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Potassium and Phosphate Uptake in Corn Roots

FURTHER EVIDENCE FOR AN ELECTROGENIC H^+/K^+ EXCHANGER AND AN OH^-/Pi ANTIPORTER[1]

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ABSTRACT

Evidence is presented that K^+ uptake in corn root segments is coupled to an electrogenic H^+/K^+-exchanging plasmalemma ATPase while phosphate uptake is coupled to an OH^-/Pi antiporter. The plasmalemma ATPase inhibitor, diethylstilbestrol, or the stimulator, fusicoccin, altered K^+ uptake directly and phosphate uptake indirectly. On the other hand, mersalyl, an OH^-/Pi antiporter inhibitor, inhibited phosphate uptake instantly but only slightly affected K^+ uptake. Collapse of the proton gradient across the membrane by (*p*-trifluoromethoxy) carbonyl cyanide phenylhydrazone resulted in immediate inhibition of K^+ uptake but only later inhibited phosphate uptake. Changing the pH of the absorption solution had opposite effects on K^+ and phosphate uptake. In addition, a 4-hour washing of corn root tissue induced a 5-fold increase in the rate of K^+ uptake with little or no lag, but only a 2- to 3-fold increase in phosphate uptake with a 30- to 45-minute lag. Collectively these differences strongly support the coupling of an electrogenic H^+/K^+-exchanging ATPase to an OH^-/Pi antiporter in corn root tissue.

Previous studies with excised corn root segments have shown that washing immediately increased K^+ uptake activity (5, 19) and membrane potential (15) with uptake increasing by 3- to 7-fold and the membrane potential increasing to a maximum of 25 mv within 4 h. In contrast to K^+, Pi uptake (13, 16) increases only 2-3-fold after a 30-min lag period. Based on these washing response data and the study of the effect of dithioerythritol on membrane potential, resistance, and proton fluxes (17), Hanson and Lin (7, 17) suggested that active salt transport and its regulation arise from the functioning of a proton-extruding, cation-carrying ATPase in the membrane, balanced by H^+/cation and OH^-/anion antiporters. If the main driving forces, $\Delta\Psi$ and ΔpH generated by electrogenic proton pumps (10) are used for K^+ and Pi uptake, respectively, it should be possible to differentiate the energy-coupling requirements for these two ions either by changing the proton gradient across the membrane or by chemically modifying the transport carrier. Accordingly, several chemical probes such as the ATPase stimulator, FC[2] (18), the inhibitor, DES (2), the OH^-/Pi antiporter inhibitor, mersalyl (8, 16), and uncoupler, FCCP, were applied to corn root segments in an attempt to demonstrate the presence of an electrogenic H^+/K^+-exchanging membrane-bound ATPase and an OH^-/Pi antiporter. The data presented strongly support such a mechanism for active K^+ and Pi uptake in corn roots.

MATERIALS AND METHODS

Corn seeds (*Zea mays* L. B73 × Missouri 17) were germinated and 2-cm (0.5–2.5 cm from the tip) root segments from 3-day-old etiolated seedlings were collected and washed as described by Leonard and Hanson (13). The term "washed tissue" in this paper refers to root tissue washed for 4 h at 30 C in a standard medium of 0.2 mM K^+-phosphate (pH 6.0) plus 0.2 mM $CaCl_2$. Pretreatment with inhibitors was carried out in the same medium for an additional 30 min. Uptake rates were measured by incubating 20 cheesecloth-bound root segments (about 0.3 g of fresh weight) for 30 min (except those in the kinetic studies as noted in figures) in 100 ml of either washing solution labeled with 10,000 cpm/ml of carrier-free ^{32}P in standard medium or ^{86}Rb in 0.2 mM KCl and $CaCl_2$ solution at pH 6.0. The incubation temperature for uptake was kept at 30 C. Washing, pretreatment, and absorption solutions were held in a water bath with constant shaking (50 cycles/min) and forced aeration. Since tissues that have been washed for 4 h, or more, are "stable" in their physiological activities (7, 20), most of the experiments were conducted using washed tissues.

In the pH experiments 1 mM Hepes was added to the absorption solution and the pH was adjusted with HCl or NaOH. For proton flux measurements, 80 root segments (about 1.2 g fresh weight) were placed directly in 200 ml of aerated standard medium at 30 C and the pH of the solution was monitored continuously. Net proton flux rate at steady-state was calculated as described by Lin and Hanson (15). Respiration of root tissue was determined with an O_2 electrode at 30 C with 10 1-cm root sections in 10 ml of air-saturated standard medium as previously described (17).

FC was a generous gift from Dr. E. Marrè, and DES, mersalyl, and PCMBS were purchased from Sigma Chemical Co. All other chemicals were ACS reagent grade.

RESULTS AND DISCUSSION

Aging of plant tissue by washing enhances many physiological activities such as ion uptake, respiration, and cell membrane potential (7, 20). In the present study, washing corn root segments in aerated K-phosphate and $CaCl_2$ solutions increased the rate of K^+ (^{86}Rb) uptake by 5-fold with little or no lag and the rate leveled off after 4 h. In contrast, washing increased phosphate uptake by only 2- to 3-fold during the same 4-h period with an initial lag of 30 to 40 min. These results agree well with previous studies on the stimulation of ion uptake by washing (5, 13, 16, 19). Kinetic studies reveal a linear increase in accumulation of both ions for at least 60 min in washed tissue, while in fresh tissue the kinetics response was complicated by the washing response with a nonlinear increase in uptake with time. The difference in the response of

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[2] Abbreviations: DES: diethylstilbestrol; FC: fusicoccin; FCCP: (*p*-trifluoromethoxy) carbonyl cyanide phenylhydrazone; PCMBS:*p*-chloromercuribenzenesulfonic acid; PD: electrical potential difference between vacuole and external solution.

K^+ and Pi uptake to the washing suggests that different pathways are involved in the transport of these two ions.

Based on the H^+/K^+- and OH^-/Pi-exchanging mechanism proposed by Hanson and Lin (7, 17), a change in the external pH should have opposite effects on K^+ and Pi uptake. When K^+ and Pi uptakes were measured independently as a function of pH, K^+ uptake increased with increasing pH whereas Pi uptake decreased (Fig. 1). The increase in K^+ uptake could be explained by either a reduction in proton back pressure thereby facilitating proton efflux and K^+ influx, or, alternatively, the rise of pH from 4 to 8 could increase plasmalemma H^+/K^+ ATPase activity (14). Two obvious possibilities also exist for the opposite effect on Pi uptake. A rise in pH or OH^- concentration could increase the back pressure to OH^- efflux and thus decrease the Pi influx rate, or the increasing pH could change the degree of Pi ionization, thereby changing the Pi species in solution, and in turn, decrease Pi uptake. In corn root segments (17) and other higher plant cells (10, 12) cell PDs were found to be stable with changing external pH. The difference in the effect of external pH on PD and ion uptake suggests that the change in K^+ and Pi uptake with pH is balanced by the change in H^+ and OH^- efflux, therefore, no net charge is accumulated and consequently PD is not altered. This further supports the involvement of an exchanging mechanism for K^+ and Pi uptake.

To test the exchanging theory further (7, 17), several proposed site-specific chemical probes were used in the following experiments. In oat roots, Balke and Hodges (2) found that DES inhibits a plasmalemma ATPase and oxidative phosphorylation, but not the mitochondrial ATPase. Figure 2 shows the effect of DES on ion uptake in washed corn root tissue. At low DES concentrations (<10 μM), uptake of K^+ and net efflux of H^+ were strongly inhibited, while Pi uptake was essentially unaffected. Furthermore, the I_{50} for DES was 10 μM for both K^+ uptake and net H^+ efflux and 35 μM for Pi uptake. DES concentrations of above 50 μM strongly inhibited both K^+ and Pi uptake but the maximum degree of inhibition was greater for K^+ than Pi. A plausible explanation for this differential effect of DES could be that DES at low concentration inhibited only plasmalemma ATPase-mediated K^+/H^+ exchange (2, 10, 18) without affecting nonplasmalemma ATPase-mediated Pi uptake (8, 11, 16, 17). The inhibitory effect of DES at high concentrations on Pi uptake could be due to the inhibition of ATP production and utilization inside the cells (2).

The kinetic studies of the effect of DES on ion uptakes (Fig. 3) showed that K^+ uptake was inhibited instantly after the addition of 50 μM DES. However, the inhibition of Pi uptake occurred only after the tissue had been exposed to a higher concentration for more than 15 min and presumably after the cellular energy metabolisms were inhibited (2). The fact that the ATP level (3) and respiration rate (data not shown) did not change significantly during the first 10 min after the addition of DES in corn root tissue suggests that DES at 50 μM is not acting as a conventional uncoupler during this initial period (2). Additionally instantaneous inhibition of net H^+ efflux was found in the same tissue. These data suggest that at low DES concentration, DES may be affecting the H^+/K^+-exchanging ATPase of the proposed model (7, 17) but not the OH^-/Pi antiporter.

In oat roots, the inhibition by DES of anion (Cl^-) uptake was always greater or equal to that of K^+ uptake (2); however, in the present study using Pi as the anion, this was not the case. One could explain this difference by assuming that not all anions are taken up by the same mechanism. In this regard, electrogenic Cl^- pumps in plant tissue have been suggested (1). Thus, the difference in the effect of DES on Cl^- and Pi could be explained if Cl^-, like K^+, is taken up by a DES-sensitive exchanging ATPase, while Pi uptake occurs by a DES-resistant OH^-/Pi antiporter which is not an ATPase.

The uncoupling of ion transport (11, 16) and electrogenic potential (10, 15) by uncoupling agents reflects the collapse of the

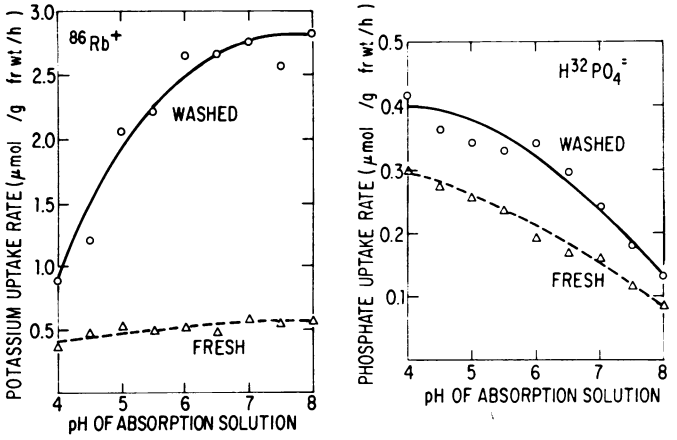

FIG. 1. Effect of pH on K^+ ($^{86}Rb^+$) and phosphate ($H^{32}PO_4^-$) uptake.

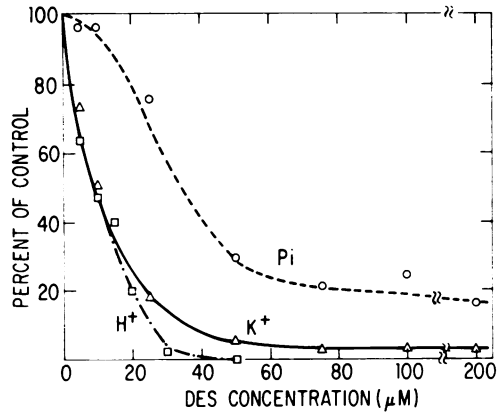

FIG. 2. Effect of DES on net proton efflux (□—·—□), K^+ uptake (△——△), and Pi uptake (○- - -○). Control rates were 1.26, 2.48, and 0.43 μmol/g fresh weight·h for H^+ efflux, K^+, and Pi uptake, respectively. Thirty-min pretreatment was used to equilibrate the tissue with DES or other inhibitors (below) and K^+ or Pi uptake was followed immediately in the presence of the same inhibitor concentration. I_{50} was 10 μM for K^+ uptake and H^+ efflux, and 35 μM for Pi uptake.

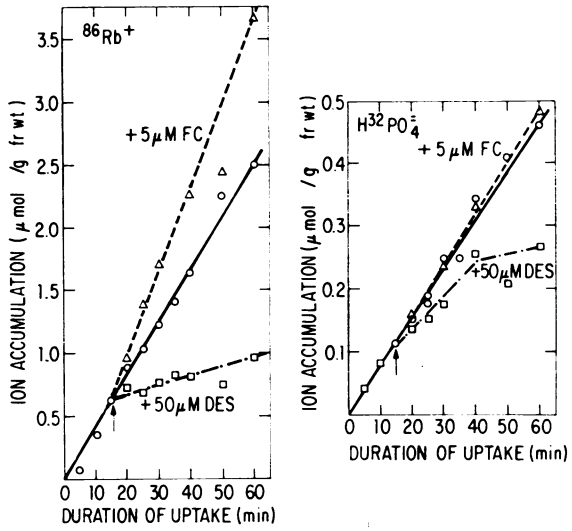

FIG. 3. Kinetics of effect of DES and FC on K^+ ($^{86}Rb^+$) and Pi ($H^{32}PO_4^-$) uptake. FC and DES were added at the time pointed by arrows.

H^+ gradient across the membrane (7, 16). Theoretically, the degree of the dependence of the two exchanging components in the proposed exchange mechanism to the H^+ gradient generated by the electrogenic H^+ pump can be estimated by the effect of the uncoupling agents on K^+ and Pi uptake. The uncoupler FCCP caused a differential inhibition of K^+ and Pi uptake (Fig. 4). The I_{50} for FCCP were 0.5, 1.0 and 1.5 μM for net H^+ efflux, K^+ and Pi uptake, respectively. The data support the proposed exchange mechanism and further suggest that K^+ uptake is more direct linked to the H^+ pump than that of Pi uptake.

FC, a fungal toxin which binds to the plasmalemma ATPase and is hypothesized to stimulate electrogenic H^+/K^+ exchange (18), rapidly stimulated K^+ uptake (Fig. 3) and doubled the net H^+ efflux rate (1.54 *versus* 3.05 μmol H^+/g fresh weight·h) within 20 min. However, at the same concentration, Pi uptake was either not affected or only slightly stimulated (Fig. 3). The extent of this stimulation was only 10% at 10 μM FC after 30 min. Because of the limited supply of FC, it was not possible to conduct a concentration study with FC analogous to that done with DES and FCCP; however, judging from the kinetic data, K^+ and Pi uptake would also be affected differently by FC concentration. These results show that FC differentially affects K^+ and Pi uptake and in a manner consistent with the H^+/K^+- and OH^-/Pi-exchanging mechanism.

Based on these studies with chemical probes, it is suggested that active K^+ influx and H^+ efflux are mediated by an active H^+/K^+ exchange ATPase, which can be separated from the Pi uptake component. According to the proposed exchange mechanism, it should also be possible to use Pi transport site-specific chemical modifier(s) to differentiate the two components. Therefore, the following experiments were conducted.

At low concentrations, the water-soluble sulfhydryl-binding mercurial, mersalyl, inhibits Pi transport in corn mitochondria (8), presumably by blocking the OH^-/Pi antiporter of the inner mitochondrial membrane. Furthermore, in corn root tissue mersalyl does partially inhibit Pi uptake (16) and enhance net proton efflux (15), but does not appear to affect the ATP level (16). In this study exposure of corn root segments to 10 μM mersalyl resulted in an immediate, but transitional, inhibition of Pi uptake, while K^+ uptake was not affected (Fig. 5). At the same concentration mersalyl did increase the net H^+ efflux within 30 min (data not shown). On the other hand, when tissues were exposed to mersalyl at higher concentrations for a longer time, both Pi and K^+ uptakes were inhibited but to a different extent (Fig. 6). The differential response of K^+ and Pi uptake to mersalyl which is opposite to that of DES, FCCP or FC, suggests that Pi uptake can be unhibited while K^+ uptake is unaffected and further supports the H^+/K^+ and OH^-/Pi exchange mechanism.

The transitional inhibition of Pi uptake (Fig. 5) could be explained by assuming that there is more than one Pi transport system in the plasmalemma, as has been suggested in mitochondria (4), with mersalyl possibly inhibiting only the OH^-/Pi site. When the OH^-/Pi site is inhibited, other Pi transport systems (4) might be turned on resulting in a partial recovery of Pi uptake. Other —SH reagents such as PCMBS did not differentially inhibit K^+

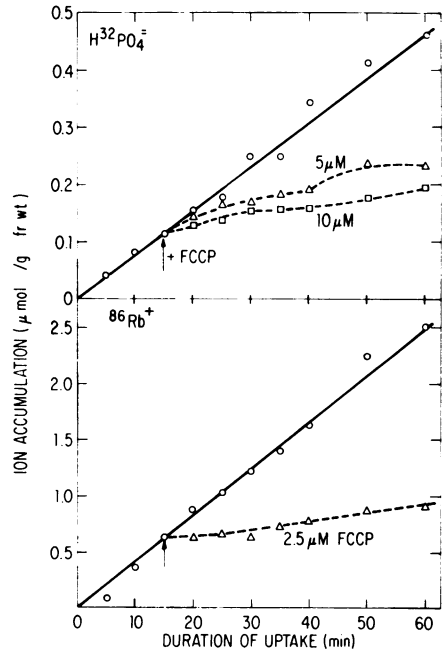

FIG. 4. Kinetics of effect of FCCP on K^+ ($^{86}Rb^+$) and Pi ($H^{32}PO_4^-$) uptake. FCCP was added at the time pointed by arrows. At 2.5 μM, FCCP had no or very little effect on Pi uptake and therefore this curve is not shown.

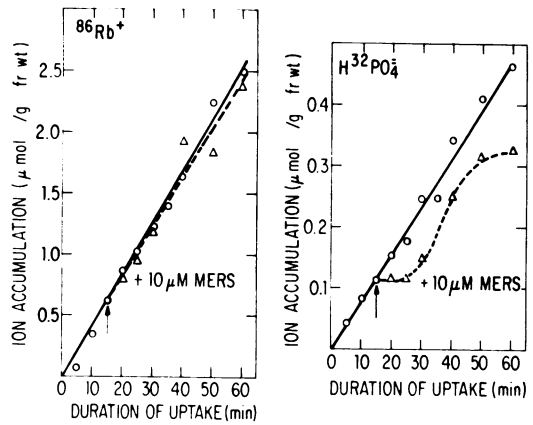

FIG. 5. Kinetics of effect of MERS (mersalyl) on K^+ ($^{86}Rb^+$) and Pi ($H^{32}PO_4^-$) uptake. Mersalyl was added at the time pointed by arrows.

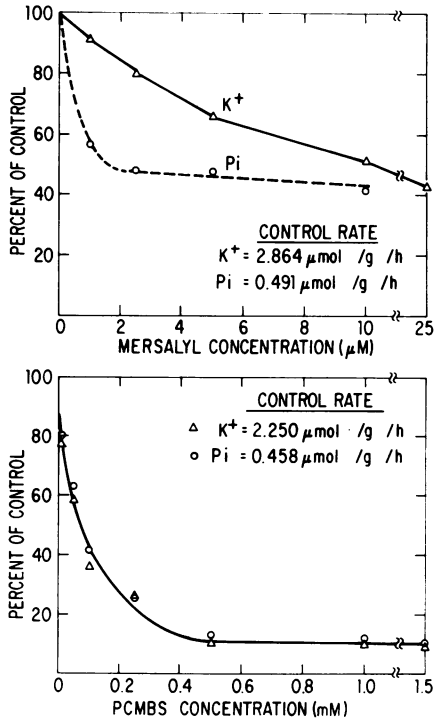

FIG. 6. Effect of mersalyl (upper panel) and PCMBS (lower panel) on K^+ ($^{86}Rb^+$) and Pi ($H^{32}PO_4^-$) uptake. Thirty-min pretreatment was added in K^+ and Pi uptake measurement (see Fig. 2 legend).

and Pi uptake (Fig. 6). Net H^+ efflux was strongly inhibited by PCMBS but increased by mersalyl although no immediate (0–15 min) change in respiration rate was found when tissue was treated with either 0.2 mM PCMBS or 20 μM mersalyl. The discrepancy in the effect of mersalyl and PCMBS on K^+ and Pi uptake is not surprising since unlike mersalyl, PCMBS has been reported to inhibit plasmalemma ATPase both *in vivo* (6) and *in vitro* (9). Mersalyl may affect only the —SH groups of the OH^-/Pi antiporter whereas PCMBS has an additional inhibitory effect on the H^+/K^+-exchanging ATPase.

CONCLUSION

The present study supports the coupling of an electrogenic H^+/K^+-exchanging ATPase to an OH^-/Pi antiporter in corn root tissue. Initially several treatments such as a change in pH or the addition of inhibitors or stimulators of uptake were found to affect one uptake component without affecting the other. However, longer treatments tended to affect both components. This is to be expected since both uptake processes are metabolically dependent and closely related to the transmembrane proton gradient and therefore any marked change in one component would also be expected to affect the other indirectly.

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