THE ROLE OF XYLULOSE 5-PHOSPHATE IN XYLOSE METABOLISM OF LACTOBACILLUS PENTOSUS

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Lampen (1) observed that cell-free extracts of Lactobacillus pentosus grown on D-xylose catalyzed the disappearance of D-xylose in the presence of ATP.1 The phosphate esters which accumulated were identified as a mixture of D-ribose 5-phosphate and D-ribulose 5-phosphate. Neither D-xylose 5-phosphate nor D-xylulose 5-phosphate was found in the reaction mixture. Mitsuhashi and Lampen (2) also demonstrated the presence of xylose isomerase in these extracts and, on the basis of the fact that D-xylulose was esterified more rapidly than D-xylose, it was postulated that the following reaction sequence takes place.

\[
\text{d-Xylose} \rightleftharpoons \text{d-xylulose} \xrightarrow{\text{ATP}} \text{xylulose 5-phosphate} \rightleftharpoons \text{ribulose 5-phosphate} \rightleftharpoons \text{ribose 5-phosphate}
\]

Hochster (3) has obtained evidence for a similar series of reactions in Pseudomonas hydrophila. With extracts of this organism D-xylose is converted to a mixture of pentose phosphates, including a phosphate ester of xylulose. Slein (4) has obtained extracts of Pasteurella pestis which contain a xylose isomerase and a xylulose kinase.

It is clear from these results (1-4) that a mechanism exists for the conversion of xylulose 5-phosphate to ribulose 5-phosphate. Since transketolase cleaves substrates with either configuration with respect to the hydroxyl group at carbon atom 3 (5), this enzyme might be expected to provide such a mechanism. Thus a cleavage of xylulose 5-phosphate to "active glycolaldehyde" and triose phosphate, followed by a recondensation of these fragments, would be expected to produce both epimeric ketose esters. However, Ashwell and Hickman (6) have described an enzyme


1 The following abbreviations are used: ADP, adenosine diphosphate; ATP, adenosine triphosphate; DPN+, oxidized diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane; Ru-5-P, n-ribulose 5-phosphate; Xu-5-P, n-xylulose 5-phosphate; PKPE, phosphoketopentepimerase; ATPase, adenosine triphosphatase; EDTA, ethylenediaminetetraacetate; TCA, trichloroacetic acid; TK, transketolase.

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preparation from mouse spleen which converts ribose 5-phosphate to a mixture of ribulose phosphate and xylulose phosphate in the absence of transketolase. They suggest the existence of a specific enzyme which catalyzes the equilibration between the two pentose phosphates.

In the present communication data are presented for the occurrence, in cell-free extracts of \textit{L. pentosus} grown on xylose, of both a specific xylulose kinase and a specific epimerase. The latter enzyme catalyzes the equilibration of D-xylulose 5-phosphate and D-ribulose 5-phosphate and will be referred to as phosphoketopentoepimerase (PKPE). The isolation and properties of D-xylulose 5-phosphate are also recorded. This ester is cleaved by purified spinach transketolase at the same rate as is ribulose 5-phosphate. It is thus established that spinach transketolase has no specificity for a particular configuration of the hydroxyl group of the 3rd carbon atom of the ketopentose.\(^2\)

\textit{Methods}

\textit{Materials}—Barium ribulose 5-phosphate was prepared by enzymatic oxidation of 6-phosphogluconate by yeast 6-phosphogluconic dehydrogenase (7). DPNH was prepared by the method of Ohlmeyer (8). D-Xylose (anhydrous) was obtained from Professor W. Z. Hassid of the University of California. Lithium phosphoenolpyruvate (91 per cent pure) was supplied by Mr. William E. Pricer of this Institute. D-Xylulose was prepared by the oxidation of D-arabitol by \textit{Acetobacter suboxydans} (9). Paper chromatographic analysis of the preparation showed it to be free of D-ribulose and D-xylose. Other substrates and cofactors were commercial preparations.

Glycerophosphate dehydrogenase and hexosephosphate isomerase were prepared from rabbit muscle extract by precipitation with ammonium sulfate (10). The fraction which precipitated between 0.2 and 0.5 saturation with ammonium sulfate was used as a source of hexosephosphate isomerase, and the fraction collected between 0.5 and 0.7 saturation was the source of glycerophosphate dehydrogenase. Crystalline aldolase was prepared according to the method of Taylor \textit{et al.} (11). Acid phosphatase was purified from potato by the method of Kornberg.\(^3\) Pyruvate kinase and

\(^2\) Recently, highly purified preparations of spinach transketolase have been obtained which show little activity with ribulose 5-phosphate unless a phosphoketopentoepimerase preparation is added. The apparent lack of specificity of the spinach transketolase preparations employed in the present work may be due to the presence of phosphoketopentoepimerase in these preparations. Srere and Racker (personal communication) have now shown that yeast transketolase is specific for xylulose 5-phosphate and other substrates with the trans configuration. The same may be true for the spinach enzyme.

\(^3\) Unpublished procedures.
lactic dehydrogenase were isolated by the method of Kornberg and Pricer (12). We are indebted to Dr. J. Hurwitz of this Institute for a generous supply of spinach phosphoribulokinase (13).

Dowex 1, 10 per cent cross-linked, was used in the formate form.

Analytical Methods—Spectrophotometric measurements were made at room temperature with a Beckman model DU spectrophotometer. Pentoses were measured by the orcinol test according to Mejbaum (14), except that heating was continued for 40 minutes.

The per cent of xylulose in a mixture of ribulose and xylulose was determined by the carbazole method (15) as modified by Ashwell and Hickman (6). The method is based on the fact that color development with ribulose in the cysteine-carbazole reaction is more rapid than with xylulose. Two readings are taken at 540 m$\mu$ in the Coleman junior spectrophotometer, the first at 15 minutes and the second at 120 minutes. The per cent of xylulose in a mixture may then be calculated by the increase in absorption in the 15 to 120 minute interval, based on the behavior of authentic standards.

Inorganic phosphate was estimated by the method of Fiske and Subbarow (16). Total organic phosphate was determined by a wet ashing procedure.

ADP was estimated by a modification of the spectrophotometric method of Kornberg and Pricer (12). Protein was determined by the turbidimetric method of Büchler (17).

For the enzymatic hydrolysis of pentose phosphates 8 $\mu$moles of organic phosphate, 0.02 ml. of 0.5 M acetate buffer at pH 5.2, 0.01 ml. of 0.1 M cysteine at pH 6.5, 0.01 ml. of 0.1 M MgCl$_2$, and 0.02 ml. of potato phosphatase (17 units) in a total volume of 0.56 ml. were incubated for 120 minutes at 37°. The inorganic phosphate liberated was removed by addition of 0.02 ml. of 1 M barium acetate and 4 volumes of ethanol. The suspension was centrifuged and the supernatant solution lyophilized. The residue containing the free pentoses was dissolved in 0.5 ml. of water.

Chromatography was carried out on Whatman No. 1 paper with water-saturated phenol by a descending method. The chromatograms were sprayed with TCA-orcinol (18) and heated at 100° for 5 minutes. Sugars were identified by comparison with the $R_f$ values of known pentoses and by the characteristic gray-blue color of xylulose, the red-orange of ribulose, and the fluorescent properties of ribulose when viewed under ultraviolet irradiation.

Enzyme Assays—Two methods were employed for the assay of xylulose kinase. The first, used in preliminary observations, depended on the disappearance of free pentose as assayed by the orcinol reaction after the phosphate esters had been removed as alcohol-insoluble barium salts. The
reaction mixture contained 4 µmoles of ATP, 2 µmoles of D-xylose, 10 µmoles of MgCl₂, 20 µmoles of Tris buffer at pH 7.5, and enzyme to a total volume of 0.8 ml. Aliquots of 0.1 ml. were removed at intervals, added to 0.5 ml. of ethanol, and treated with 0.01 ml. of 20 per cent barium acetate. The barium salts were removed by centrifugation, and the supernatant solution was assayed directly for total pentose content. This assay was employed with crude enzyme extracts containing xylulose isomerase, which permitted the use of D-xylose as a substrate.

The second method was based on the appearance of ADP with D-xylose as the substrate and was employed when xylose isomerase was absent. The spectrophotometric procedure of Kornberg was followed with the inclusion of proper controls for ATPase activity. The assay system consisted of 0.6 µmole of phosphoenolpyruvate, 0.45 µmole of ATP (Sigma, crystalline), 0.5 unit of pyruvate kinase, 2 units of lactic dehydrogenase, 8 µmoles of phosphate buffer at pH 7.5, 1 µmole of magnesium chloride, 1 µmole of cysteine at pH 7.5, and 0.24 µmole of DPNH. The final volume was 1.2 ml. Xylulose kinase and 0.3 µmole of D-xylose were added rapidly. Xylulose was omitted from one cuvette; measurements of DPNH oxidation in this cuvette reflect ATPase activity. 1 unit was defined as the amount of enzyme required for a decrease of optical density of 1.0 per minute under the conditions of the test, corrected for ATPase activity. ATPase units were similarly defined. Specific activity is represented by the number of units per mg. of protein.

Spinach transketolase was assayed by the method of Horecker et al. (5).

**Purification of Xylulose Kinase**

*Preparation of Cell-Free Extracts—L. pentosus strain 124-2 (ATCC 8041)* was grown on a xylose-enriched medium from a culture provided by Professor H. A. Barker of the University of California. The culture medium contained 0.4 per cent Difco yeast extract, 1 per cent Difco nutrient broth, 1 per cent sodium acetate, 1 per cent D-xylose, 0.1 per cent glucose, 0.02 per cent MgSO₄·7H₂O, 0.001 per cent NaCl, 0.001 per cent FeSO₄·7H₂O, and 0.001 per cent MnSO₄·4H₂O. D-Xylose was autoclaved separately as a 10 per cent solution and then added aseptically to a sterilized medium. Incubations were for 24 hours at 37°. The cells were harvested at 2° with a Sharples supercentrifuge, washed with 0.02 M NaHCO₃, and stored at -16° as a thick paste. The frozen paste could be stored indefinitely with little loss in kinase activity. The yield was approximately 1.2 gm. per liter of medium. A suspension of 10 gm. of cells (wet weight) in 20 ml. of 0.01 M NaHCO₃ was exposed to a 9 kc. Raytheon sonic oscillator for 1 hour. Cell-free extracts were obtained by centrifuging the treated cells for 1 hour at 13,000 × g in the International
refrigerated centrifuge. The amber-colored extract (Table I) was stored at 0° for several months with little loss in kinase activity. It contains xylulose isomerase, phosphoriboisomerase, ATPase, xylulose kinase, transketolase, and phosphoketopentose-epimerase.

**Manganese Step**—The cell-free extract was treated with 0.05 volume of 1 M MnCl₂, kept for 30 minutes at 4°, and centrifuged at 13,000 × g for 5 minutes. The precipitate containing nucleoprotein and most of the ATPase activity was discarded.

**Calcium Phosphate Gel Step**—To the clear supernatant solution were added 3 volumes of calcium phosphate gel (19) (17.9 mg., dry weight, per ml.). The suspension was centrifuged at 0° and washed once with 3 volumes of water, after which inactive proteins were eluted with 20 ml. of 0.05 M potassium phosphate buffer, pH 7.7. (Trials should be conducted at this elution step with phosphate buffer solution of varying concentration, since occasionally the kinase is eluted with more dilute buffer.) The first eluate was discarded and the enzyme eluted from the gel with 20 ml. of 0.1 M phosphate buffer at pH 7.7.

**First (NH₄)₂SO₄ Step**—To the eluate (20 ml.) at 0° were added 30 ml. of saturated (at room temperature) ammonium sulfate solution, and the precipitate was discarded. 4.7 gm. of ammonium sulfate were added to the supernatant solution, and the precipitate was dissolved in water to a final volume of 5 ml.

**Acid-Heat Step and Ammonium Sulfate Step**—The solution was adjusted to pH 5.4 by the addition of 0.5 M KH₂PO₄. It was then warmed to 53° in 2 minutes and maintained at this temperature for 5 minutes. Higher temperatures produced considerable loss of activity. The heated solution was cooled to 4° and treated with 2.5 volumes of cold saturated ammonium sulfate. The heavy precipitate was discarded, and 1.9 gm. of ammonium

### Table I

#### Purification of Xylulose Kinase

<table>
<thead>
<tr>
<th>Step</th>
<th>Total volume</th>
<th>Units per ml.*</th>
<th>Specific activity*</th>
<th>Over-all per cent recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>20</td>
<td>(21)†</td>
<td>(0.24)</td>
<td></td>
</tr>
<tr>
<td>Mn supernatant solution</td>
<td>20</td>
<td>21</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Calcium phosphate gel eluate</td>
<td>20</td>
<td>16</td>
<td>5.09</td>
<td>80</td>
</tr>
<tr>
<td>1st (NH₄)₂SO₄ ppt</td>
<td>5</td>
<td>27</td>
<td>5.0</td>
<td>32</td>
</tr>
<tr>
<td>Heat treatment and (NH₄)₂SO₄ ppt...</td>
<td>5</td>
<td>21</td>
<td>16.0</td>
<td>25</td>
</tr>
</tbody>
</table>

* As defined in the text.
† Estimated as a minimal value.
sulfate were added to the supernatant solution. The precipitate was collected, dissolved in 5 ml. of water, and stored at 0°. This preparation contains 21 units of kinase, 1 unit of ATPase, 0.15 unit of transketolase per ml. of enzyme, and PKPE activity.

**Properties of Xylulose Kinase**—The purified preparations were stored at 0° for several months with little loss in activity. On exposure to 53° for 5 minutes, the following losses were observed: at pH 8, 66 per cent; pH 7.0, 60 per cent; pH 6.2, 50 per cent; pH 5.7, 30 per cent; and pH 5.4, 26 per cent. At 55°, pH 5.4, losses were much greater, and at 60° all activity disappeared.

L-Xylulose, D-ribulose, L-erythrulose, D-xylose, and D-ribose are inert as substrates. Since the enzyme is specific for D-xylulose, it can be employed for the spectrophotometric determination of small concentrations of D-xylulose in the presence of other sugars.

The pH optimum lies on a rather broad plateau between pH 7.0 and 7.9. The enzyme is activated by a number of divalent cations. At a final concentration of 0.005 M, the following quantities of ADP (in micromoles) were formed in 5 minutes at 25°: Mg++, 0.17; Mn++, 0.16; Fe++, 0.19; and Zn++, 0.17. Without addition of metal 0.08 μmole of ADP was found. The reaction mixture in these experiments contained 0.3 μmole of xylulose, 0.4 μmole of ATP, and 0.01 mg. of xylulose kinase in 0.68 ml. of 0.1 M Tris buffer, pH 7.5. Sulfhydryl compounds did not increase the rate.

**Preparation and Properties of D-Xylulose 5-Phosphate**

When the purified kinase preparations were incubated with D-xylulose and ATP, a mixture of xylulose phosphate and ribulose phosphate resulted, indicating the presence of PKPE. The mixture of xylulose phosphate and ribulose phosphate was separated from other reaction products by ion exchange chromatography, but these two components could not be separated from each other. In order to obtain the xylulose ester free of ribulose phosphate, the mixture of the two esters was further incubated with an excess of ATP and phosphoribulokinase (13), which converts Ru-5-P to the diphosphate ester. From this final mixture Xu-5-P is readily isolated by ion exchange chromatography.

The initial reaction mixture contained 8 mg. of xylulose kinase (105 units), 450 μmoles of D-xylulose, 480 μmoles of ATP, 100 μmoles of cysteine, in 15 ml. of 0.05 M NaHCO₃, containing 0.01 M MgCl₂. The gas phase was 95 per cent nitrogen and 5 per cent carbon dioxide; the temperature, 37°. The reaction was followed manometrically in the Warburg apparatus until the evolution of carbon dioxide ceased, indicating the completion of the phosphorylation reaction. The reaction mixture was cooled to 4° and placed on a 12 cm. X 2.5 sq. cm. Dowex 1-formate column, washed with
25 ml. of water, and eluted by gradient elution with 200 ml. of water in
the mixing chamber and 0.4 N formic acid containing 0.1 M sodium formate
in the reservoir. The volume of each fraction collected was 10 ml. The
fractions containing the phosphate esters (Fractions 32 to 42) were adjusted
to pH 6.2 with 8 ml. of 0.6 N barium hydroxide and 25 ml. of 2 N KOH
and precipitated with 4 volumes of ethanol. The precipitate was kept at
0° for 30 minutes, collected by centrifugation, washed with 10 ml. of 70
per cent ethanol, and dried in vacuo. The yield based on organic phosphate
was 390 μmoles of ester phosphate, containing approximately 175 μmoles
of D-xylulose 5-phosphate.

The barium salts were dissolved in 5 ml. of 0.05 N acetic acid, 0.8 ml. of
0.57 M Na₂SO₄ was added, and the barium sulfate removed by centrifuga-
tion. The phosphate esters were then incubated with 20 ml. of 0.05 M
NaHCO₃ containing 0.01 M MgCl₂, 100 μmoles of cysteine, 250 μmoles of
ATP, and 0.03 ml. of phosphoribulokinase (120 units, 0.48 mg. of protein).
The chromatographic procedure used in separating the mixed esters was
the same as that employed above. The fractions containing xylulose 5-phosphate (Fractions 30 to 34) were pooled and adjusted to pH 6.2 with
6 ml. of 0.6 N barium hydroxide and 13 ml. of 2 N KOH and precipitated
with 4 volumes of ethanol. The precipitate was collected by centrifuga-
tion, washed with ethanol, and dried in vacuo. The yield based on organic
phosphate was 167 μmoles.

The ketopentose moiety was characterized as follows: The barium salt
was converted to the sodium salt with sodium sulfate and 6 μmoles were
hydrolyzed with potato phosphatase as described in "Methods." The
dephosphorylated product was assayed directly by employing (a) xylulose
kinase and the ADP spectrophotometric assay system, and (b) the cysteine-
carbazole reaction as modified by Ashwell and Hickman (6) for estimating
the per cent of xylulose in the dephosphorylated reaction mixture. Ac-
cording to the former method xylulose accounted for 82 per cent of the
organic phosphate, while the colorimetric assay showed 96 per cent of the
ketopentose to be xylulose. (c) Paper chromatography as described in
"Methods" revealed only one component in the dephosphorylated product,
having the same Rf as authentic xylulose and giving the characteristic
gray-blue color when sprayed with the TCA-orcinol reagent. (d) A com-
parison of the absorption spectra given by authentic xylulose and the de-
phosphorylated product in the orcinol reaction is shown in Fig. 1. The
curves obtained with xylulose and the product of dephosphorylation were
very similar, but quite different from that given by ribulose. For xylulose
5-phosphate the absorption at 540 mμ was considerably depressed. The
extinction coefficient at 670 mμ was somewhat higher than that of free
xylulose. (e) As indicated previously, the rate of color development in
the cysteine-carbazole reaction is characteristic for xylulose, which requires more than an hour for full development and differentiates it from ribulose, which is completely converted in less than 10 minutes. The dephosphorylated product behaves quantitatively like xylulose in this test (Fig. 2).

Periodate oxidation of the phosphate ester gave somewhat low results, only 67 per cent of the theoretical quantity of periodate being consumed.

**Fig. 1.** Absorption spectra in the orcinol reaction. Xylulose 5-phosphate was the chromatographed reaction product. Experimental xylulose was the same substance after enzymatic dephosphorylation.

**Activity of Xylulose 5-Phosphate with Transketolase**—Since transketolase catalyzes the transfer of "active glycolaldehyde" from a number of ketol compounds to suitable acceptors, it was of interest to compare the reactivity of D-xylulose 5-phosphate and D-ribulose 5-phosphate, which differ only in the position of the hydroxyl group on the 3rd carbon atom.

Several assay systems were employed to test the reactivity of these substrates. The first may be described by the accompanying equations.

\[
\begin{align*}
\text{Xylulose 5-phosphate} & \xrightarrow{\text{TK}} \text{"active glycolaldehyde"} + \text{ribose phosphate} \\
\text{Sedoheptulose 7-phosphate} + \text{ribose phosphate} & \xrightarrow{\text{TK}} \text{ribulose 5-phosphate} & \xrightarrow{\text{isomerase}} \text{ribose 5-phosphate}
\end{align*}
\]
With the formation of triose phosphate as a measure of substrate activity, it was found that xylulose 5-phosphate was a good substrate for trans-

![Graph showing rate of color development in the cysteine-carbazole reaction.](image)

**Fig. 2.** Rate of color development in the cysteine-carbazole reaction. O represents an authentic sample of xylulose; X, the dephosphorylated product of keto-pentose phosphate prepared by the enzymatic phosphorylation of xylulose; ■, authentic sample of ribulose.

### Table II

**Pentose Phosphates As Substrates for Transketolase**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Triose phosphate formed per 10 min. $\mu$ mole $\times 10^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribose 5-phosphate</td>
<td>3.1</td>
</tr>
<tr>
<td>Ribulose 5-phosphate</td>
<td>3.4</td>
</tr>
<tr>
<td>Xylulose 5-phosphate</td>
<td>1.9</td>
</tr>
</tbody>
</table>

The assay system contained 0.5 $\mu$ mole of pentose phosphate, 0.01 ml. of DPNH, 1 mg. of glycerophosphate dehydrogenase, 0.9 ml. of 0.01 m glycylglycine-cysteine buffer at pH 7.5, 3 units of spinach transketolase (contains phosphoriboisomerase), and 1 $\mu$ mole of MgCl$_2$. The oxidation of DPNH was measured at 340 $\mu$ of a 1 ml. cuvette. The temperature was 25°.

To confirm these findings, the reaction mixture was dephosphorylated, chromatographed on paper, and sprayed with the TCA-orcinol reagent. In the complete system the formation of triose phosphate was accompanied by the appearance of a large spot having the characteristic light blue color of heptuloses and the same $R_F$ as sedoheptulose. No heptulose spot could be detected in the control with boiled transketolase.
In the second system employed, transketolase activity was measured in the following manner.

\[
\text{Sedoheptulose 1,7-diphosphate} \xrightarrow{\text{aldolase}} \text{tetrose 4-phosphate triose + phosphate}
\]

\[
\text{Xylulose 5-phosphate} \xrightarrow{\text{TK}} \text{"active glycolaldehyde" + triose phosphate}
\]

\[
\text{"Active glycolaldehyde" + tetrose phosphate} \xrightarrow{\text{TK}} \text{fructose 6-phosphate}
\]

\[
2 \text{ triose phosphate} \xrightarrow{\text{aldolase}} \text{fructose diphosphate}
\]

\[
\text{Sedoheptulose 1,7-diphosphate + xylulose 5-phosphate} \rightarrow \text{fructose diphosphate + fructose 6-phosphate}
\]

Hexose monophosphate formation was followed by the reduction of TPN to TPNH in the presence of glucose 6-phosphate dehydrogenase and glucose phosphate isomerase (Fig. 3). The rate of reaction with xylulose phosphate was somewhat greater than with ribulose phosphate, although the reaction stopped at about 63 per cent of the expected completion.

**Evidence for Phosphoketopentoepimerase**

Crude extracts of *L. pentosus* were found to contain high transketolase activity, and it was postulated that the transformation of xylulose 5-phosphate to ribulose 5-phosphate was due to the presence of this enzyme. It was therefore assumed that inactivation of transketolase would permit xylulose 5-phosphate to accumulate. Accordingly, an experiment was carried out with a crude extract which had been heated at 55° for 5 minutes at pH 7.7. This treatment resulted in a 77 per cent loss in kinase activity and a complete loss in transketolase activity. In this experiment β-xylulose and ATP gave rise to a mixture of pentose phosphates, which was dephosphorylated with potato phosphatase and analyzed by the cysteine-carbazole reaction and by paper chromatography. The results always indicated a 45:55 mixture of xylulose 5-phosphate and ribulose 5-phosphate.

A more detailed study showed that a new enzyme, phosphoketopentosepimerase, was present which catalyzed the epimerization reaction. The preparation and properties of PKPE will now be considered.

**Preparation**—5 ml. of crude bacterial extract were treated with 0.05 volume of 1 M MnCl₂ as described previously. The suspension was centrifuged, and the clear supernatant solution was heated at 65° for 5 minutes and then fractionated with ammonium sulfate. 2 ml. of saturated ammonium sulfate were added, and the precipitate was discarded; 4.5 ml. of saturated ammonium sulfate were then added to the supernatant solution,
The utilization of xylulose phosphate and ribulose phosphate by transketolase. The complete system contains 1 μmole of sedoheptulose diphosphate, 32 μmoles of triethylamine at pH 7.5, 8 μmoles of EDTA, 0.16 μmole of TPN, 0.12 mg. of aldolase, 1 unit of spinach transketolase, 1.1 mg. of muscle phosphohexoisomerase preparation, 0.2 mg. of Zwischenferment, 0.5 μmole of magnesium chloride, and 0.091 μmole of xylulose phosphate or 0.067 μmole of ribulose phosphate. See the text for a description of the reaction sequence.

**TABLE III**

*Equilibration of Xylulose 5-Phosphate and Ribulose 5-Phosphate Catalyzed by Phosphoketopentoepimerase*

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Substrate</th>
<th>Xylulose formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>No enzyme</td>
<td>Ribulose 5-phosphate</td>
<td>0 per cent</td>
</tr>
<tr>
<td>Boiled enzyme</td>
<td>&quot;</td>
<td>0 per cent</td>
</tr>
<tr>
<td>Enzyme</td>
<td>&quot;</td>
<td>49 per cent</td>
</tr>
<tr>
<td>No enzyme</td>
<td>Xylulose 5-phosphate</td>
<td>94 per cent</td>
</tr>
<tr>
<td>Boiled enzyme</td>
<td>&quot;</td>
<td>97 per cent</td>
</tr>
<tr>
<td>Enzyme</td>
<td>&quot;</td>
<td>43 per cent</td>
</tr>
</tbody>
</table>

0.05 ml. of PKPE (0.9 mg. of protein per ml.), 0.11 μmole of xylulose 5-phosphate or 0.1 μmole of ribulose 5-phosphate, 0.1 ml. of 0.1 M Tris buffer at pH 7.5. Incubation time 20 minutes at 37°. At the end of the incubation, 0.02 ml. of 0.5 M acetate buffer at pH 5.2 was added. The mixture was heated to 100° for 5 minutes, cooled, and 0.01 ml. of potato phosphatase (10 units) was added. The system was incubated for 60 minutes at 37° and then tested directly with the cysteine-carbazole reagents. See "Methods" for additional details.
and the precipitate containing PKPE was collected and dissolved in 1 ml. of water. Since in some cases ATPase activity was still detectable, the final fraction was heated to 70° for 5 minutes. Although no attempt is made here to define the activity of PKPE in quantitative terms, the experiments to be described permit a partial characterization of the enzyme.

**Properties**—With either ribulose 5-phosphate or xylulose 5-phosphate as substrate, an equilibrium mixture of both esters is rapidly attained (Table III).

\[
\text{Xylulose 5-phosphate } \rightleftharpoons \text{ ribulose 5-phosphate}
\]

The equilibrium value for the ratio of ribulose 5-phosphate to xylulose 5-phosphate was approximately 1.20. Under the experimental conditions, with ribulose 5-phosphate as substrate the reaction had reached equilibrium in 20 minutes and was more than 60 per cent complete in 5 minutes (Fig. 4). The products of the reaction have been dephosphorylated and subjected to paper chromatography. In the experiment with ribulose 5-phosphate and boiled PKPE, no xylulose spot was present; with active PKPE, a strong xylulose spot was observed. No heptulose spot was detected. The enzyme does not appear to require metal ions for activity. It was not inhibited by EDTA (final concentration 10^{-2} M), nor was it affected by the addition of sulfhydryl compounds such as cysteine or glutathione. Ribulose 1,5-diphosphate did not serve as a substrate.
Fig. 5. Rate of DPNH oxidation as a function of the epimerization of xylulose phosphate to ribulose phosphate by PKPE. The complete system (1.5 ml., 25°) contained 0.45 μmole of ATP (Sigma, crystalline), 2 μmoles of cysteine at pH 7.5, 2 μmoles of magnesium chloride, 6 μmoles of phosphate buffer at pH 7.5, 0.6 μmole of phosphoenolpyruvate (PEP), 0.5 unit of pyruvate kinase, 2 units of lactic dehydrogenase, 0.24 μmole of DPNH, 0.065 μmole of xylulose 5-phosphate, 20 units of phosphoribulokinase, and 0.05 ml. of PKPE (0.9 mg. per ml.). Control reaction mixtures are indicated in the figure.

The epimerization can be studied spectrophotometrically with the aid of phosphoribulokinase and the ADP assay system.

\[
\begin{align*}
\text{Xylulose 5-phosphate} & \xrightarrow{\text{PKPE}} \text{ribulose 5-phosphate} \\
\text{Ribulose 5-phosphate} & \xrightarrow{\text{ATP}} \text{ribulose 1,5-diphosphate + ADP} \\
\text{ADP + phosphoenolpyruvate} & \rightarrow \text{ATP + pyruvate} \\
\text{Pyruvate + DPNH + H}^+ & \rightarrow \text{lactate + DPN}^+
\end{align*}
\]

As indicated in Fig. 5, no oxidation of DPNH occurred in the absence of PKPE, phosphoenolpyruvate, or phosphoribulokinase. In the presence of the ribulose 5-phosphate trapping system, all of the xylulose 5-phosphate is utilized. No transketolase activity could be detected in the PKPE system by the usual assay procedures.

**DISCUSSION**

With the identification of xylulose kinase and PKPE in extracts of *L. pentosus*, it is now possible to formulate a sequence of reactions leading
from xylose to ribose 5-phosphate. A (xylose isomerase) has been found in *L. pentosus* (2), in *P. hydrophila* (3), and recently in *P. pestis* (4). B (xylose kinase) has been described in some detail in this paper. The enzyme is highly specific and catalyzes the phosphorylation of d-xylose by ATP to yield xylulose 5-phosphate. This ester participates in at least two reactions. It can be converted to ribulose 5-phosphate by the relatively stable enzyme PKPE (C). This enzyme not only occurs in extracts of *L. pentosus*, but has also been found in spleen extracts (6) and in

\[
\text{Xylose} \xrightarrow{\Delta} \text{xylulose} \xrightarrow{B} \text{xylulose 5-phosphate} \xrightarrow{D} \text{ribulose 5-phosphate}
\]
muscle fractions. The mechanism of epimerization is as yet unknown. However, on the basis of evidence for a 3-ketopentose phosphate in their reaction products, Ashwell and Hickman (6) have postulated the sequence represented in the accompanying diagram. With the L. pentosus PKPE preparations no evidence for the 3-keto sugar is available.

An additional possibility for epimerization is the observation that xylulose 5-phosphate serves as a substrate for transketolase (D). As yet the experimental conditions have not been found for the accumulation of ribulose 5-phosphate from a reaction involving xylulose 5-phosphate and transketolase. With the purified spinach preparations sufficient phosphoriboisomerase is present to produce ribose 5-phosphate, which serves as an acceptor in the transketolase reaction. Thus, the formation of sedoheptulose 7-phosphate is always observed, whether the initial substrate is xylulose 5-phosphate or ribulose 5-phosphate.

In xylose-adapted L. pentosus cells these reactions are involved in the utilization of xylose (1, 2). It is of interest that they are also present in mammalian cells. This circumstance suggests that the xylulose ester may play a wider rôle in carbohydrate metabolism than was hitherto suspected.

SUMMARY

Xylulose kinase has been isolated from xylose-adapted Lactobacillus pentosus cells and has been purified some 50-fold. Of the several substrates tested, only D-xylulose is reactive. The enzyme is metal-activated.

The kinase was employed for the preparation of D-xylulose 5-phosphate. Xylulose 5-phosphate participates as substrate with two enzymes: (a) transketolase, which together with phosphoriboisomerase converts xylulose 5-phosphate to heptulose 7-phosphate, and (b) a newly described enzyme, phosphoketopentosepimerase, which catalyzes the epimerization of xylulose 5-phosphate to ribulose 5-phosphate.

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