13. **Pfeffer PE, Tu S-I, Gerasimowicz WV, Boswell RT** (1987) Role of the vacuole in metal ion trapping as studied by *in vivo* ^{31}P-NMR spectroscopy *In* B Marin, ed, Plant Vacuoles, Their Importance in Solute Compartmentation in Cells and Their Applications in Plant Biotechnology. Plenum Press, New York, pp 349–359
14. **Pfeffer PE, Tu S-I, Gerasimowicz WV, Cavanaugh JR** (1986) *In vivo* ^{31}P NMR studies of corn root tissue and its uptake of toxic metals. Plant Physiol **80**: 77–84
15. **Pitman MG** (1982) Transport across plant roots. Q Rev Biophys **15**: 481–554
16. **Racker E** (1979) Transport of ions. Account Chem Res **12**: 338–344
17. **Rebeille F, Bligny R, Douce R** (1982) Regulation of Pi uptake by *Acer pseudoplatanus* cells. Arch Biochem Biophys **219**: 371–378
18. **Rebeille F, Bligny R, Martin J-B, Douce R** (1983) Relationship between the cytoplasm and the vacuole phosphate pool in *Acer pseudoplatanus* cells. Arch Biochem Biophys **225**: 143–148
19. **Roberts JKM, Callis J, Jardetzky O, Walbot V, Freeling M** (1984) Cytoplasmic acidosis as a determinant of flooding intolerance in plants. Proc Natl Acad Sci USA **81**: 6029–6033
20. **Roberts JKM, Callis FJ, Wemmer D, Walbot V, Jardetzky O** (1984) Mechanism of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. Proc Natl Acad Sci USA **81**: 3379–3383
21. **Roberts JKM, Jardetzky O** (1981) Monitoring of cellular metabolism by NMR. Biochim Biophys Acta **639**: 53–76
22. **Roberts JKM, Linker CS, Benoit AG, Jardetzky O, Nieman RH** (1984) Salt stimulation of phosphate uptake in maize root tips studied by ^{31}P nuclear magnetic resonance. Plant Physiol **75**: 947–950
23. **Roberts JKM, Ray PM, Wade-Jardetzky N, Jardetzky O** (1980) Estimation of cytoplasmic and vacuolar pH in higher plant cells by ^{31}P NMR. Nature **283**: 870–872
24. **Roberts JKM, Ray PM, Wade-Jardetzky N, Jardetzky O** (1981) Extent of intracellular pH changes during H^+ extrusion by maize root-tip cells. Planta **152**: 74–78
25. **Roberts JKM, Wade-Jardetzky N, Jardetzky O** (1981) Intracellular pH measurements by ^{31}P nuclear magnetic resonance. Influence of factors other than pH on ^{31}P chemical shifts. Biochemistry **20**: 5389–5394
26. **Roberts JKM, Wemmer D, Ray PM, Jardetzky O** (1982) Regulations of cytoplasmic and vacuolar pH in maize root tips under different environmental conditions. Plant Physiol **69**: 1344–1347
27. **Sasaki Y, Okabo A, Murakami T, Arima Y, Kumazawa K** (1987) Radial transport of phosphate in corn roots (II). J Plant Nutr **10**: 1263–1271
28. **Schibeci A, Henry RJ, Stone BA, Brownlee RTC** (1983) ^{31}P NMR measurement of cytoplasmic and vacuolar pH in endosperm cells of ryegrass (*Lolium multiflorum*) grown in suspension culture. Biochem Int **6**: 837–844
29. **Tu S-I, Sliwinski BJ** (1985) Mechanistic investigation on the temperature dependence and inhibition of corn root plasma membrane ATPase. Arch Biochim Biophys **241**: 348–355
30. **Waterton JC, Bridges IG, Irving MP** (1983) Intracellular compartmentation detected by ^{31}P-NMR in intact photosynthetic wheat-leaf tissue. Biochim Biophys Acta **763**: 315–320