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Effect of pH on Orthophosphate Uptake by Corn Roots[1]

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ABSTRACT

Orthophosphate (Pi) influx in washed corn roots was studied with experimental conditions allowing a distinction of pH effects on Pi ionization in the medium and on the transport system itself. There appeared to be no relationship between the pH dependencies of membrane potential, H^+ secretion, and ^{32}Pi influx. The Pi uptake *versus* pH curves were compared to the calculated ones describing the concentrations of the different ionized Pi forms in the medium and in the cell walls; the latter were obtained using the theoretical model described by Sentenac and Grignon (1981 Plant Physiol 68: 415–419). The conclusion was that the transported form is $H_2PO_4^-$ and the concentration sensed by the transport system is the local one. The ionic compositions of experimental media were manipulated to ensure constant pH and various $H_2PO_4^-$ concentrations, or constant $H_2PO_4^-$ concentration and various pH values in the walls. The kinetic analysis of the results in the micromolar range showed that the transport system has an intrinsic sensitivity to pH, and is switched from a low activity state at pH > 6 to a high activity one at pH < 4 (pH in the walls). This change could be triggered by the protonation of a group with pK 5.5.

For Pi (2) as for other ions (10), the influx kinetics are complex when examined in an extended concentration range (0 to a few 10s mM). In the μM range, which corresponds to the normal natural conditions in cultivated soils (1), they are generally hyperbolic (2, 14, 24), with a K_m about 5 μM.

Pi uptake is thought to involve the $H_2PO_4^-$ form. This conclusion has been mainly suggested by the observation of a good correspondence between the effects of pH on Pi uptake rate and on the relative concentration of the $H_2PO_4^-$ form (2). The more striking correspondence has been observed with barley, but it was not absolute (23). The proposed explanation was that the pH at a charged surface is different from the bulk one. However, with the same species, the hypothesis that $H_2PO_4^-$ is the transported form was at variance with the fact that the kinetics of Pi uptake *versus* Pi concentration in the μM range were monophasic at pH 4, 5, 6 but became biphasic when the pH and, consequently, HPO_4^{2-} concentration were increased (11); the proposed explanation was the existence of two different transport systems specific for $H_2PO_4^-$ and for HPO_4^{2-}, respectively.

Apart from fixing the concentration of the transported form, pH may affect Pi uptake in two ways. First, the transport may be kinetically controlled via the intrinsic sensitivity of the carrier to pH. Second, a thermodynamic control by pH may be expected if Pi is cotransported with H^+. Pi uptake by corn roots is dependent upon metabolic energy but does not seem to be directly linked to ATP concentration in tissues (18). It is stimulated by acidification of the external medium and inhibited by mersalyl, a sulfhydryl reagent known to block the Pi/H^+ cotransport (or Pi/OH^- antiport) of the inner mitochondrial membrane (16). This set of data is the main experimental support of the hypothesis of a Pi/H^+ cotransport on the plasma membrane in corn roots. The stoichiometry of this hypothesized symport is unknown. The strong hyperpolarization observed in white clover (4, 9) and sunflower (5) during Pi uptake may be the sign of an unbalanced stoichiometry ($H_2PO_4^-/H^+ > 1$), although it may also be due to the acceleration of the electrogenic proton pump in response to H^+ entry, or even to a primary electrogenic Pi pump (3). In *Lemna*, the plasma membrane is depolarized during Pi uptake (22). This would indicate that the symport mediates the transfer of a net positive charge in this species.

We investigated the pH dependence of Pi uptake by corn roots. We took into account the fact that plasma membranes of root cells do not directly perceive the ionic conditions of the external solution. Instead, they sense a modified medium, the ionic composition of which is affected by electrostatic interactions between the external solution and the cell wall fixed charges (21). We chose the experimental conditions in order to identify the possible different effects of pH on Pi transport.

MATERIALS AND METHODS

Plant Material. Roots were obtained from 5-d-old corn seedlings (*Zea mays* L. var INRA 508) grown in the dark at 25°C on aerated 0.2 mM $CaSO_4$. Three-cm segments of primary roots were taken 1 to 4 cm from the tip and washed for 12 h in aerated, frequently renewed 2 mM $CaSO_4$.

Composition of the Media. Pi absorption solutions contained 2 mM $CaSO_4$ and 0 to 2 mM KH_2PO_4. K^+ concentration was fixed at 2 mM by adding K_2SO_4. The pH was adjusted at various values between 3.00 ± 0.02 and 8.00 ± 0.02 with H_2SO_4 or $Ca(OH)_2$ and maintained with the help of a pH-stat system (see below).

Flux Experiments. Pi influx was measured by incubating 30 root segments (about 1 g fresh weight) for 5 min in 40 ml of a solution labeled with ^{32}Pi (1.7 $MBq \cdot \mu mol^{-1}$). The experimental device was a modified Büchner funnel with a porous fritted glass plate. Vigorous bubbling was assured by injecting CO_2 free air through the plate. After incubation, the solution was pulled under vacuum, and the root segments were rinsed for 3 min with flowing 2 mM $CaSO_4$. They were blotted, weighed, and treated for 24 h with toluene-ethanol-water (1-4-95, v/v/v) (24). The extracts were assayed for ^{32}P with a scintillation counter using the Cerenkov effect.

The rates of net H^+ exchanges were measured with the same device, from the recorded automatic delivery of 0.01 N $Ca(OH)_2$ or 0.01 N H_2SO_4 (Metrohm pH-stat).

In some experiments, the effects of root pretreatment by rose bengal or iodoacetate on Pi uptake and O_2 consumption were investigated. In the case of the rose bengal experiments, the roots

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were pretreated for 15 min with solutions containing 25 mM K_2SO_4, 0.2 mM $CaSO_4$, 50 mM Tris-H_2SO_4 (pH 8.3), with or without 10 mg·l^{-1} rose bengal (Sigma). These solutions were vigorously bubbled and exposed to a white light source (Osram, HQIT 250 w). In the experiments with iodoacetate, the roots were pretreated for 1 min with aerated solutions containing 10 mM K_2SO_4, 2 mM $CaSO_4$, 2 mM Mes-KOH (pH 5.6), with or without 20 mM K^+-iodoacetate. At the end of the pretreatments, the roots were rinsed for 3 min with flowing 2 mM $CaSO_4$ and then were kept for 12 min in this solution. Pi influx was measured in 2 mM KH_2PO_4 plus 2 mM $CaSO_4$ and 5 mM Mes-KOH (pH 5.6). O_2 consumption was manometrically determined in the same medium (Warburg apparatus).

Membrane PD[2] Measurements. The micro salt-bridges were made from Clark E.I. GC200F glass tubes. They were filled with 3 M KCl and selected for low tip potential and impedance about 10 MΩ, and connected to a high impedance electrometer (WPI 725) via Ag-AgCl electrodes. The washed root segments were maintained in a plexiglass cell under flowing 2 mM $CaSO_4$. A micro salt-bridge was inserted under microscopic observation in an outer cortical cell with the help of an automatic step-to-step micromanipulator (Narishige MO 81). Data shown are steady values of membrane PD reached within 5 min after changing the washing 2 mM $CaSO_4$ solution for the measurement medium.

Ion Distribution in Cell Walls. The cell walls of root segments were isolated with Triton X-100 and equilibrated for 12 h with $CaCl_2$ plus KCl or $MgCl_2$ as described elsewhere (21). Ions were extracted with 0.1 N HCl and assayed by flame photometry.

Calculations. The equilibrium concentrations of the various ionic species in the solutions were calculated from the appropriate mass action law expressions using the following parameters: pK values of the first two H_3PO_4 functions, 2.12 and 7.20; Ca^{2+} dissociation constants for $CaH_2PO_4^+$, $CaHPO_4$, and $CaSO_4$, 83 mM (8), 6.3 mM, and 5.12 mM (20), respectively. The concentrations inside the walls were calculated using our theoretical model (21). This model describes the ionic atmosphere in the cell walls as a result of simultaneous Donnan interactions between free ions and fixed charges, and specific association equilibria. The calculations were performed with a 64 kbytes computer.

RESULTS

Ion Distribution in Cell Walls. Isolated root cell walls were used to estimate ionic concentrations at the cell surface. For experimental reasons, it is more difficult to measure anion exclusion than cation accumulation in cell walls. Direct measurements of Pi in equilibrium conditions were not possible. For this reason, ionic conditions in the cell walls were first determined from Ca^{2+}-K^+ and Ca^{2+}-Mg^{2+} exchange experiments, which revealed a slight selectivity in favor of Ca^{2+} (Fig. 1). However, the accumulation ratios for K^+ were close to the square roots of those for Ca^{2+} and the discrimination between Ca^{2+} and Mg^{2+} was not important. Taking into account this slight selectivity is not worthwhile for the subsequent work and corn cell walls are therefore described as an ideal Donnan system in which selectivity for ions other than H^+ is purely valency dependent. A quantitative fit to the experimental ionic exchange data by the Donnan model was used to determine the values of the structural parameters (21). These values were 105 μeq·g^{-1} cell wall dry weight for the fixed anionic sites, 3.2 for their pK, and 0.45 ml·g^{-1} cell wall dry weight for the volume of the polyelectrolytic phase. The theoretical curves are plotted in Figure 1. The fact that the cell wall K^+ content in the absence of Ca^{2+} is only 50 μmol·g^{-1} dry weight (Fig. 1A) is because about 50% of the sites are still occupied by protons. The theoretical curves are straight lines in Figure 1B because the cell wall selectivity in favor of Ca^{2+} was neglected. The above values of the structural parameters were introduced in the model (21), which was then applied to the various experimental conditions selected for Pi influx measurements. This procedure gave the local theoretical concentrations of the ions implied in the functioning of the Pi transport system.

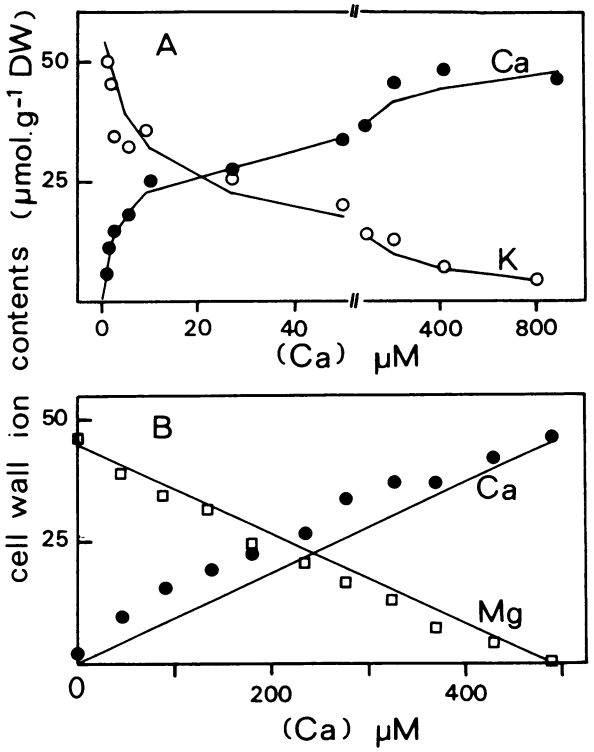

FIG. 1. Cation exchanges in corn cell walls isolated from 3-cm (1–4 cm from the tip) primary root segments. A, K^+-Ca^{2+} exchange isotherms; the media contained 1 mM KCl with varying concentrations of $CaCl_2$ (pH 5.5). B, Ca^{2+}-Mg^{2+} exchange; the media contained $CaCl_2$ + $MgCl_2$, total concentration 500 μM (pH 5.5). The lines join the theoretical points obtained using a model of the ionic atmosphere in cell walls (Sentenac, Grignon 1981 Plant Physiol 68: 415–419); values of the structural parameters of the model: 105 μeq·g^{-1} cell wall dry weight for the fixed anionic sites, 3.2 for their pK, and 0.45 ml·g^{-1} cell wall dry weight for the volume of the polyelectrolytic phase. These values are used in the subsequent work for computing the theoretical data.

Pi Influx Preliminary Experiments. ^{32}Pi absorption by 1 or 3 cm root segments taken at various distances from the tip was measured. The higher absorption rates were found between 0 and 4 cm from the tip, irrespective of the Pi concentration used. The effect of washing time on ^{32}Pi absorption at 40 μM, 200 μM, and 2 mM was examined for 36 h. The classical enhancement of Pi influx (15) was observed during the first 10 h. The influx then remained constant or slowly decreased. The presence of 5 mM glucose had no effect. It was verified that ^{32}Pi absorption was linear with time for more than 5 min in the various pH conditions and Pi concentrations used in this work.

Effects of Pi and pH on Membrane PD and Net Proton Transport. Membrane PD and net H^+ transport were measured at various pH values in 2 mM $CaSO_4$, or 2 mM $CaSO_4$ plus 1 mM K_2SO_4, or 2 mM $CaSO_4$ plus 2 mM KH_2PO_4 (Fig. 2; Table I). Pi induced a slight hyperpolarization. An increase in pH was found to hyperpolarize the membrane and to accelerate the net H^+ transport, from a net influx below pH 4.5 to a net efflux above this value. Such a pH dependency was described by Lin and Hanson (19) for H^+ transport but not for PD. They found that membrane PD was independent of pH between pH 4 and 7.

[2] Abbreviation: PD, potential difference.

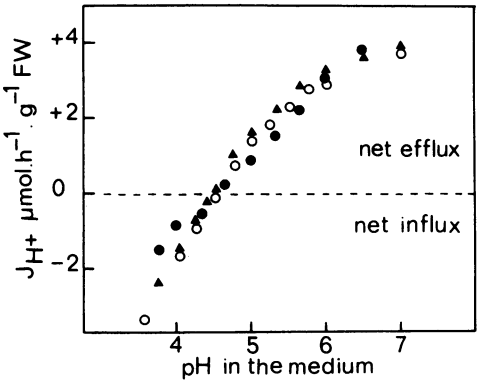

FIG. 2. Effect of Pi and of pH on net H^+ transport by corn roots. The media contained 2 mM $CaSO_4$ (○), 2 mM $CaSO_4$ + 1 mM K_2SO_4 (▲), or 2 mM $CaSO_4$ + 2 mM KH_2PO_4 (●). The pH was adjusted with H_2SO_4 or $Ca(OH)_2$ and a Metrohm pH-stat system. Root segment preparation: see the caption of Table I.

Table I. *Effect of Pi and of pH on Membrane PD of Corn Root Cells*

Three-cm (1–4 cm from the tip) segments of primary roots were washed for 12 h in 2 mM $CaSO_4$. A micro salt-bridge was inserted in an outer cortical cell and steady membrane PD was measured within 5 min after changing the washing solution for the measurement medium. A, the measurement media contained 2 mM $CaSO_4$; B, 2 mM $CaSO_4$ + 1 mM K_2SO_4; C, 2 mM $CaSO_4$ + 2 mM KH_2PO_4. The pH was adjusted with H_2SO_4. The values of membrane PD (mv) are the mean of four or five values (given with their 95% confidence limits).

Treatment	Membrane PD at Following pH in the Medium			
	3	3.75	4.75	5.75
			mv	
A ($CaSO_4$)	−58 ± 7	−67 ± 6	−95 ± 10	−138 ± 15
B ($CaSO_4$ + K_2SO_4)	−30 ± 5	−39 ± 5	−64 ± 4	−76 ± 9
C ($CaSO_4$ + KH_2PO_4)	−37 ± 6	−52 ± 7	−72 ± 6	−86 ± 6
C − B	−7	−13	−8	−10

Effect of pH on Pi Uptake. The dependency of ^{32}Pi influx on pH at 40 μM and 2 mM external Pi (Fig. 3) was compared with the calculated curves describing the relative concentrations of the different forms of Pi in the external solution (Fig. 4A) and in the cell walls (Fig. 4B). The differences between the $H_2PO_4^-$ curves in Figure 4, A and B, will be discussed later. Clearly, the shape of the experimental influx curves is different from that of any curve of Pi forms in the medium, but is reminiscent of the curve for $H_2PO_4^-$ in the walls.

Effect of Pi Concentration on Pi Uptake at Various pH Values. ^{32}Pi influx was measured at pH 3, 4, 5, and 7 for concentrations of the $H_2PO_4^-$ form in the medium ranging from 0 to about 60 μM. When plotted *versus* this concentration (Fig. 5A), the results were in accordance with those of Figure 3: the absorption rates were highest at pH 3, similar to each other at pH 4 and 5, and lowest at pH 7. When the calculated concentrations of $H_2PO_4^-$ in the cell walls were taken into account (Fig. 5B), the isotherms were quite similar at pH 3, 4, and 5. Hence Pi uptake appeared to be closely dependent upon local $H_2PO_4^-$ concentration, unaffected by variations in pH in the range pH 3 to 5, but reduced at pH 7.

Effect of pH in the Walls on Pi Uptake at Constant $H_2PO_4^-$ Local Concentration. The values of pH and of total Pi concentration in the external media were chosen so as to obtain theoretical pH values in the walls varying from pH 3 to 7 and simultaneously a constant theoretical local concentration for $H_2PO_4^-$ (Table II). This latter was fixed about 2.5 μM, since the

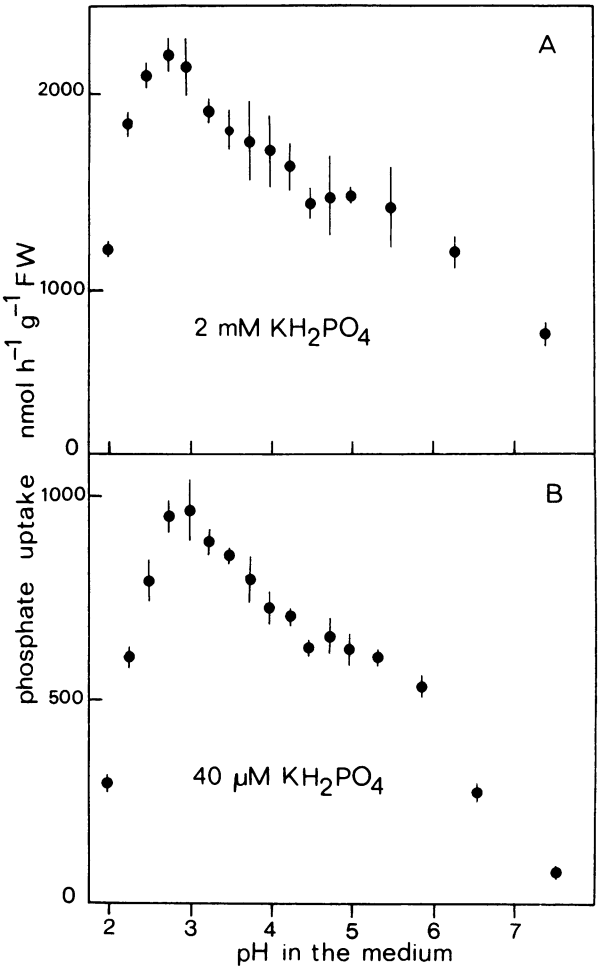

FIG. 3. pH dependency of ^{32}Pi uptake by corn root segments. Media contained: A, 2 mM KH_2PO_4 + 2 mM $CaSO_4$; B, 40 μM KH_2PO_4 + 2 mM $CaSO_4$ + 0.98 mM K_2SO_4. The pH was adjusted and maintained with H_2SO_4 or $Ca(OH)_2$ and a pH-stat system. For root segment preparation, see the caption of Table I; 5 min incubation; mean values of five experiments and 95% confidence limits.

absorption isotherms showed a quasi-plateau at this value (Fig. 5B). The results (Fig. 6) are plotted as a function of the external medium pH (curve A), and of the pH in the walls (curve B). This representation reveals the direct effect of H^+ on Pi uptake system since concentration of the transported Pi form was constant. The curves presented a sigmoid shape, reminiscent of titration of an ionized group with a pK near 6.4 (curve A) or 5.5 (curve B). Among amino acids, only histidine has a group with a pK close to these values. Root segments were pretreated with classical histidine reagents (7, 26). Rose bengal and iodoacetate reduced Pi uptake at pH 5.6 by 47% and 38%, respectively. In the same conditions, O_2 consumption was reduced by 14% (rose bengal) and 50% (iodoacetate).

DISCUSSION

The kinetic analysis of ^{32}Pi influx as a function of the total Pi concentration in the medium cannot give the intrinsic characteristics of the Pi transport system for several reasons. First, if only one of the different ionized forms is transported, it would be necessary to use the concentration of this form as the variable. Second, care should be taken of the fact that local concentrations rather than bulk ones are probably of significance for the transport. Furthermore, some important parameters such as ionic

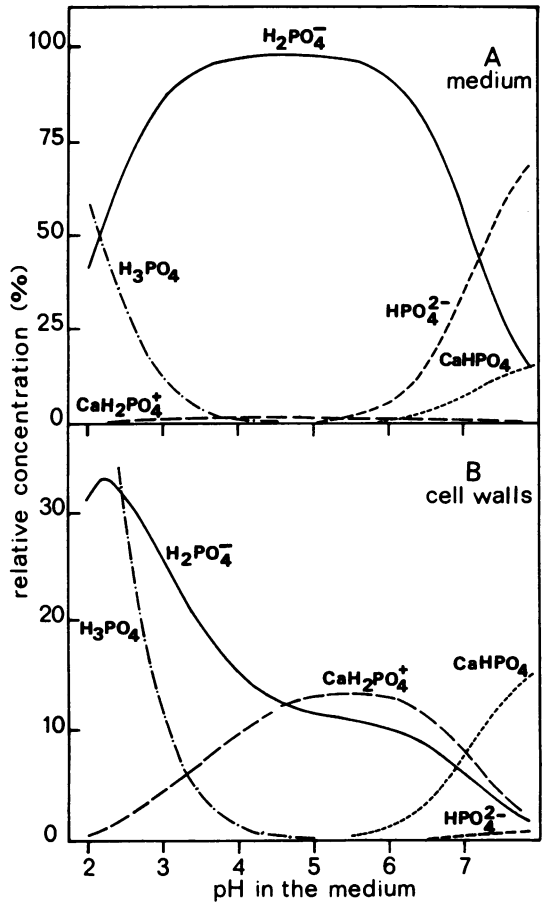

FIG. 4. Effect of pH on the concentrations of the different Pi forms in the medium and in the walls, expressed as per cent of Pi concentration in the medium (100%). A, Medium: it contains 40 μM KH_2PO_4, 2 mM $CaSO_4$, and 0.98 mM K_2SO_4; pK values for H_3PO_4 and $H_2PO_4^-$, 2.12 and 7.2; dissociation constants for $CaH_2PO_4^+$, $CaHPO_4$, and $CaSO_4$ are 83 mM, 6.3 mM, and 5.12 mM, respectively. B, Cell walls at equilibrium with the medium; theoretical model of the cell wall ionic atmosphere and values of the parameters: see the caption of Figure 1. Changing KH_2PO_4 concentration from 40 μM to 2 mM induces only slight changes in the relative concentrations.

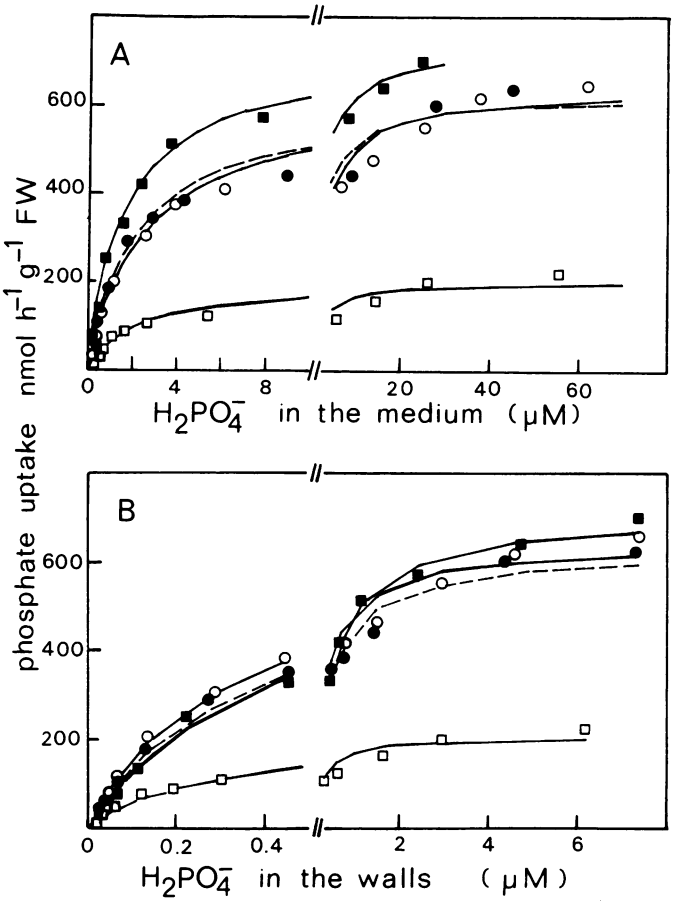

FIG. 5. Kinetics of ^{32}Pi uptake by corn root segments at various pH. The data are plotted *versus* $H_2PO_4^-$ concentration in the medium (A) or in the walls (B); computations of $H_2PO_4^-$ concentrations: see Figure 4. Lines: Michaelis-Menten hyperbolae obtained by direct least-square adjustments; the values of J_{max} and K_m are listed in Table III. The absorption media contained 0 to about 60 μM KH_2PO_4, 2 mM K^+ (adjusted with K_2SO_4), and 2 mM $CaSO_4$, pH 3 (■), pH 4 (●, dashed line), pH 5 (○), or pH 7 (□), adjusted and maintained with H_2SO_4 or $Ca(OH)_2$ and a pH-stat system. Root segment preparation: see the caption of Table I; 5 min incubation; means of six experiments.

strength, K^+-stimulated proton extrusion, and electrical transmembrane PD should be maintained as constant as possible in the full range of the tested Pi concentration. By the same token, the available data on pH dependency of Pi uptake (9) were not obtained in experimental conditions authorizing a precise distinction between the effects of pH on Pi dissociation and on the transport system itself.

In the experiments reported here (Figs. 2, 3, 5, and 6; Table I), the ionic strength was between 10 and 13 mM and the Ca^{2+} and K^+ concentrations were maintained close to 2 mM. Neither the net H^+ flux nor the membrane PD were markedly affected by the Pi concentration. This latter point is exemplified by Figure 3 and Table I which show the effects of Pi observed at the highest used Pi concentration (2 mM). Furthermore, the pH dependency of Pi uptake (Fig. 3) is related neither to the effects of pH on PD (Table I), contrary to what was observed with white clover (9), nor to those on H^+ transport (Fig. 2). Thus, in these experiments, it is unlikely that pH controls Pi uptake via its effects on the thermodynamic electrical constraint or on the H^+ delivery by the proton pump.

When compared with the calculated curves on Figure 4, A and B, the response of Pi influx to pH (Fig. 3) suggests both that $H_2PO_4^-$ is the transported form and that the concentration in cell walls is the effective one. The curves describing the $H_2PO_4^-$ concentration in the medium (Fig. 4A) and in the walls (Fig. 4B) as a function of the pH in the medium are markedly different. In particular, the $H_2PO_4^-$ concentration increases in the walls from pH 4.5 to 3 while it decreases in the medium, due to the formation of H_3PO_4 at the expense of $H_2PO_4^-$ (pK = 2.12). This takes place in the walls too, but it is overwhelmed by the attenuation of the Donnan exclusion of $H_2PO_4^-$ due to the protonation of cell wall fixed groups (pK = 3.2). In our work, the problems of the nature of the transported form and of the local *versus* bulk concentration may not be dissociated. Lin investigated the effects of pH on Pi uptake by corn root segments (16) and corn root protoplasts (17). Unfortunately, the experimental conditions used were not proper for a precise comparison of the two situations, and the effects of the walls cannot be discussed from these data.

When analyzed as a function of the $H_2PO_4^-$ concentration in the medium as well as in the walls, the influx isotherms in the 0 to 2 mM total Pi concentration range at pH 3, 4, 5, and 7 (data not shown) exhibited the complex kinetics characteristic of the so-called dual mechanism (6, 14, 23). The following discussion will be restricted to the high-affinity mechanism.

In the range 0 to 60 μM ($H_2PO_4^-$ in the medium) or 0 to 6 μM

Table II. *Values of pH and of Pi Concentration for the Experiment Described in Figure 6*

Computation of $H_2PO_4^-$ concentrations in the media and in the walls, and of pH in the walls: see Figure 4.

Medium			Cell Walls	
pH	Total Pi	$H_2PO_4^-$	pH	$H_2PO_4^-$
	μM			μM
4.00	15.3	14.8	3.21	2.34
4.80	19.9	19.4	3.89	2.41
5.60	21.8	20.8	4.66	2.39
6.10	23.2	20.8	5.15	2.36
6.60	28.1	21.2	5.65	2.40
6.80	32.4	21.6	5.85	2.44
7.00	36.9	20.7	6.05	2.34
7.20	46.3	20.7	6.25	2.34
7.45	70.0	21.0	6.50	2.50
7.65	91.1	22.0	6.70	2.32
7.80	137	23.3	6.85	2.64
7.85	135	24.0	6.90	2.38
8.00	180	20.7	7.05	2.34

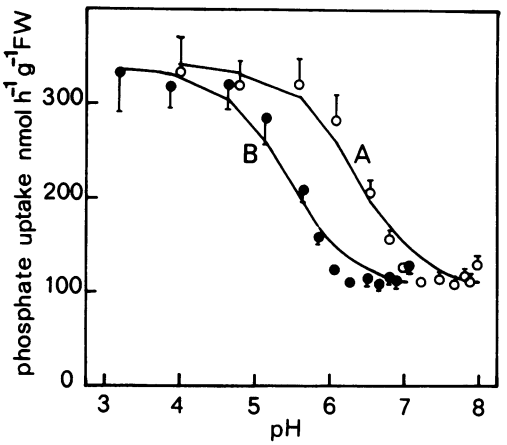

FIG. 6. ^{32}Pi uptake by corn root segments at various pH values with $H_2PO_4^-$ concentration constant in the walls. The data are plotted *versus* the pH in the medium (○, and curve A) or in the walls (●, and curve B). Lines: theoretical titration curves for groups with pK 6.4 (curve A) or 5.5 (curve B): these pK values were obtained by direct least square adjustment. The values of total Pi concentrations in the absorption media were chosen so as to fix the concentration of $H_2PO_4^-$ in the walls at a constant value, about 2.5 μM; they are listed in Table III, together with the values of pH and of $H_2PO_4^-$ concentrations in the media and in the walls. In addition to KH_2PO_4, all the media contained 2 mM $CaSO_4$, and 2 mM K^+ (adjusted with K_2SO_4); their pH was adjusted and maintained with H_2SO_4 or $Ca(OH)_2$ and a pH-stat system. Root segment preparation: see Table I; 5 min incubation; means of six experiments and 95% confidence limits.

($H_2PO_4^-$ in the walls), typical hyperbolic kinetics were observed, although there was a slight contribution of the low-affinity mechanism to the experimental points with a weight increasing with Pi concentration. This contribution limited the quality of the fitting but remained small enough to authorize a kinetic analysis of the high-affinity mechanism (theoretical curves in Fig. 5, A and B). The J_{max} and K_m parameters obtained by direct least square adjustment are listed in Table III. The affinity constant of the transport system for $H_2PO_4^-$ is about 2 to 3 μM or 0.3 to 0.5 μM, according to whether the concentration in the medium or in the walls was taken into account. The former values compare well with those published for corn (6, 19), but they may underestimate the intrinsic affinity of the transport system as the latter ones suggest.

Table III. *K_m and J_{max} Values of ^{32}Pi Uptake by Corn Roots in the Low Concentration Range for the Experiment Described in Figure 5*

Kinetics Parameters	pH in the Medium (pH in the walls)			
	3 (2.48)	4 (3.2)	5 (4.07)	7 (6.05)
Fig. 5A (medium)				
J_{max} (nmol·h^{-1}·g^{-1} fresh wt)	734	631	638	207
K_m (μM)	1.8	2.4	2.8	2.4
Fig. 5B (cell walls)				
J_{max} (nmol·h^{-1}·g^{-1} fresh wt)	712	623	637	207
K_m (μM)	0.49	0.37	0.32	0.27

Acidifying the medium increased Pi influx (Fig. 5). This effect occurred mainly between pH 7 and pH 5 (between pH 6 and pH 4 in the walls). This is due to a 300% increase of J_{max}; in the same pH range, the affinity is little affected (20% increases for K_m) (Table III). The effect of pH on J_{max} may be analyzed with Figure 6, which shows Pi influx measured in such conditions that the calculated $H_2PO_4^-$ concentration in the walls was fixed at 5-fold the highest value of K_m in Table III. The shape of the curves in Figure 6 suggests that the transport system may be switched from a low-activity state at pH > 7 to a high-activity one at pH < 5, and that the conversion between the two states corresponds to the ionization of a group with a pK near pH 5.5 (pH in the walls), or pH 6.4 (pH in the medium). In other words, the system behaves as if it had an affinity constant for H^+ about 3 μM (walls) or 0.4 μM (medium). This behavior could result from the protonation of histidine imidazol. The experiments with iodoacetate were inconclusive. Nevertheless, the effect of the nonpermeating rose bengal was in accordance with the above hypothesis.

The activation of the Pi transport by H^+ may correspond to a catalytic effect or to the use of H^+ as a substrate for cotransport with $H_2PO_4^-$. The fact that Pi influx does not vanish at pH > 6 (Fig. 6) may be the sign that in these conditions H^+ is captured by a high-affinity site (pK > 8) or is furnished by a source other than the medium. In the former case, Figure 6 would picture the transition of the Pi transport system from a uniprotonated, low activity form, to a biprotonated, high activity form, or from a 1 H^+:1 Pi cotransport to a 2 H^+:1 Pi one. In the latter case, local H^+ delivery by the proton pump, which is high at pH > 6 (Fig. 2), could energize the Pi uptake (13, 22, 25). Another explanation would be that the Pi influx at pH > 6 is catalyzed by a system independent of H^+. Finally, the pH effect in Figure 6 could also indicate the functioning of a reversible $H_2PO_4^-/HCO_3^-$ antiport, since bicarbonate pK is in the range of the pK values estimated from the activation of Pi transport.

The pH dependency of Pi uptake has been considered as a proof of the specificity of the transport system for $H_2PO_4^-$ in barley (23), or as an indication of a Pi/OH^- antiport in corn (16), where this hypothesis was independently suggested by the inhibiting effect of mersalyl. However, the possibility of simultaneous involvement of both these effects was not investigated. Our results show that pH affects Pi uptake by corn roots in two ways. First, it determines the concentration of the transported Pi form. Second, at a constant $H_2PO_4^-$ concentration, it acts by controlling the intrinsic kinetic properties of the transport system (J_{max}, principally), and/or an hypothetical cosubstrate concentration (H^+, for cotransport, OH^- or HCO_3^- for antiport). These two hypothesis cannot be discriminated either on the basis of the present results or of published data.

The analysis which led to these conclusions was based on the assumption of walls at equilibrium with the medium rather than

in a stationary state. This assumption is likely to be valid in most cases in our work since Pi and H^+ net transports are rather slow. As mentioned above, the validity of the equilibrium hypothesis is less clear at pH > 6 because in this situation a high rate of H^+ extrusion coincides with a low H^+ concentration in the medium.

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