

D-Xylulokinase and D-Ribulokinase in Higher Plants^{1, 2, 3, 4}

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Although D-xylose and D-ribose can be utilized in respiration (3, 7, 27) or in polysaccharide formation by higher plants, the initial metabolic reactions by which they are introduced into the general metabolic stream have not been firmly established. Several workers (2, 3, 14, 17) have suggested that these aldopentoses are converted to intermediates of the pentose phosphate pathway, probably to D-xylulose 5-phosphate and D-ribulose 5-phosphate, respectively.

Previous work in this laboratory (19, 20) has demonstrated the presence of xylose isomerase in plants. A partially-purified preparation of the enzyme catalyzes the isomerization of D-xylose and D-ribose to the corresponding 2-pentuloses. Phosphorylation of D-xylulose or D-ribulose, catalyzed by the appropriate kinase, would complete the sequence of reactions necessary for the introduction of D-xylose or D-ribose into the pentose phosphate pathway in higher plants.

This paper presents evidence that higher plants contain D-xylulokinase. The enzyme has been obtained substantially free of xylose isomerase. Both D-xylulose and D-ribulose can be phosphorylated by an enzyme preparation from pea meal.

Materials and Methods

Substrates. D-Xylose, D-ribose, ATP, P-enolpyruvate (tricyclohexylammonium salt), and NADH were obtained from commercial sources. D-Xylulose was prepared according to Touster (26) and D-ribulose according to Glathaar and Reichstein (8). Both ketopentoses were further purified by paper chromatography.

D-Xylulose-1-C¹⁴ was prepared by enzymic isomerization of D-xylose-1-C¹⁴ and used without separation from the remaining aldose. A typical reaction mixture contained 0.05 M Tris-maleate buffer, pH 7.4, 0.001 M cysteine, 0.005 M MnCl₂, 0.056 M D-xylose-1-C¹⁴, and 1.5 units of wheat germ xylose isomerase (20) in a total volume of 0.60 ml. The mixture was

incubated for 6 hours at 30° and then stored frozen. The mixture, which contained 10 to 15 % of the pentose as xylulose, is referred to elsewhere in this paper as the isomerization mixture.

Enzymes. Xylulokinase was extracted from pea meal or wheat germ with 4 parts (w/v) of 0.05 M Tris-maleate buffer, pH 7.4, 0.001 M cysteine, for 60 to 75 minutes. All steps were carried out at 0 to 5°. The suspension was centrifuged at 3000 × g for 10 minutes to remove debris. Seedlings were ground with sand in Tris-maleate buffer in a chilled mortar for 2 minutes, and the pulp was strained through cheesecloth prior to centrifugation. Endogenous low-molecular weight materials were removed by gel filtration through small columns of Sephadex G-50.

Xylose isomerase was prepared as described previously (20). Lactate dehydrogenase and pyruvate kinase were purchased from Sigma Chemical Company.

Separation of Xylose Isomerase and D-Xylulokinase from Pea Meal. Pea meal extract (see above) was adjusted to pH 7.4 with alkali. Solid ammonium sulfate (35 g/100 ml of extract) was added to bring the mixture to 55 % saturation with the salt (based on the solubility at 25°). The precipitate was removed by centrifugation for 15 minutes at 12,000 × g. The supernatant was taken to 75 % saturation with additional ammonium sulfate (14 g/100 ml). The precipitate was collected by centrifugation and dissolved in 0.05 M Tris-maleate buffer, pH 7.4, 0.001 M cysteine.

The fraction which precipitated between 55 and 75 % saturation with ammonium sulfate was stable for at least 5 weeks at -15° and could be frozen and thawed repeatedly without appreciable loss. However, most of the kinase activity was lost within 4 days at -15° after removal of the ammonium sulfate by dialysis or gel filtration.

Analytical Methods. Xylose isomerase and ribose isomerase were assayed as described previously (20).

Two general methods of assays were used to demonstrate kinase activity: A) For relatively crude and slightly active preparations it was advantageous to use C-14 labeled D-xylulose (as generated from D-xylose-C¹⁴ with xylose isomerase). The phosphorylated product was isolated 1 of 2 ways as described below, as the alcohol-insoluble Ba salt in the precipitation assay, or by separation on an ion-exchange column in the column assay. B) The second general method, (Spectrophotometric assay) suitable

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for more active preparations, was based on the well-established procedure of measuring liberated ADP by coupling to pyruvate kinase and lactic acid dehydrogenase. In several cases phosphorylation was followed by measuring release of H^+ , as a confirmatory measure, using a pH-stat.

In the precipitation or column assays the kinase assay mixture consisted of 0.028 M Tris-maleate buffer, pH 7.4, 0.002 M cysteine, 0.0019 M $MnCl_2$ (from the isomerization mixture), 0.007 M each of $MgCl_2$, ATP, and NaF, and 0.018 to 0.024 M D-xylose plus D-xylulose in a final volume of 0.14 ml except as otherwise indicated. The reaction was usually carried out at 30° for 2 hours unless otherwise stated. ATP was omitted from the controls until the end of the incubation. In the precipitation assay, the reaction mixtures were deproteinized with 10% trichloroacetic acid and fructose 6-P (5 μ moles per tube) was added as a carrier. The supernatants were made alkaline (pink to phenolphthalein) and the phosphate esters were precipitated by the addition of 100 or 200 μ moles of barium acetate and 4 volumes of ethanol. The mixtures were chilled overnight and the precipitates were collected by suction filtration and washed with 80 to 95% ethanol and acetone. The filter papers carrying the precipitates were transferred to aluminum planchets and counted. In the column assay, which was adapted from the galactokinase assays of Horowitz (9) and Starlinger (24), the reaction mixtures were diluted with 0.4 ml of water. The reactions were stopped by heating the tubes for 2 minutes in a boiling water bath, and the mixtures were cooled and centrifuged. Aliquots (400 μ l) of the supernatants were placed on Dowex-1-Cl ($\times 8$, 50–100 mesh) microcolumns (0.3 \times 4 cm). The columns were washed with five 0.4-ml portions of water to remove pentoses. Phosphate esters were eluted onto plastic phanchets with three 0.2-ml portions of 3 N HCl. The samples were air-dried and counted.

Preparations which were virtually devoid of xylose isomerase, i.e., the 55 to 75% ammonium sulfate fraction from pea meal, were assayed with D-xylulose instead of a mixture of D-xylose and D-xylulose as the starting substrate. In the spectrophotometric assay the reaction mixture contained 0.07 M Tris-HCl buffer, pH 7.5, 0.0014 M cysteine, 0.005 M $MgCl_2$, 0.003 M ATP, 0.040 M D-xylulose (omitted from the controls) and enzyme in a total of 0.50 ml. Because the reaction mixture was sometimes turbid and of high OD, it was not feasible to attempt continuous measurements, as is usually done in the analogous pentokinase reactions previously described (1, 4, 25). The reaction mixture, after incubation at 30° for the desired period, was heated to deproteinize it as described for the column assay. Samples of the supernatants were analyzed for ADP by the coupled pyruvate kinase-lactate dehydrogenase method. The ADP assay mixture contained a suitable aliquot of the deproteinized kinase reaction mixture and final concentrations of the other substances

as shown: 0.057 M Tris-HCl buffer, pH 7.5, 0.001 M cysteine, 0.006 M $MgCl_2$, 0.00075 M P-enolpyruvate, 0.0003 M NADH, and 5 to 10 μ g (1.6–3.3 units) of lactate dehydrogenase in 1.0 ml. The initial absorbance at 340 m μ was measured ($OD_{340} = 0.6$ – 0.8), and excess pyruvate kinase was added (4–10 μ g, or 1.0–2.5 units). The OD_{340} was followed at 25° until the value reached a minimum.

Protein was estimated as previously described (20). Radioactive samples were counted with a Nuclear-Chicago Model D-47 Geiger tube fitted with a Micro-mil window. Radioactivity on paper chromatograms was detected with a Forro or a Vanguard (Model 880) scanner.

Paper Chromatography. The following solvents were used for paper chromatography of sugars: ethyl acetate-water-acetic acid (10:6:5, v/v) (15), ethyl acetate-pyridine-water (8:2:1, v/v) (28), phenol-water (4:1, v/v) (18), and *n*-butanol-pyridine-water (10:3:3 v/v) (10). Sugars were detected by means of a modified $AgNO_3$ -NaOH dip (23), or by orcinol-trichloroacetic acid spray (12) followed by *N,N*-dimethyl-*p*-phenylenediamine spray (13, 22).

Results

Incorporation of C¹⁴-Pentose into Phosphate Esters. Radioactivity derived from C¹⁴-xylose or xylulose was incorporated into products which formed ethanol-insoluble barium salts, or could be retained on anion-exchange columns, when the isomerization mixture was incubated with ATP, $MgCl_2$, and extracts of pea meal or wheat germ. The incorporation was substantially reduced when ATP was omitted. However, ATP was added to the controls at the end of the incubation as a control for the possible occlusion of radioactive pentoses in the precipitate. When crude enzyme extracts were used, this method probably measured a mixture of phosphate esters, because the D-xylulose-5-phosphate formed initially was undoubtedly utilized in other reactions of the pentose phosphate pathway.

The incorporation of C¹⁴-pentose was maximal between pH 7.0 and 7.6 (fig 1). This pH optimum is similar to the values reported for most pentokinases (5). Boiled extracts did not catalyze incorporation. The extent of incorporation increased linearly with time of incubation (fig 2) and with enzyme concentration (fig 3). These results indicate that this assay is a valid measure of the activity of an enzyme. The incorporation was stimulated by about 25% when 0.03 M inorganic phosphate was present in the reaction mixture.

Enzyme Sources. Several plant tissues were assayed for D-xylulokinase activity (table I). Readily demonstrable levels of activity were present in pea meal, spinach leaf acetone powder, wheat germ and wheat seedling acetone powder. Assays of seedling extracts gave inconclusive results. Wheat germ xylose isomerase contained demonstrable

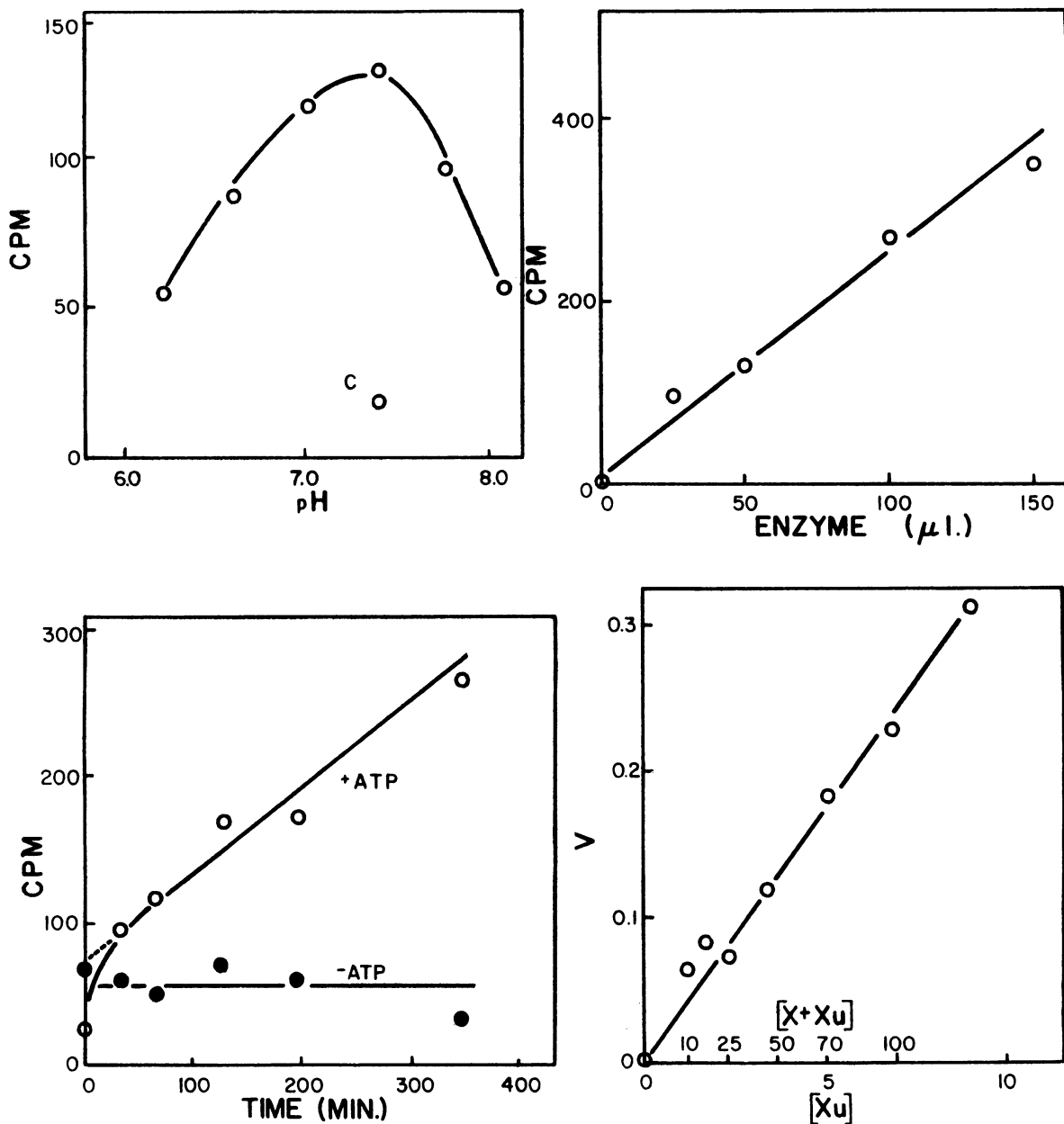


FIG. 1. (*upper left*). Effect of pH on incorporation of C^{14} -pentose into Ba-ethanol precipitate. Reaction mixtures included 5 μ moles of Tris-maleate buffer (pH as indicated) plus 1.5 μ moles at pH 7.4, 1.9 μ moles xylose plus xylulose (10,000 cpm), and 0.05 ml of crude wheat germ extract (pH adjusted to values shown) in 0.12 ml. C: control (ATP omitted).

FIG. 2 (*lower left*). D-Xylulokinase activity as function of time. The reaction mixtures contained 20 μ moles of pentose (isomerization mixture, 31,000 cpm), $MgCl_2$ and 0.25 ml of pea meal extract in a total volume of 0.70 ml. Aliquots (0.10 ml) were removed for analysis by the precipitation method. Conditions: 30°, pH 7.4.

FIG. 3 (*upper right*). D-Xylulokinase activity as function of enzyme concentration. Reaction mixtures contained 5 μ moles of pentose (isomerization mixture, 15,000 cpm), crude pea meal extract, $MgCl_2$, ATP and buffer in a total volume of 0.28 ml. The control values (ATP omitted) were the same in the presence or absence of enzyme, and averaged 78 cpm. Corrected cpm values are shown. The precipitation method was used. Reaction conditions: 80 minutes, 30°, pH 7.4.

FIG. 4 (*lower right*). D-Xylulokinase activity as function of substrate concentration. Reaction mixtures contained 15,000 cpm of C^{14} -pentose (isomerization mixture) and 0.10 ml of crude pea meal extract in a total volume of 0.28 ml. Assay conditions: 135 minutes, 30°, pH 7.4. The column method was used. Legend: V-velocity (μ moles of pentose incorporated in 135 minutes); X, D-xylose; X + Xu, D-xylose plus D-xylulose. Substrate concentrations: $M \times 10^3$.

Table I. Occurrence of D-Xylulokinase in Plant Tissues

The precipitation assay was used. Wet weights for fresh tissues; dry weights for acetone powders or wheat germ. Extractions were made with 4 to 6 volumes of buffer.

| Tissue | Pentose incorp. μ moles/g per hr | Relative* activity |
|---|---|-----------------------|
| Expt. A (1.65 μ moles pentose, 60000 cpm) | | |
| Pea meal extract | 0.75 | 0.6 |
| Wheat germ extract | 0.41 | 1.0 |
| Spinach acetone powder | 0.27 | 0.4 |
| Expt. B (4.0 μ moles pentose, 15000 cpm) | | |
| Pea meal extract | 2.64 | |
| Wheat seedling (3 day) acetone powder | 2.17 | |
| Corn (3-day seedlings) | 0.31 | |

* The relative activity of wheat germ extract is taken as 1.0.

Table II. Cation Requirements for D-Xylulokinase

All reaction mixtures contained 0.0018 M Mn^{++} (See Materials and Methods), and 4 μ moles of C^{14} -pentose (15,000 cpm), added as the isomerization mixture. Pea meal extract was filtered through Sephadex G-50 to remove endogenous cations. The column assay was used.

| Salt added (0.007 M) | Kinase activity | |
|-------------------------|--|-------------|
| | Net incorp. of C^{14} pentose cpm | μ moles |
| $MgCl_2$ (ATP omitted) | 0 | 0 |
| $MgCl_2$ | 137 | 0.030 |
| $MnCl_2$ | 77 | 0.021 |
| $CoCl_2$ | 109 | 0.029 |
| $ZnSO_4$ | 33 | 0.009 |
| None | 65 | 0.017 |
| " + 0.0014 M EDTA | 39 | 0.010 |

amounts of xylulokinase even after 20-fold purification.

Cation and Thiol Requirements. The xylulokinase activity was stimulated by Co^{++} , Mg^{++} , and perhaps slightly by Mn^{++} ions, but was depressed by Zn^{++} ions (table II). Endogenous cations were

removed from the pea meal extract by gel filtration through Sephadex G-50 prior to testing. However the assay mixtures contained 0.0018 M Mn^{++} introduced with the isomerization mixture. Some activity was observed with no further addition of divalent cations. Inhibition of the kinase activity by 0.0014 M EDTA also pointed to a metal requirement. Enzyme activity obtained on extracting pea meal with 0.05 M, pH 7.4 buffer (phosphate or Tris-maleate) was reduced by 80 % if 0.002 M EDTA was present.

Cysteine (0.002 M) and GSH (0.002 M) both stimulated the xylulokinase activity of a crude wheat germ extract, but *p*-chloromercuribenzoate (0.0015 M) completely inhibited the activity. These results showed that the xylulokinase activity depends on reduced sulfhydryl groups.

Separation of Xylulokinase from Xylose Isomerase. Xylulokinase could be separated from xylose isomerase by fractionation with ammonium sulfate (table III). The xylose isomerase activity was almost completely precipitated below 55 % saturation with ammonium sulfate (76 % recovery of the isomerase activity) together with some xylulokinase activity. Between 55 and 75 % saturation with ammonium sulfate, a fraction was obtained which contained 16 % of the xylulokinase but negligible xylose isomerase. Since removal of the isomerase was the primary objective, no further attempts to improve the recovery of the kinase were made at this time. The failure to obtain a fraction having increased specific activity of the kinase may be due to inactivation during the fractionation and subsequent dialysis.

Effect of Substrate Concentration. With a crude enzyme preparation from pea meal, incorporation of C^{14} -pentose (supplied as the isomerization mixture) was directly proportional to the substrate concentration at the xylulose concentrations used (fig 4). With the 55 to 75 % ammonium sulfate fraction from pea meal, the xylulokinase was saturated at approximately 0.08 M D-xylose, under the conditions employed in this experiment (0.005 M ATP).

No experiments to determine the optimum ATP and Mg^{++} concentrations were carried out with the enzyme preparation at this stage of purification.

Table III. Ammonium Sulfate Fractionation of Pea Meal Extract

Preparations were dialyzed for 4 hours at 2° against 0.05 M Tris-maleate buffer, pH 7.4, 0.001 M with respect to cysteine, before assay. The column assay was used for D-xylulokinase.

| Fraction | D-Xylulokinase units* | Total activity | | Total protein mg | Kinase specific activity units mg protein |
|-----------------------|--------------------------|--------------------------|-------------------------------|------------------------|---|
| | | D-Xylulokinase units* | Xylose isomerase units* | | |
| 1. Crude extract | 47.8 | | 154 | 3,930 | 12.0 |
| 2. 0-55 % saturation | 5.0 | | 117 | 1,670 | 3.0 |
| 3. 55-75 % saturation | 7.2 | | 0 | 980 | 7.4 |
| 4. 75-90 % saturation | 0.5 | | 0 | 220 | 2.3 |

* Units are defined as follows: D-xylulokinase: 1 μ mole of D-xylose phosphorylated per hour; xylose isomerase: 1 μ mole of D-xylose formed per hour (20).

Substrate Specificity. Several pentoses and fructose were tested as possible substrates with the 55 to 75% ammonium sulfate fraction from pea meal (table IV). D-Xylulose and D-ribulose both served as substrates; however, their respective aldose isomers, D-xylose and D-ribose, were not utilized. D-Fructose had only slight, if any, activity.

Since D-xylose and D-ribose are apparently isomerized by the same enzyme in higher plants (20), it is interesting to know whether the subsequent phosphorylation of the corresponding 2-pentuloses is catalyzed by the same kinase. Nonadditive formation of ADP in a mixed substrate reaction (table V) suggests that the same kinase catalyzes the phosphorylation of D-xylulose and D-ribulose in higher plants.

Acid Production. Net increase in acidity during the reaction was shown by means of a titrimetric assay at pH 7.5. D-Xylulose was omitted from the controls. The release of titratable hydrogen ions is consistent with, but not proof of, the presence of a xylulokinase in this system.

Identification of the Reaction Product. D-Xylulose (1.08 mmoles) was incubated with 750 μ moles of $MgCl_2$, 300 μ moles of ATP, 15 μ moles of cysteine, 125 μ moles of Tris-HCl Buffer, pH 7.5, and 5 ml of xylulokinase (55-75% ammonium sulfate fraction from pea meal, filtered through Sephadex G-50) in a total volume of 10 ml at 30°. The pH was maintained between 6.8 and 7.6 by the addition of 0.1 N KOH. After 6 hours, the reaction was stopped by adjusting the pH to 4.1 with acetic acid. Protein was removed by centrifugation and the product was isolated according to the procedure of Anderson and Wood (1). After being stored for several days at -15°, the barium salts were filtered, washed with ethanol, and air-dried.

The product was dissolved in dilute HCl, treated with Dowex-50 (H^+) to remove barium ions, and dephosphorylated with prostatic acid phosphatase at

pH 5 for 5 hours, and again deionized with Dowex-50 (H^+). Chromatography of the product in ethyl acetate-pyridine-water (8:2:1, v/v) (28), followed by dipping the paper in $AgNO_3$ -NaOH reagent (23), revealed a spot having a mobility similar to xylulose. This component was not apparent on chromatograms of the product which had not been subjected to phosphatase treatment. The product prior to dephosphorylation remained at the origin on chromatography in this solvent, as has been observed for other phosphate esters.

The sugar obtained by dephosphorylation of the product showed the same rate of color development in the cysteine-carbazole reaction (2,6) as did authentic xylulose. The sugar also gave a positive ketose test with the orcinol-trichloroacetic acid spray reagent (12) and a purple color similar to that produced with xylulose by the *N,N*-dimethyl-*p*-phenylenediamine reagent (22).

Discussion

The earlier demonstration that plants can convert D-xylose to D-xylulose (19,20) supports the hypothesis that D-xylose is metabolized in higher plants via the pentose phosphate pathway before it is utilized in respiration or in polysaccharide formation. D-Xylulose probably enters the pentose phosphate pathway by being converted to D-xylulose-5-phosphate in the xylulokinase reaction. Plants contain D-ribulose-5-phosphate 3-epimerase, phosphoriboisomerase and transketolase which catalyze the conversion of D-xylulose-5-phosphate to other intermediates of the pentose phosphate pathway.

Both the characteristics of the enzyme reaction and of the product indicate that the enzyme described here is a xylulokinase. The enzyme activity was detected in several different plants, but no survey has been made to determine the extent of its occurrence in higher plants. Kinases for both isomers of xylulose (1, 5, 16, 25) and ribulose (4, 5, 11, 21, 22) have been found in a variety of animal and microbial tissues.

Comparison of the specificity of the plant xylulokinase with those from other sources is of interest because our results suggest that the enzyme from pea meal also catalyzes the phosphorylation of D-ribulose. Final proof must await purification of the enzyme. If the plant xylulokinase is nonspecific for the configuration on carbon atom C-3 of the ketopentose, it is unlike other xylulokinases for which substrate specificities have been determined. The L-isomers have not yet been tested. Some ribulokinases can utilize both D- and L-ribulose (4, 21); however, xylulokinase appear to be active on only 1 enantiomorph of xylulose (1, 5, 25) and inactive against ribulose.

The mechanism of ribose utilization in plants may differ from that in other organisms, some of which can phosphorylate ribose directly by the action of ribokinase (5). In such cases, ribose-5-phosphate is

Table IV. *Substrate Specificity of D-Xylulokinase*

Reaction mixture contained 0.20 ml of a Sephadex G-50 eluate of the 55 to 75% ammonium sulfate fraction from pea meal and 1.5 μ moles of ATP (0.003 M). The spectrophotometric assay was used. Conditions: 60 minutes, 30°, pH 7.5.

| Pentose | Conc (M) | ADP formed μ moles |
|-------------------------|----------|------------------------|
| None | ... | 0.29 |
| D-Xylulose | 0.040 | 0.91 |
| D-Xylose | 0.053 | 0.22 |
| D-Xylose | 0.16 | 0.23 |
| D-Ribulose | 0.10 | 0.72 |
| D-Ribose | 0.050 | 0.20 |
| D-Ribose | 0.15 | 0.23 |
| D-Xylulose and D-Xylose | 0.040 | |
| D-Xylose | 0.053 | 0.95 |
| D-Xylose and D-Ribulose | 0.040 | |
| D-Ribulose | 0.10 | 0.83 |

the first intermediate of the pentose phosphate pathway. The failure to detect ribokinase in plants supports the idea that plants need ribose isomerase and ribulokinase for the introduction of ribose into the pentose phosphate pathway. No instances of the direct phosphorylation of xylose have been demonstrated.

The assay methods employed in this study demonstrated the presence of a kinase in several ways: A) incorporation of C¹⁴-xylulose into products which formed ethanol-insoluble barium salts or could be retained on anion-exchange columns, B) increase in the formation of ADP from ATP in the presence of xylulose or ribulose, and C) production of increased titratable hydrogen ion in the reaction.

The reaction product from D-xylulose was isolated and partially characterized. The product behaved on chromatography in ethyl acetate-pyridine-water (8:2:1, v/v) similar to an organic phosphate ester. The position of the phosphate group, which remains to be established, is assumed to be carbon atom C-5, because all known xylulokinases are xylulo-5-kinases, and because phosphoxylulomutases for the conversion of xylulose-1-phosphate to the 5-phosphate are unknown. The principal sugar obtained after dephosphorylation of the product was identified as xylulose A) by its chromatographic mobility, B) by its rate of color development in the cysteine-carbazole reaction, C) and by its color reactions with spray reagents which differentiate xylulose from other ketoses.

It seems therefore, that D-xylose and D-ribose could both be introduced into the pentose phosphate pathway in higher plants by isomerization to D-xylulose and D-ribulose, respectively, which would then be subsequently phosphorylated. If, as it seems reasonable to expect, phosphorylation would occur at the hydroxyl group on carbon atom C-5, the resulting products would be D-xylulose-5-phosphate and D-ribulose-5-phosphate, both of which are intermediates of the pentose phosphate pathway.

Summary

D-Xylulokinase occurs in tissues of higher plants, including pea seed, spinach leaf, wheat germ, and wheat seedling. The enzyme can be obtained substantially free of xylose isomerase by fractionation with ammonium sulfate. Wheat germ D-xylulokinase shows optimal activity between pH 7.0 and 7.6. The enzyme from pea meal requires divalent cations (Mg⁺⁺, Mn⁺⁺, or Co⁺⁺) and sulfhydryl groups for maximum activity. Both D-xylulose and D-ribulose are phosphorylated, but D-xylose and D-ribose are not phosphorylated by the kinase.

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Movement of Tritiated Water in the Root System of *Helianthus annuus* in the Presence and Absence of Transpiration¹

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Ordin and Gairon (7) have studied the effect of osmotic substrate on the diffusion rate of tritiated water into root sections of *Vicia* and *Zea*. They found that while osmotic stress administered with mannitol had no effect on the equilibration of tritiated water in *Zea* an enhancement was observed in *Vicia* roots. Since the above results were obtained with excised roots the objective of this paper was to establish the pattern with which tritiated water equilibrates in root systems in the presence and absence of transpiration from the shoots.

Materials and Methods

Plant Preparation. The plants were grown and treatments carried out in a controlled-environment chamber equipped with a ceiling bank of 14 high-intensity, power-groove, fluorescent tubes 2 m long and spaced at intervals of about 13 cm, with nine 100-w incandescent bulbs interspersed to furnish red and yellow spectral wavelengths.

All plants were grown at 20°, 3000 ft-c of light, with 12-hour days and 12-hour nights.

The nutrient solution used, half-strength Hoagland's No. 2 (3), included the A-Z micronutrient solution except that 1 ml of sodium iron sequestrene

(21 g/liter) instead of iron tartrate was used per liter of nutrient solution.

Tritium was supplied as liquid tritiated water (THO), with a specific activity of 0.1 $\mu\text{C}/\text{ml}$.

Seeds of sunflower (*Helianthus annuus*, Advance) were selected for uniform size and barely covered with distilled water in the dark for 24 hours. Seeds with a visible radicle were then planted in vermiculite in a porous clay pot on a bench in the controlled-environment chamber. The pot was irrigated daily with nutrient solution and allowed to drain. When the cotyledons were fully expanded (about 7 days), the seedlings were reselected for uniformity, maximum size, deep-green color, and morphological similarity of roots and shoots.

The root-stem transition was marked with indelible red ink, and each seedling was transferred to a 1-liter aluminum-foil wrapped glass jar containing nutrient solution. The seedlings were secured by a wad of unwettable cotton in a slot in a paraffin-coated cork fitted snugly into the top of the jar. Two holes in the cork permitted gas exchange between the solution and the atmosphere. The root-stem transition was held about 2 cm above the surface of the solution. Solution volume was restored daily with distilled water.

Plant Selection. At time of treatment (15-30 days) the plants were selected for morphologic uniformity. Water use by each plant was determined by measuring weight loss over a 12-hour light period

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