Plant Physiol. (1967) 42, 1407–1412

Effects of Temperature on Orthophosphate Absorption by Excised Corn Roots[1]

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Received June 2, 1967.

Summary. The uptake of orthophosphate (^{32}P) by excised corn roots, *Zea mays* L. was studied using roots grown on 0.2 mM $CaSO_4$. Nine concentrations of KH_2PO_4 from 1 to 256 μM were used at temperatures of 20°, 30°, and 40°. Enzyme kinetic analysis was applied to the data obtained. Two apparent mechanisms (sites) of phosphate uptake were observed, 1 dominating at high P concentrations and 1 at low P concentrations. A Km of 1.36×10^{-4} and a Vmax of 177×10^{-9} moles per gram of roots per hour at 30° was calculated for the mechanism dominating at high P concentrations. Similar calculations gave a Km of 6.09×10^{-6} and a Vmax of 162×10^{-9} moles per gram of roots per hour at 30° for the mechanism dominating at low P concentrations. The Q_{10} for both mechanisms was approximately 2. Calculation of thermodynamic values from the data gave $\triangle F$ of −5200 cal, $\triangle H$ of −950 to −1400 cal, and a enthalpy of activation (A) of 10,300 to 13,800 cal per mole for the mechanism dominating at high P concentrations. Similar calculations from the data for the mechanism dominating at low P concentrations gave a $\triangle F$ of −7300 cal, $\triangle H$ of −10,700 to −8200 cal, and a A of 9300 to 18,900 cal per mole. If the dual mechanism interpretation of this kind of data adequately describes this system, then both mechanisms of P absorption by corn roots involve chemical reactions.

Kinetic studies have lead to the postulation of a number of rather precise relationships with respect to the mechanism of phosphate uptake by excised barley roots (10, 14, 15). Most significant has been the interpretation that 2 apparent sites or mechanisms of P absorption are involved. The dual mechanism data have been interpreted to imply separate sites for the absorption of the 2 phosphate ionic species, $H_2PO_4^-$ and HPO_4^{2-} (8, 9). Likewise, the enhancement of Ca^{2+} on P uptake is an important observation. The effect of temperature on absorption of orthophosphate by excised corn roots from solutions of a wide range of phosphate concentrations was examined to test the hypothesis that the apparent dual mechanism of phosphate uptake may be due to the combined action of physical and metabolic components. Enzyme kinetic analysis was applied to the data obtained and certain thermodynamic values were calculated.

Materials and Methods

Seeds of *Zea mays* L, var. OH10 × 51A × B_8, supplied by the Department of Plant Breeding, Cornell University, were placed on paper towelling covering a 1.5 cm layer of acid washed sand in 33 × 22 cm pyrex baking dishes. The sand was moistened with 0.2 mM $CaSO_4$. Dishes were covered with saran wrap (punctured for aeration) and placed in an incubator in the dark at 23° for 90 to 95 hours.

Roots (approximately 6.5 cm long) were cut off adjacent to the seed, blotted dry, and weighed into lots of approximately 1 gram for each treatment. They were then placed into 500 ml wide mouthed jars containing 0.2 mM $CaSO_4$ and aeration tubes. The jars were placed in a temperature controlled water bath and allowed to come to equilibrium for 30 minutes. The $CaSO_4$ solution was removed by suction and ^{32}P labeled KH_2PO_4 solution added. The concentrations of phosphate solutions used were 1, 2, 4, 8, 16, 32, 64, 128, and 256 μM KH_2PO_4. These solutions had previously been adjusted to pH 4, brought to the required temperature and contained 0.2 mM $CaSO_4$. The temperatures used were 20°, 30°, and 40°. Each set of experiments was replicated 3 times and the data presented are the average of 3 replicates.

[1] Department of Agronomy Paper No. 773. The work was supported in part by the Division of Agricultural Relations, Tennessee Valley Authority, Wilson Dam, Alabama.

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At the end of the experimental time period the radioactive phosphate solution was removed by suction and roots washed 6 times in distilled water, transferred to metal planchets, dried under an infra-red lamp, and counted with an end window Geiger tube. Time curves were run in conjunction with all experiments to ensure that steady state linear uptake was being obtained over the experimental period of up to 4 hours.

Preliminary experiments where roots were washed 6 times with distilled water and then placed in unlabeled phosphorus solution containing 0.2 mM $CaSO_4$ showed that phosphate uptake was irreversible and all ^{32}P adhering to root surfaces was removed by rinsing 6 times in distilled water.

The total phosphorus content was measured by using the chlorostannous reduced molybdophosphoric blue method (19) for solutions of low phosphate concentration and the vanadomolybdophosphoric yellow method in nitric acid (1) for high phosphate concentration solutions.

Preliminary experiments showed that cutting roots into 2 cm lengths did not affect phosphate absorption and this was adopted as a general practice to facilitate placing the roots on metal planchets for counting. A 0.03 M sucrose solution was also found to have no effect on P uptake by excised corn roots.

Results

Kinetic treatment of results requires a steady state of phosphate uptake be maintained throughout the experiment. Time curves at 30° and 40° and at low and high phosphate concentration were linear over a 3 hour time period (fig 1) indicating that steady state conditions existed in these experiments. Positive intercepts were not found at either temperature. Thus, in the presence of Ca^{2+}, steady state metaboli-

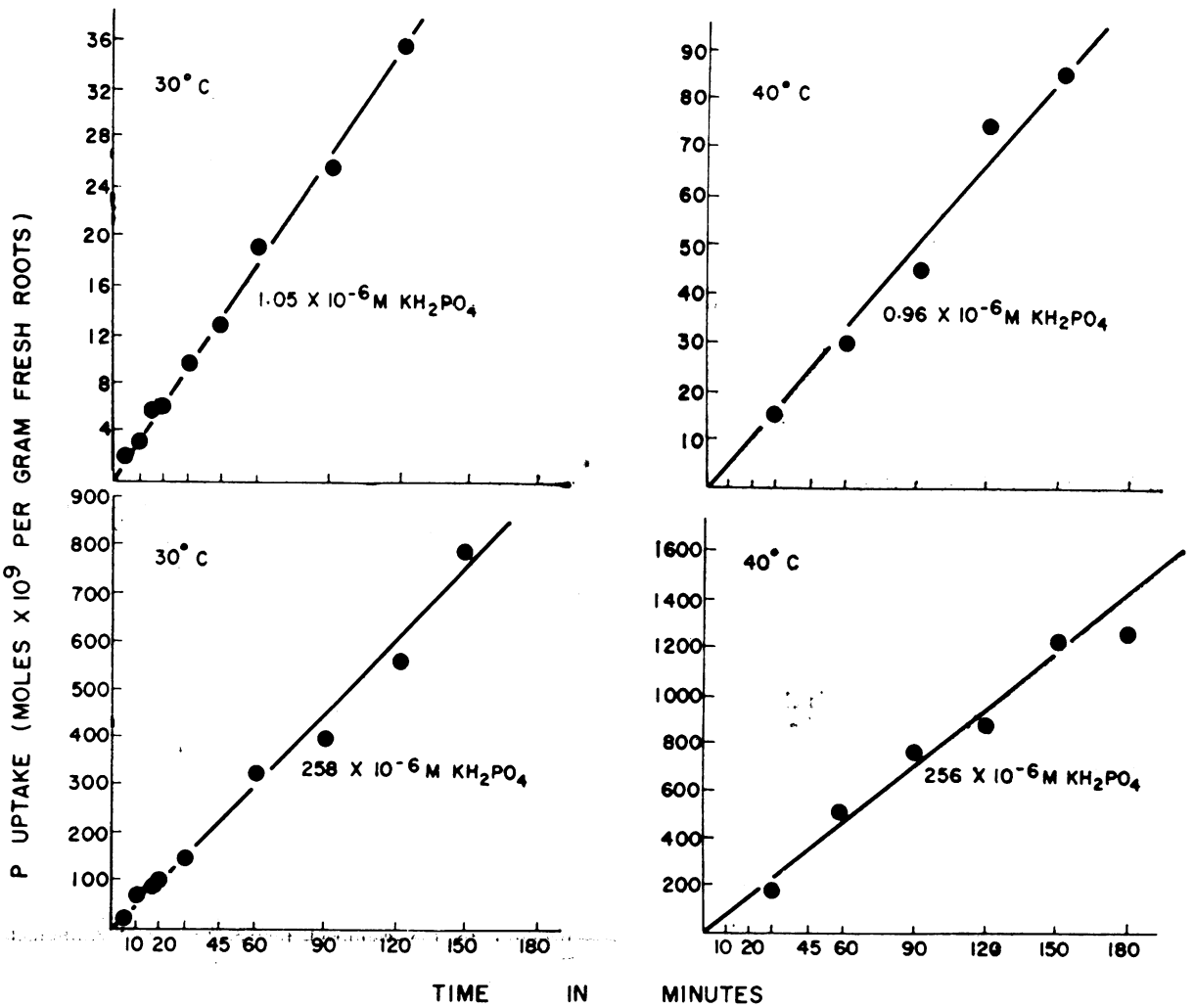

FIG. 1. Time curves showing the effect of 30° and 40° temperatures on the rate of P uptake from solutions of high and low KH_2PO_4 concentration by excised corn roots.

Table I. *Apparent Km and Vmax Values for Phosphate Absorption by Excised Corn Roots at pH 4 from KH_2PO_4 Solutions at 20°, 30°, and 40°*

Temp	Vmax		Km	
	Site a	Site b	Site a	Site b
	moles/gm root/hr			
20°	100×10^{-9}	62×10^{-9}	1.3×10^{-4}	3.56×10^{-6}
30°	177×10^{-9}	62×10^{-9}	1.36×10^{-4}	6.09×10^{-6}
40°	335×10^{-9}	62×10^{-9}	1.46×10^{-4}	9.19×10^{-6}

cally mediated absorption of phosphate occurred immediately and no preliminary period of equilibration was observed. The conclusions of Epstein et al. (5) concerning the absorption of rubidium by barley roots likely apply here also.

In experiments at 20°, 30°, and 40° with 9 different KH_2PO_4 concentrations, using a 1 hour uptake period, the rate of phosphate uptake continued to increase even at the highest concentration for all temperatures (fig 2A). When these data are examined on a kinetic plot (fig 2B) similar to that of Eadie (4) and Hofstee (12), the dual uptake mechanism similar to that reported (9, 15) is observed.

From the straight line components (fig 2B), values for Vmax and Km for reaction site a (high P) and reaction site b (low P) were calculated (table I).

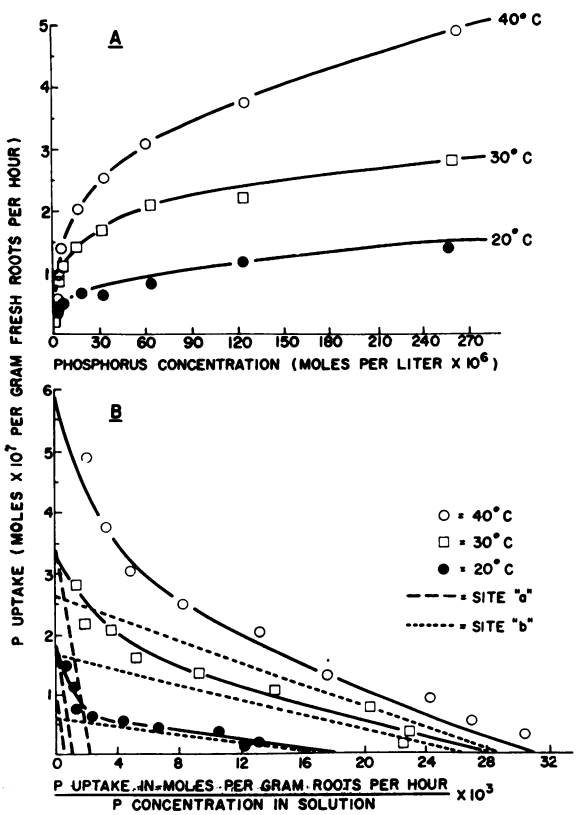

FIG. 2. A) Phosphate absorption by excised corn roots as a function of KH_2PO_4 concentration at temperatures of 20°, 30°, and 40°. B) A kinetic plot of the phosphate absorption data of figure 2A.

The Q_{10} values (table II) for site a were similar for both 10° intervals. The relative increase in uptake due to the b reaction site was much lower in the 30° to 40° interval than in the 20° to 30° interval. Using the integrated form of the Arrhenius equation (6), A can be calculated. The values for A given in table II, are a measure of the standard enthalpy of activation. Since the values for A are in fact calculated from the Q_{10} values they are both a measure of the nature of the reaction that occurs.

In the strict sense using the Arrhenius equation, A must be a constant. From the data of table II, it is obvious that A, particularly at reaction site b, was not a constant. Therefore A is only an approximate measure of the enthalpy of activation and possibly varies continuously over the temperature range used.

Table II. *Q_{10} Values and Enthalpy of Activation Values Calculated for Reaction Uptake Sites of Corn Roots*

Temp interval	Reaction site a		Reaction site b	
	Q_{10}	A, cal/mole	Q_{10}	A, cal/mole
20°–30°	1.8	10,300	2.6	18.900
30°–40°	2.0	13,800	1.6	9300

Other calculations also can be made from the effect of temperature on Km values. If the rate constants k_1 and k_2 are large in relation to k_3 (9) and the reaction is essentially irreversible (5), which is consistent with our data, then Km is approximately equal to a dissociation constant and $\frac{1}{Km}$ becomes the formation constant for the reaction:

$$M + R \underset{k_2}{\overset{k_1}{\rightleftarrows}} MR.$$

Then the change in free energy, ΔF, and the heat of reaction, ΔH, can be calculated:

$$\Delta F = -RT \ln \frac{1}{Km} = RT \ln Km, \text{ and}$$

$$\log \frac{Km_1}{Km_2} = \frac{\Delta H}{4.576} \frac{1}{T_1} - \frac{1}{T_2}$$

Utilizing these equations, ΔF and ΔH have been calculated for the 2 reaction sites and are given in table III.

Discussion

The Km values found in these experiments were of a similar order of magnitude to those previously found for the 2 sites of phosphate uptake by barley roots (9, 16). Mitochondrial studies using corn root mitochondria (2) indicated an optimum temperature of around 40° for oxidative phosphorylation. The Q_{10} values for oxidative phosphorylation in corn root

Table III. *ΔF and ΔH Values Calculated for Reaction Uptake Sites of Corn Roots*

Temperature	ΔF, cal Site a	Site b	Temp interval	ΔH, cal Site a	Site b
20°	—5200	—7300	20°–30°	—950	—10,700
30°	—5400	—7200	30°–40°	—1400	—8200
40°	—5500	—7200			

Table IV. *Summary of Data from the Literature and Results Obtained in the Current Experiments Indicating the Characteristics of Sites a and b of Phosphate Uptake by Plant Roots*

Species and Ref ( )	Site a	Site b
Barley (8, 9, 10) Corn*	1. Dominates at high P conc (256 μM KH_2PO_4)	1. Dominates at low P conc (1 μM KH_2PO_4)
Barley (15)	2. Not affected by Ca	2. Strongly activated by Ca
Barley (8)	3. Km strongly affected by pH OH^- competitively inhibits absorption.	3. Km strongly affected by pH OH^- competitively inhibits absorption.
Barley (8)	4. Involves $H_2PO_4^-$	4. Involves HPO^{2-}_4
Barley (8)	5. Rate limiting breakdown involves cleavage of an R-O bond.	5. Rate limiting breakdown involves cleavage of an R-O bond.
Barley (9)	6. Unaffected by nembutal.	6. Inhibited by 5 mM nembutal, affects Vmax but not Km.
Barley (9)	7. Relatively unaffected by 5 μM P-P or ADP.	7. Competitively inhibited by 5 μM P-P and ADP, Vmax unchanged, Km reduced.
Barley (10)	8. Unaffected by paraphenylenediamine, napthoquinone, methylene blue and 2, 3 dimercapto-propanol.	8. Paraphenylenediamine napthoquinone, methylene blue and 2, 3 mercaptopropanol stimulate HPO^{2-}_4 uptake by increasing Vmax, Km unaffected.
Barley (9)	9. Acetoacetate decrease Vmax.	9. Acetoacetate decrease Vmax.
Barley (8)	10. Carrier is cytochrome b.	10. Carrier is NADH
Corn*	11. ΔF = —5200 cal. ΔH = — 950 cal. —1400 cal. A = 10,300 cal. 13,800 cal.	11. ΔF = —7300 cal. ΔH = —10,700 cal. —8200 cal. A = 18,900 cal. 9300 cal.
Barley (9)	12. Km a = 2.6×10^{-4}, Vmax a = 26×10^{-9} moles P/g/hr.	12. Km b = 4.17×10^{-6}, Vmax b = 5×10^{-9} moles P/g/hr.
Corn*	13. Km a = 1.36×10^{-4}, Vmax a = 177×10^{-9} moles P/g/hr.	13. Km b = 6.09×10^{-6}, Vmax b = 162×10^{-9} moles P/g/hr.
Barley (13)	14. Glucose -1-^{32}P and uridine diphospho-glucose accumulate first.	14. ^{32}Pi accumulated first.
Barley (16)	15. Pi and sugar esters at high P.	15. Nucleic acids at low P concentration.
Barley (16)	16. After 15 secs. exposure ATP and ADP contained most ^{32}P after 30 secs. Hexose phosphates contained max ^{32}P after mins.	

* Results reported in this paper.

mitochondria were approximately 2 in the 25° to 35° range. The Q_{10} values and the values for A calculated for both reaction sites in these experiments are indicative of chemical reactions. In comparison an A value of 11,700 calories was found for the respiration of pea roots (17). The $\triangle H$ values for the a (high P conc) reaction site, however, suggests very weak bonding energy between ion and carrier. Boszormenyi (3) in his experiments found that the rate of bromide uptake as a function of temperature expressed in the Arrhenius plot does not always result in a straight line. He suggested that possibly the limiting step in ion absorption may be different at different temperatures. Likewise, the A values calculated from our experiments could be interpreted to signify that the rate limiting step is different at different temperatures.

Before discussing these results further, table IV is presented summarizing the results reported here and those reported in the literature regarding the nature of the 2 apparent mechanisms of P uptake.

Low phosphate excised roots grown in 0.2 mM $CaSO_4$ and maintained in solutions containing Ca so as to avoid removal of Ca and damage to the outer plasma membrane absorb phosphate under steady state conditions. It may be doubtful that ion absorption experiments made in the absence of Ca bear any relation to normal ion uptake by plant cells (10, 11, 20). Of all the essential elements for plant growth, none causes such complete and abrupt cessation of plant growth as transferring a plant to a medium devoid of Ca. Leakage of previously absorbed ions is likely to occur only when lack of Ca causes a breakdown of cell membranes or high concentrations of other ions such as Na (7) cause rupture of the outer cell membrane. Thus, when these corn roots were maintained in $CaSO_4$ solutions and studies were over relatively short periods of up to 4 hours, phosphate uptake was irreversible.

On the basis of the results reported in this paper and those of other workers (table IV) it is suggested that phosphate uptake by plant roots involves 2 reactions. One of these reactions, which dominates at low external phosphate concentration, appears highly dependent on Ca (15) and is possibly located in the outer plasma membrane of the cytoplasm. It may be linked to NADH and possibly involves combination of ADP with Pi at the membrane surface, movement through the membrane, and release at the inner surface of Pi.

The second mechanism which operates at high external phosphate concentrations may involve movement of glucose-1-P into a membrane and release of Pi on hydrolysis at the inner surface. This may be into the vacuole of vacuolated cells. Undirectional transfer through a membrane has been established for other cell systems. The involvement of ATP and glucose-1-P is suggested by $\triangle F$ values (table III) and identification of initially labeled compounds (13, 16).

Provided the importance of calcium and the occurrence of 2 reactions, 1 at the outer membrane surface and 1 at the inner membrane surface, are considered the data in table IV appears to be explained reasonably well. While our data afforded no information on the ionic species involved in P uptake our experiments were made at pH 4 where one would reasonably expect $H_2PO_4^-$ to be the only ion of consequence involved. The explanation offered by Torii and Laties (18) for their results is pertinent and strongly supports our interpretation of the results of these experiments. The temperature studies reported in this paper indicate that with both reactions, phosphorus uptake involves chemical reaction.

Acknowledgments

The authors acknowledge the use of laboratory facilities provided by the U. S. D. A. Federal Nutrition Laboratory. They especially acknowledge the advice and help of the late Dr. T. R. Nielsen with these experiments.

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