PRODUCTS OF ORTHOPHOSPHATE ABSORPTION BY BARLEY ROOTS[1]

PATRICIA C. JACKSON[2] AND C. E. HAGEN[3]

AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

The rate-limiting step of orthophosphate absorption by barley roots has been shown by kinetic studies (5) to be coupled to two of the sites of oxidative phosphorylation associated with isolated mitochondria. One of the reactions is coupled to reduced cytochrome b and the other to reduced diphosphopyridine nucleotide (DPNH). The present paper describes the identification of some of the products of the rate-limiting absorption step. The significance of the distribution of absorbed phosphate among these products and the rate of appearance in the products is discussed.

MATERIALS AND METHODS

The experimental plant material consisted of excised roots from 6-day old seedlings of barley (*Hordeum vulgare*, var. Atlas 46). The seedlings had been grown in the dark in continuously aerated 10^{-4} M $CaSO_4$, essentially as described by Epstein and Hagen (2). Roots were excised from the seedlings just prior to the absorption procedure, rinsed three times with 50 volumes of distilled-demineralized water, and suspended in 100 volumes of continuously aerated distilled-demineralized water.

Solutions for absorption experiments were single salt solutions of orthophosphate containing P^{32}, either in the absence or presence of various reagents under study. Sufficient volumes of the absorption solutions per mass of roots were used so that the concentration of phosphate and the reagents remained constant during the absorption. Hydrogen ion concentration was maintained to within 0.1 unit of pH. The excised roots were blotted with clean dry cheesecloth, weighed, and again rinsed three times with distilled-demineralized water before the absorption solutions were added. Absorption was terminated by rapid decantation of the solutions, followed by four rinses of the roots with 50 volumes of distilled-demineralized water.

The P^{32}-labelled components associated with the roots after absorption were extracted by grinding with four volumes of 95 % ethanol with a Pyrex mortar and pestle. The resulting root homogenate was centrifuged and aliquots of the supernatant fraction were taken for chromatography. Where concentration of the supernatant fraction was desirable, it was evaporated at room temperature under vacuum to approximately one-tenth the original volume.

Descending chromatograms were run at room temperature on 17 × 20 inch sheets of Whatman #1 or #3 filter paper. The filter paper had been washed by irrigation with a 20 : 50 : 30 parts, by volume, mixture of acetic acid, ethanol, and water, followed by irrigation with distilled-demineralized water, and dried before use. Both one- and two-dimensional chromatograms were run with various combinations of solvents. Seventeen different solvents were tried, of which the two listed below were the most satisfactory for separation of the P^{32}-labelled compounds in the root extracts.

I. *n*-butyl alcohol : proprionic acid : water—45 : 23 : 32 % by volume (1).

II. Iso-butyric acid : ammonium hydroxide : water —57 : 4 : 39 % by volume (9).

For identification of the P^{32}-labelled fractions, the extract was applied as a 1 cm wide band across one end of a sheet of Whatman #3 paper and chromatographed with one of the above solvents. The separated bands of P^{32}-labelled fractions were then located on the paper by means of radioautographs, cut out, and eluted with water. For separation of the fractions for kinetic studies of phosphate incorporation into the components, the extracts were applied to the chromatographic paper as 1 cm diameter spots. Radioactive spots on the chromatograms were located, cut out, and counted in an internal proportional counter to determine the amount of P^{32}.

Nucleotides were located on the chromatograms by visual observation under a mineralight ultraviolet lamp. Sugar compounds were located after spraying the chromatograms with aniline phthalate (10). Identification of the sugar compounds was aided by color reactions with the spray that were characteristic of the type of sugar. Compounds containing phosphorus were located by spraying with an ammonium molybdate (6) or an iron salicylate solution (12).

The eluted P^{32}-labelled components were further characterized by the following chemical and physical criteria: A. Ultraviolet spectra in acid, alkaline, and neutral solutions. B. Co-chromatography with a known compound in several solvents. C. Rates of hydrolysis in 0.1 *N* or 1 *N* HCl at 100° C. D. Stepwise decomposition of the components. E. Identification of the decomposition products. The known compound was carried through the same procedure as the root components for direct comparison.

Specific activities of the eluted P^{32}-labelled components were also determined. The P^{31} content was determined essentially by the method of Truog and Meyer (13) after the components were hydrolyzed.

RESULTS AND CONCLUSIONS

IDENTIFICATION OF ABSORPTION PRODUCTS. Extracting the roots with 95 % ethanol shows that 75 % of the total phosphate absorbed in 10 minutes is soluble in ethanol. Water or 10 % trichloroacetic acid

[1] Received July 24, 1959.

[2] Chemist, Mineral Nutrition Laboratory, ARS, USDA.

[3] Deceased.

(TCA) extract additional P^{32} from the ethanol insoluble fraction. However, a constant amount of activity is not extractable by water or TCA, irrespective of the absorption time. Thus, the activity associated with the insoluble fraction contains up to 16 % of the total absorbed P^{32} after 15 seconds absorption, but less than 2 % after 10 minutes absorption.

The ethanol soluble components were separated by paper chromatography. Chromatograms of the ethanol extracts show that P^{32}-labelled phosphate is incorporated into five major fractions (fig 1), which contain 95 % of the total P^{32} extracted by ethanol. The remaining 5 % of the ethanol soluble activity is distributed among at least ten components. The distribution of P^{32} among the five fractions is the same whether the ethanol extraction is carried out at 0° C or at room temperature. The additional activity extracted by water or TCA is identical to fraction IV of the ethanol soluble fractions. Thus, 80 to 95 % of the total absorbed phosphate appears in these five fractions. Although the extraction by water and TCA is somewhat more efficient, considerable hydrolysis of fractions I and III is associated with the use of these solvents. Two-dimensional chromatograms, using the same solvent in both directions, reveal that appreciable hydrolysis of some of the components occurs during chromatography with many of the strongly acidic irrigating mixtures. Thus, consideration was given to formation of artifactual components from hydrolysis of the fractions.

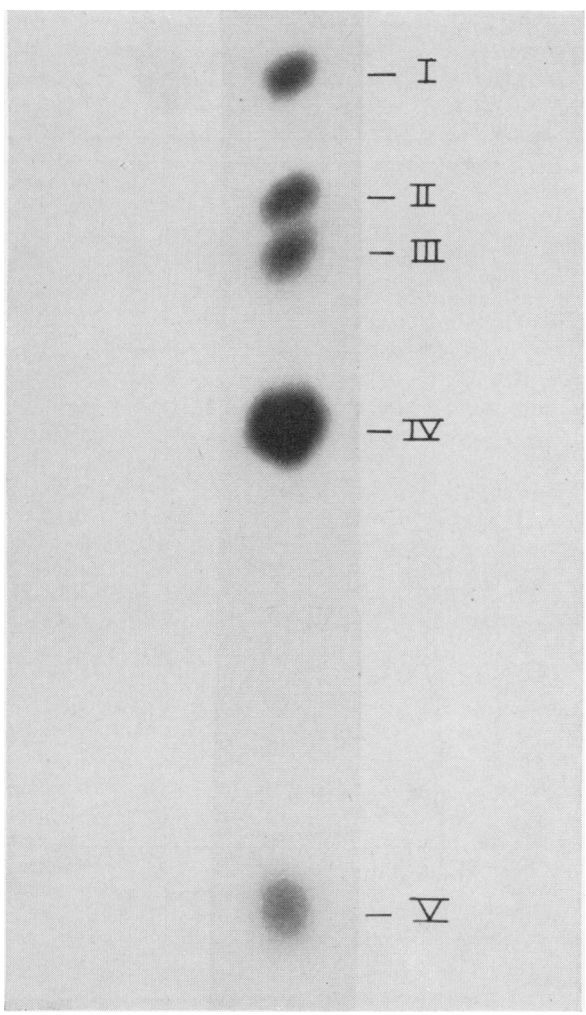


FIG. 1. Radioautograph of a chromatogram showing separation of P^{32} labeled components in excised barley roots. The solvent was a mixture of 57 % iso-butyric acid, 4 % ammonium hydroxide, and 39 % water.

The isolated P^{32}-labelled root fractions eluted from band chromatograms were chromatographed with some 40 phosphorus compounds commonly occurring in biological systems. Co-chromatography of a known compound with a root fraction is interpreted as only a tentative identification of the fraction since a pair of dissimilar compounds may co-chromatograph quite precisely in many different solvents. Failure of a fraction to co-chromatograph in any one solvent with a known compound is proof of dissimilarity of the compounds.

Fraction I (fig 1) co-chromatographs with uridine diphosphate glucose (UDPG) in all of the solvent mixtures tested. UDPG is identical with this fraction in ultraviolet absorption spectra in acid, alkaline, and neutral solutions, and in rates of hydrolysis. Products of partial decomposition of fraction I are the same as those of UDPG; namely, uridine diphosphate (UDP), uridine monophosphate (UMP), glucose, and inorganic phosphate. The terminal phosphate of the UDPG fraction contains 90 % of the total P^{32} of fraction I, with 10 % remaining in the phosphate of the UMP moiety.

Fraction III (fig 1) co-chromatograms with glucose-1-phosphate (G-1-P), mannose-6-phosphate, and galactose-1-phosphate. Separation of these compounds by other solvent systems was not attempted. However, the rate of hydrolysis of fraction III corresponds to that of G-1-P rather than that of mannose-6-phosphate or galactose-1-phosphate. Further, the formation of glucose upon decomposition rather than mannose or galactose indicates that most of fraction III is G-1-P.

Fraction IV (fig 1) was identified chemically (11) as well as chromatographically, as inorganic orthophosphate (P_i). Some of the P_i in fraction IV appears to be an artifact arising from hydrolysis of UDPG and, particularly, G-1-P during extraction of the roots and chromatography. The identity of fractions II and V remains unknown. Neither fraction co-chromatographed in all solvent mixtures with any of the known phosphate compounds, nor were the individual fractions further separable in any of the solvents. Only 10 % of either fraction hydrolyzes in 1 *N* HCl at 100° C in 4 hours.

No particular effort was made to identify any of the minor P^{32}-labelled components or unlabelled phosphate compounds in the roots. In the course of identification of the major components, however, a small fraction of P^{32} was found to be associated with uridine triphosphate. The root extracts contain some un-

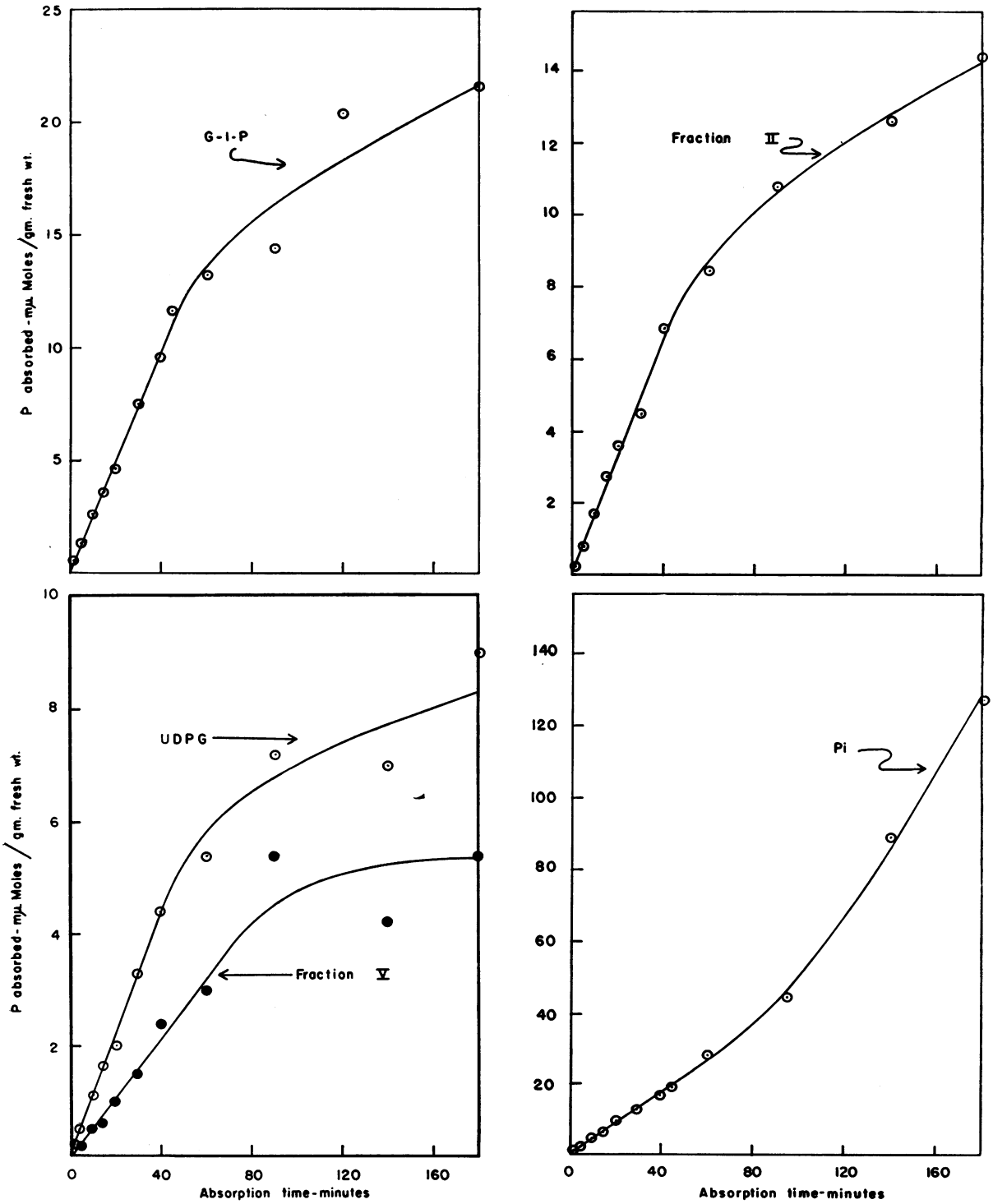


FIG. 2. Time course of P^{32} incorporation into the major phosphate absorption products in excised barley roots. Absorption was from 10^{-5} M phosphate at pH 4.

labelled adenosine diphosphate (ADP) and adenine, but no P^{32}-labelled adenosine phosphates. No measurable amounts of adenosine triphosphate (ATP) are present. In contrast to the roots, the leaves of barley plants contain relatively large amounts of adenosine monophosphate, ADP, and even greater amounts of ATP.

KINETICS OF P^{32} DISTRIBUTION IN ROOT COMPONENTS. The rate of phosphate absorption by the roots is constant for at least 4 hours. Figure 2 shows the time course of P^{32} incorporation into the five root fractions. The rate of the P^{32} appearance in UDPG and G-1-P is apparently constant for 40 minutes. Thereafter, the rate decreases. The incorporation rate into fraction II decreases after 50 minutes and, in fraction V, it decreases after 70 minutes. The time sequence of the rate decrease in these four fractions was always the same; i.e., UDPG and G-1-P, followed by fraction II, and last, fraction V. However, the rate of P^{32} appearance in P_i increased after 50 minutes of absorption with the rate increase compensating for the decreases in the rates in the other compounds. The long period of steady-state incorporation of P^{32} into the fractions excludes the possibility that any of them are initial sites or compounds formed prior to the rate-limiting absorption step. Thus, the five fractions must be products of phosphate absorption.

The decrease observed in the rate of P^{32} incorporation into four of the five fractions can be accounted for in two ways. One is that the amounts of the phosphate fractions increase significantly during phosphate absorption and the rate decreases when a mass equilibrium saturation is attained. In such a case, the time sequence of the rate decreases need have no relation to the sequence of formation of the fraction. The other possibility is that the fractions are in a dynamic equilibrium and do not increase significantly in amount during phosphate absorption. The appearance of P^{32} in the fractions, then, is a function of exchange of P^{31} for P^{32}. The fractions are in a sequence of reactions and the rate decrease occurs when the specific activity of the fractions approaches that of the incoming phosphate. The decrease in rate would occur in the same sequence of time as the sequence of incorporation.

To determine which of the two possibilities discussed occurs during phosphate absorption, the specific activity of the five separated fractions was determined after various absorption periods. Phosphate was absorbed by the roots from 10^{-5} M solution at pH 4 for intervals from 10 to 180 minutes. The amounts of the separated fractions were determined by chemical determination of phosphate and, in the case of UDPG, also by ultraviolet absorption at 260 mμ.

UDPG and G-1-P show no increase in total phosphate content in 180 minutes of absorption. The total phosphate of fractions II and V, and P_i increases by 10 to 20 %. In contrast, the specific activity of all fractions increases with time (table I). The rate of P^{32} incorporation into the five fractions is constant where the specific activity is below approximately 15 % of the external phosphate specific activity. Thereafter, as the specific activity increases further, the incorporation rate decreases. In fraction V, the specific activity is only 14 % of the external phosphate specific activity after 180 minutes absorption and the rate is constant. The rate of P^{32} incorporation in P_i and its rate of specific activity increase increases with time.

TABLE I

EFFECT OF ABSORPTION TIME ON SPECIFIC ACTIVITY OF P^{32}-LABELED ROOT FRACTIONS [1,2]

ABSORPTION TIME MIN	P^{32} FRACTION				
	UDPG	II	G-1-P	P_i	V
10	2.7	3.6	6.9	0.54	1.5
30	8.4	10.3	13.5	1.33	2.4
60	12.7	14.8	16.5	2.0	4.8
90	27	23	53	4.7	7.0
180	33	27	58	11.4	14.4

[1] Roots had absorbed P^{32} from 10^{-5} M orthophosphate at pH 4.
[2] Percent of external phosphate specific activity.

It is apparent from this experiment that the break-off of the time curves of P^{32} appearance in G-1-P, UDPG and fractions II and V occurs because the specific activities of the fractions approach that of the incoming phosphate. The break-off appears to occur when the fraction specific activity exceeds 15 % of the external specific activity. Other experiments show that the time at which the break-off occurs is a function of the phosphate concentration and the specific activity of the absorption solution. The specific activity was always highest in UDPG, G-1-P and fraction II, lower in fraction V and lowest in P_i.

A study of the effect of P^{31} absorption on the distribution of P^{32} in the root fractions was carried out also to determine the sequence of the P^{32} incorporation into the five fractions. The roots had absorbed P^{32} for 60 minutes, a time at which equilibration of P^{32} among the fractions is just becoming apparent (fig 2). Under these conditions, the P^{31} absorbed would be expected to move P^{32} from the most primary components into later or final products. Roots which had absorbed P^{32} from a 10^{-5} M phosphate solution at pH 4 for 60 minutes were rinsed four times with distilled-demineralized water and placed in a 10^{-5} M solution of P^{31} phosphate at pH 4 for various intervals up to 2 hours. The same procedure was performed with phosphate solutions at pH 8, also. The amount of P^{32} absorbed was the same for all root samples at any one hydrogen ion concentration. Under these conditions, the roots continue to absorb P^{31} at the same rate as they have absorbed P^{32} without any loss of P^{32} from the roots. Results from the determination of the amount of P^{32} in each of the separated fractions (e.g., fig 3) show that the P^{32} is transferred from UDPG and G-1-P into fraction II and P_i. Fraction II initially gains P^{32} displaced from

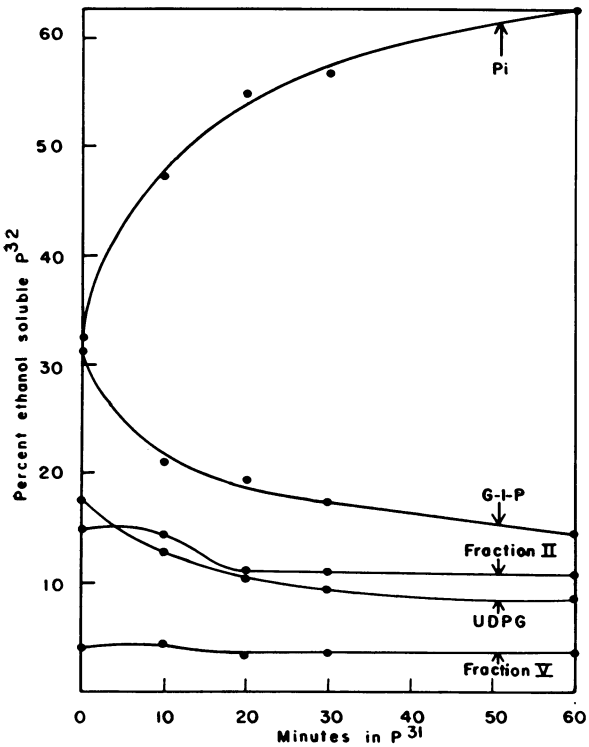
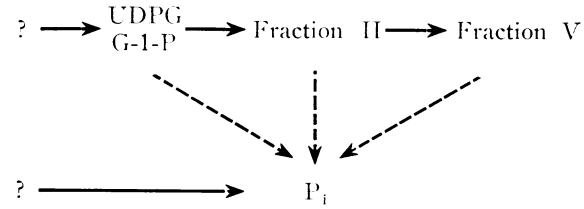


FIG. 3. Effect of P^{31} absorption on the P^{32} distribution in the root components. The roots had absorbed P^{32} for 60 minutes from a 10^{-5} M phosphate solution at pH 4, after which they were rinsed and placed in a 10^{-5} M P^{31} solution at pH 4.

one or both of the fractions, UDPG and G-1-P. Subsequently, activity in fraction II is also transferred to P_i. Continued incorporation of P^{31} has little or no effect on the P^{32} associated with fraction V, in this experiment. Fraction V eventually loses P^{32}, but in short times, little effect is apparent.

The influence of several sugars, namely, sucrose, fructose, glucose, mannose, galactose, and ribose, was studied to determine how much reactions subsequent to the rate-limiting absorption step alter the P^{32} distribution among the five fractions. Typical results are shown in table II. Previous experiments with C^{14}-labelled sucrose had shown that the sugar was absorbed and metabolized rapidly, with no effect on the accompanying phosphate absorption. None of the sugars tested in the present experiment alter the total amount of phosphate absorbed. The sugar concentration was 10^{-2} M in solutions of 10^{-6} or 5×10^{-4} M phosphate at pH 4. A pronounced shift in the distribution of P^{32} is produced by mannose, galactose, and ribose at either concentration of phosphate in the external solution. All of the sugars, except ribose, decrease the proportion of P^{32} in P_i with a proportionate increase of P^{32} in fraction II or G-1-P. Ribose decreases the P^{32} distribution in fraction V with a proportionate increase in fraction II. The P^{32} distribution in all of the five major fractions, except UDPG, is markedly affected by the sugars. The change in UDPG is relatively small and inconsistent. The sugars apparently form no new P^{32}-labelled fractions and only shift the distribution of P^{32} among the five major fractions. Thus, the effect of the sugars shows conclusively that the distribution of P^{32} among the five major fractions, with the possible exception of UDPG, is greatly influenced by reactions subsequent to the rate-limiting absorption step.

Experiments on the effect of P^{31} and the specific activity determinations show that the amount of P^{32} in the fractions is a function of P^{32}/P^{31} exchange. Therefore, a sequence of incorporation is inferred by the sequence of the breaks in the time curves. Thus:



All four fractions appear to contribute P^{32} to P_i. Incorporation into UDPG and G-1-P is so rapid that experiments do not show which of these two compounds precedes the other in sequence. There is a suggestion that UDPG may be the first by its relative insensitivity to the effect of sugars. Incorporation of P^{32} into fraction II is shown by the influence of P^{31} absorption (fig 3) to be subsequent to the incorporation into UDPG and G-1-P. That it is a product of UDPG and G-1-P rather than a precursor is suggested also by the effect of the sugars (table II), inasmuch as the P^{32} incorporation into this fraction can be increased without influencing incorporation into either UDPG or G-1-P. Fraction V is eliminated as a primary product by its relatively long period of steady-state incorporation, coupled with its low specific activity. The effect of ribose suggests that fraction V arises from fraction II. Fraction V does not

TABLE II

EFFECT OF SUGARS ON P^{32} DISTRIBUTION IN BARLEY ROOTS*

PERCENT OF ETHANOL SOLUBLE P^{32}

TREATMENT	P^{32} FRACTION				
	UDPG	II	G-1-P	P_i	V
Control	12	22	18	40	8
10^{-2} M glucose	15	27	20	30	8
10^{-2} M fructose	11	28	18	37	6
10^{-2} M mannose	8	6	70	12	4
10^{-2} M galactose	16	20	37	24	3
10^{-2} M ribose	13	30	19	38	1

* Roots had absorbed P^{32} from 10^{-6} M orthophosphate at pH 4 for 15 minutes.

appear to be an end product because eventually its rate of incorporation also decreases. Although some P^{32} in the P_i fraction comes from the other four fractions, short time experiments indicate that P^{32} appears in this fraction as soon as in UDPG and G-1-P.

Phosphate absorption occurs through two sites. The amount of phosphate absorbed through one of the sites, relative to the other is dependent upon the phosphate concentration and the pH of the external solution (4). Thus, if the products from one site differ from the other, the P^{32} distribution among the products should be in varying ratios dependent upon the limitations imposed by the absorption solution on the two individual sites. Further, the sequence of the P^{32} incorporation into the five major products suggests that UDPG, G-1-P and fractions II and V all are products of the same absorption site.

The influence of phosphate concentration and hydrogen ion concentration on the distribution of P^{32} among the root fractions was studied for comparison with the results of the kinetics of phosphate absorption. Short times of absorption were used to minimize the effects of equilibration among the products. Roots absorbed phosphate for 10 minute periods from solutions of 10^{-6} M to 5×10^{-4} M phosphate at pH 4 to 8. A decrease in the relative amounts of activity in UDPG, G-1-P and fractions II and V occurs with increasing phosphate concentration and increasing hydrogen ion concentrations (table III). According to the analysis of the kinetics of phosphate absorption (5), this decrease would be expected if all four of these fractions had arisen from the absorption coupled to cytochrome b. The proportion of P_i increases under these conditions, reflecting the absorption through the site that is apparently coupled to DPNH (5).

The effects of several inhibitors and activators on the distribution of P^{32} among the fractions were studied under conditions in which phosphate absorption coupled to cytochrome b would be affected. The inhibitors included pyrophosphate and nembutal, and the activators were calcium, succinate, and *p*-phenylenediamine. Representative results are shown in table III. Pyrophosphate and nembutal decrease the relative activity in UDPG, G-1-P and fraction II. Succinate, *p*-phenylenediamine and calcium increase the relative activities of these fractions, which again respond as if the absorption were coupled to cytochrome b. For example, the 30 % increase in total phosphate absorption induced by succinate, in table III, is entirely accounted for by the increase in the amount of P^{32} in UDPG, G-1-P and fraction II. The amount of P^{32} incorporated into P_i is unaffected. With all of the reagents, P_i responds as would absorption coupled to DPNH. The effects of the inhibitors and activators on fraction V were not as consistent with absorption through either site. This is not too surprising since fraction V is a later product than the others in sequence of P^{32} incorporation.

Some of the P^{32} incorporation into P_i by the roots arises from the release of phosphate from UDPG, G-1-P and fractions II and V, as shown by the relative changes in specific activity of the fractions with increasing absorption time (table I). This is also shown by the effect of P^{31} absorption on the distribution of P^{32} among the fractions (fig 3), and the decreased rates of P^{32} incorporation into UDPG, G-1-P and fractions II and V with a concomitant increase in the rate of incorporation into P_i (fig 2). However, not all of the activity in P_i arises from these compounds since P_i reflects the kinetics of absorption through the DPNH-linked site while UDPG, G-1-P and fraction II reflect the kinetics of the cytochrome b-linked site. These compounds qualitatively reflect the absorption kinetics but the shift in P^{32} distribution is not always as great as expected from the kinetics. This can be attributed in large measure to the transfer of P^{32} to P_i from the other four fractions. It is apparent, also, from the sugar experiments that the relative amounts of P^{32} incorporated into the fractions are greatly influenced by reactions subsequent to the rate-limiting absorption step.

TABLE III

EFFECTS OF VARIOUS AGENTS ON P^{32} DISTRIBUTION IN BARLEY ROOTS*

TREATMENT	% ETHANOL SOLUBLE P^{32}					TOTAL ABSORPTION
	P^{32} fraction					*mμ M/min*
10^{-6} *M Phosphate*	*UDPG*	*II*	*G-1-P*	*P_i*	*V*	*g fresh wt*
pH 4	13	21	21	38	7	1.0
pH 8	14	22	24	32	8	0.8
pH 4. 5×10^{-3} M calcium	15	24	24	34	3	1.2
pH 4. 10^{-3} M succinate	16	29	25	30	1	1.3
pH 4. 10^{-3} M nembutal	11	20	21	41	7	0.6
pH 4. 5×10^{-4} M pyrophosphate	8	11	7	68	6	0.5
5×10^{-4} *M Phosphate*						
pH 4	9	16	13	56	6	20.0
pH 8	13	19	14	46	8	12.0
pH 4. 5×10^{-3} M calcium	11	26	17	43	3	22.0
pH 4. 10^{-3} M nembutal	7	8	7	74	3	15.0

* Roots had absorbed P^{32} for 10 minutes.

Discussion

Miettinen and Savioja (8) found that roots of 6-week old pea plants grown in a nutrient solution incorporated P^{32} into ATP, UTP, and GTP in 10 seconds. Loughman and Russell (7) consider five nucleotides as the earliest products of 5 minute P^{32} absorption by young barley plants. They identified ATP and ADP as two of the nucleotides, but the others remain unknown. In the present experiments with etiolated barley roots, the most primary products of phosphate absorption among five major components which incorporate P^{32} within 15 seconds are UDPG, G-1-P and P_i. These results present no particular disagreement with those of Miettinen and Savioja or Loughman and Russell in as much as experimental factors differ greatly. UDPG was found by Ginsburg, Stumpf, and Hassid (3) to be one of the major nucleotide components of 5-day old mung bean seedlings. The amount of UDPG they found calculates to be approximately 5×10^{-8} M/g fresh weight. By comparison, barley roots in our experiments contain 7×10^{-8} M/g fresh weight. Ginsburg et al also found an approximately equal amount of AMP but only a fourth as much ADP as UDPG. This agrees with the relatively low level of adenosine compounds found in the barley roots.

Phosphate absorption by barley roots is coupled to oxidative phosphorylation. Since ATP has been shown to be the primary product of oxidative phosphorylation, its presence was expected to be among the P^{32}-labelled components of the root extracts. Yet, no P^{32}-labelled ATP was found. The explanation of this lies in consideration of the absorption kinetics. Analysis of the kinetics of phosphate absorption indicates that the absorption rate is not limited by the rate of ATP turnover; that ATP turnover must be as fast or faster than the rate-limiting absorption step. As a result, the concentration of ATP would be maintained at a low level.

The large percentage of the absorbed P^{32} appearing as inorganic orthophosphate is derived as a product of internal metabolism and breakdown of a labile compound. The fact that the P_i fraction is not exchangeable with phosphate in the external absorption solution and is apparently a product of DPNH-linked absorption suggests that it must have been in some high energy combination. Teleologically, the formation of only P_i from a high energy phosphate combination seems inconsistent unless energy is conserved through formation of a labile compound such as acetyl phosphate. It is apparent from the present studies, however, that the turnover rates for the individual products of phosphate absorption are so rapid that the initial precursor can not be identified by observation of the labelling of the phosphate esters.

Summary

Orthophosphate absorbed by barley roots is incorporated into five major compounds which incorporate 80 to 90 % of the total phosphate absorbed in periods from 15 seconds to 2 hours. The kinetics of the incorporation into each of the compounds shows that all five are products of the rate-limiting absorption step. UDPG, G-1-P and P_i appear to be the earliest compounds among the five in sequence of phosphate incorporation. Four of the compounds, UDPG, G-1-P and two unknowns arise from absorption through one site and P_i arises from another. This is shown by the sequence of P^{32} incorporation and the differential effects of phosphate concentration, pH and various inhibitors and activators. Further, the specific effects of the activators and inhibitors suggest that UDPG, G-1-P and the two unknowns arise from absorption coupled to cytochrome b of the respiratory chain. P_i is apparently a product of DPNH-linked absorption.

Acknowledgements

The authors wish to express gratitude to Dr. S. B. Hendricks and Mr. J. E. Leggett for their interest, criticism, and assistance.

Literature Cited

1. Benson, A. A. 1957. Sugar phosphates, paper, and column chromatography. In: Methods in Enzymology III, S. Colowick, ed. 110–129.
2. Epstein, E. and C. E. Hagen 1952. A kinetic study of the absorption of alkali cations by barley roots. Plant Physiol. 27: 457–474.
3. Ginsburg, V., P. K. Stumpf, and W. Z. Hassid 1956. The isolation of uridine diphosphate derivatives of D-glucose, D-galactose, D-xylose and L-arabinose from mung bean seedlings. Jour. Biol. Chem. 233: 977–983.
4. Hagen, C. E. and H. T. Hopkins 1955. Ionic species of orthophosphate absorption by barley roots. Plant Physiol. 30: 193–199.
5. Hagen, C. E., J. E. Leggett, and P. C. Jackson 1957. The sites of orthophosphate uptake by barley roots. Proc. Nat. Acad. Sci. 43: 496–506.
6. Hanes, C. S. and F. A. Isherwood 1949. Separation of the phosphoric esters on the filter paper chromatogram. Nature 164: 1107.
7. Loughman, B. C. and R. S. Russell 1957. The absorption and utilization of phosphate by young barley plants. IV. The initial stages of phosphate metabolism in roots. Jour. Exp. Bot. 8: 280.
8. Miettinen, J. K. and T. Savioja 1958. Uptake of orthophosphate by normal and P-deficient pea plants. Suomen Kemistilehti 31: 84–90.
9. Pabst Laboratories. 1956. Ultraviolet absorption of 5′ ribonucleotides. Circular OR-10, p. 20.
10. Partridge, S. M. 1949. Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. Nature 164: 443.
11. Pons, W. A. and J. D. Guthrie 1946. Determination of inorganic phosphorus in plant materials. Ind. Eng. Chem., Anal. Ed. 18: 184–186.
12. Runeckles, V. C. and Gleb Krotkov 1957. The separation of phosphate esters and other metabolites by ionophoresis and chromatography on paper. Arch. Biochem. Biophys. 70: 442–453.
13. Truog, E. and A. H. Meyer 1929. Improvements in the Deniges colorimetric method for phosphorus and arsenic. Ind. Eng. Chem., Anal. Ed. 1: 136–139.