A Coupled Reaction Catalyzed by the Enzymes Transketolase and Transaldolase

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The formation of pentose phosphate from hexose monophosphate involves either the oxidative pathway from glucose 6-phosphate through 6-phosphogluconate or the series of nonoxidative transfer reactions catalyzed by transketolase and transaldolase, in which sedoheptulose 7-phosphate is an intermediate (1). In mammalian tissues the nonoxidative pathway appears to play a major role (2-5), accounting for the synthesis of more than 50% of the ribose of ribonucleic acid and the acid-soluble nucleotides.

Pentose phosphate synthesis from hexose monophosphate by the nonoxidative pathway has been presumed to involve the following sequence of reactions (1):

\[
\text{Fructose 6-phosphate} + \text{glyceraldehyde 3-phosphate} \xrightarrow{\text{transketolase}} \text{xylulose 5-phosphate} + \text{erythrose 4-phosphate}
\]

\[
\text{Sedoheptulose 7-phosphate} + \text{glyceraldehyde 3-phosphate} \xrightarrow{\text{transketolase}} \text{xylulose 5-phosphate} + \text{ribose 5-phosphate}
\]

\[
\text{Sedoheptulose 7-phosphate} + \text{glyceraldehyde 3-phosphate} \xrightarrow{\text{transaldolase}} \text{xylosyl 4-phosphate} + \text{erythrose 4-phosphate}
\]

\[
\text{Sedoheptulose 7-phosphate} + \text{glyceraldehyde 3-phosphate} \xrightarrow{\text{transaldolase}} \text{xylulose 5-phosphate} + \text{ribose 5-phosphate}
\]

Thus the synthesis of pentose phosphate from hexose monophosphate should require stoichiometric quantities of triose phosphate. On the other hand only catalytic or priming amounts of either triose phosphate or tetrose phosphate should be required for the accumulation of heptulose phosphate (Reactions 1 and 2).

The first indication that this formulation of the role of triose phosphate might require modification came from the work of Bonsignore et al. (6, 7) who found that thoroughly dialyzed rat liver extracts catalyzed an active synthesis of heptulose phosphate from hexose monophosphate alone, in the absence of any detectable quantities of triose phosphate. In fact, added triose phosphate failed to alter the course of the reaction, although the presence of enzymes which catalyzed the removal of triose phosphate during the reaction led to a reduction in the quantity of heptulose produced (8).

The conversion of hexose monophosphate to heptulose phosphate can be shown to occur as well with mixtures of purified transketolase and transaldolase, in the absence of detectable quantities of triose phosphate or other acceptor. The results reported here suggest a coupled attack of transketolase and transaldolase upon 2 moles of fructose 6-phosphate, the former producing erythrose 4-phosphate and the latter glyceraldehyde 3-phosphate, each of these cleavage products serving as acceptor for the other enzyme.

EXPERIMENTAL PROCEDURE

Materials and Methods

Transketolase was prepared from spinach leaves and assayed as previously described (9). The preparations showed specific activity between 60 and 90 units per mg. Transaldolase was prepared from Torula yeast kindly provided by the Lake States Yeast Corporation, Rhinelander, Wisconsin. The details of the method are given below. This yeast was also used for the purification of 6-phosphogluconic dehydrogenase, by the use of the procedure previously described (10). The preparation is rich in glucose 6-phosphate dehydrogenase and was used when the latter enzyme was required. Hexosephosphate isomerase was an ammonium sulfate fraction from rabbit muscle, collected between 52 and 70% saturation according to the method of Taylor et al. (11). The same preparation was employed as a source of D-glycerothosphate dehydrogenase and triosephosphate isomerase. Crystalline D-glycerothosphate dehydrogenase, free of triosephosphate isomerase, was purchased from Boehringer and Söhne. Crystalline preparations of triosephosphate isomerase and aldolase, prepared by the method of Beisenherz et al. (12), were kindly provided by Dr. B. Bloom of the National Institutes of Health. Xylulose 5-phosphate-3-epimerase and phosphoketolase were purified from extracts of Lactobacillus plantarum (13). Pentosephosphate isomerase was purified from spinach by the method of Hurwitz et al. (14).

Fructose 6-phosphate was a commercial preparation, purified by paper chromatography as described below. Sedoheptulose 7-phosphate was prepared as previously described (15). D-Erythrose 4-phosphate dimethyl acetal, as the crystalline cyclohexylamine salt, was kindly provided by Dr. C. Ballou of the University of California. Glucose 6-phosphate was the crys-
talline barium salt (16). Other substances were obtained from commercial sources.

The dialyzed liver supernatant fraction was prepared as described by Bonsignore et al. (6).

Routine assays for transketolase and transaldolase were carried out as previously described (15, 17). Triose phosphate was determined enzymatically by the method of Racker (18). Glucose 6-phosphate and fructose 6-phosphate were determined with glucose 6-phosphate dehydrogenase, with or without the addition of phosphoglucone isomerase. Pentose was determined by the orcinol reaction (19) and heptulose by the cysteine-carbazole method of Dische (20). Sedoheptulose was determined as standard for the colorimetric procedures was kindly provided by Dr. N. K. Richtmyer of the National Institutes of Health.

For the assay of pentose phosphate in the presence of sugars which interfered with the orcinol test, a more active preparation than that described previously for brewers' yeast (Table I). The dried yeast preparation (250 g) was reduced to a fine powder by treatment for 2 minutes in a brewers' yeast (Table I). The dried yeast preparation (250 g) was adjusted to pH 4.6 to 4.7 with about 5 ml of 5 ml). The clear extract was divided into 4 equal parts and fractionated with acetone. Each aliquot (900 ml) was adjusted to pH 7.4 (gel fraction, 15 ml).

Second Acetone Fractionation—To the gel fraction was added 34.6 ml of 0.25 M glycylglycine buffer, pH 7.4, to bring the protein to 10 mg per ml; this solution was diluted with 56.4 ml of 0.1 M potassium acetate buffer, pH 5.0. Three fractions were collected by the successive addition of 54.6, 22.8, and 22.8 ml of acetone, in the same way as before. The third fraction, containing the bulk of the activity, was dissolved in 8.0 ml of 0.25 M glycylglycine buffer, pH 7.4. From this solution (10.4 ml) the activity was precipitated with ammonium sulfate. The solution was treated with an equal volume of saturated ammonium sulfate (saturated at room temperature, pH adjusted to 7.5 with NH4OH). The small precipitate was removed by centrifugation and the supernatant solution treated with 4 g of solid ammonium sulfate. The precipitate was collected by high speed centrifugation and dissolved in 5 ml of 0.25 M glycylglycine buffer, pH 7.4. Merecaptoethanol (0.005 ml) was added to the solution (acetone Fraction B, 6.0 ml).

Fractionation with Ammonium Sulfate at Room Temperature—The gel fraction or acetone Fraction B were used in most experiments; these were stable at −10° for several months when merecaptoethanol was present. Further purification of acetone Fraction B was carried out with small aliquots. In the experiment shown in Table I, 1.1 ml of acetone Fraction B were diluted with 0.4 ml of water; the protein concentration was 12.4 mg per ml and the ammonium sulfate saturation (by conductivity measurement on a diluted aliquot) was 0.16. This solution was then warmed to room temperature for 30 to 60 minutes and centrifuged. Three more fractions were collected in the same way by the successive addition of 0.4-ml quantities of room temperature, saturated ammonium sulfate. The last two fractions, containing 65 to 100% of the original activity, were dissolved in a total of 1.0 ml of glycylglycine buffer, pH 7.4, and treated with 0.001 to 0.002 ml of merecaptoethanol (ammonium sulfate Fraction B).

The preparations were stored at −10°. From time to time, with repeated thawing, additional merecaptoethanol was added.

<table>
<thead>
<tr>
<th>Step</th>
<th>Total units</th>
<th>Specific activity (μL/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast autolysate</td>
<td>21,600</td>
<td>0.52</td>
</tr>
<tr>
<td>Acetone Fraction A</td>
<td>13,200</td>
<td>12.1</td>
</tr>
<tr>
<td>Gel fraction</td>
<td>11,400</td>
<td>20.8</td>
</tr>
<tr>
<td>Acetone Fraction B</td>
<td>7,000</td>
<td>60.5</td>
</tr>
<tr>
<td>Ammonium sulfate Fraction B</td>
<td>1,170</td>
<td>180.0</td>
</tr>
</tbody>
</table>

* This step was carried out with 1.1 ml (1160 units) of acetone Fraction B.
Formation of Heptulose from Fructose 6-Phosphate

Comparison of Liver Extract and Purified Enzymes—The formation of heptulose phosphate from fructose 6-phosphate proceeds at identical rate with either the dialyzed liver supernatant fraction or a mixture of purified spinach transketolase and yeast transaldolase (Fig. 1). The quantities of transketolase and transaldolase added in this experiment were exactly equal to those found by analysis to be present in the liver preparation.

With glucose 6-phosphate as substrate, hexosephosphate isomerase was required in addition to transketolase and transaldolase; this was not the case when glucose 6-phosphate was replaced by fructose 6-phosphate (Fig. 2). In the absence of isomerase the rate of heptulose phosphate formation was approximately seven times greater with fructose 6-phosphate in place of glucose 6-phosphate. The decrease in activity which resulted when hexosephosphate isomerase was added to the vessel containing fructose 6-phosphate suggested that high levels of this substrate were necessary to saturate the system (see below).

Levels of Transketolase and Transaldolase—In the absence of either transketolase or transaldolase no heptulose phosphate was formed from fructose 6-phosphate or glucose 6-phosphate (Fig. 3). When these enzymes were tested in varying proportions it was found that transketolase was limiting even though in terms of equivalent units it was present in excess (4.0 units of transketolase compared with 1.5 units of transaldolase). The activity of the system was maximal when the ratio of transaldolase units to transketolase units reached about 0.24; only with a large excess of the latter enzyme did the quantity of transaldolase tend to become limiting.

Stoichiometry of Reaction—The disappearance of hexose monophosphate was accompanied by the formation of heptulose phosphate as the major product (Table II). For each mole of

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Heptulose formation from glucose 6-phosphate with liver extract and with a mixture of purified transketolase (TK) and transaldolase (TA). The reaction mixtures (2.7 ml) contained 10 μmoles of glucose 6-phosphate, 0.2 μmole of thiamine pyrophosphate, 4 μmoles of MgCl₂, and 125 μmoles of glycylglycine buffer, pH 7.4. The liver extract, assayed for transketolase and transaldolase, was found to contain 4 units per ml of the former and 1.5 units per ml of the latter. The tests with purified enzymes contained 0.36, 0.72, and 1.44 units of transketolase and 0.15, 0.30, and 0.60 unit of transaldolase, respectively. In addition, 0.01 ml of hexosephosphate isomerase was added to the purified enzyme tests. Incubation was at 37°C. Aliquots for heptulose assay were heated at 100°C and filtered.

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Activity of the transketolase-transaldolase mixture with glucose 6-phosphate and fructose 6-phosphate as substrates. The reaction mixtures were as described in the legend to Fig. 1, with 0.96 unit of transketolase and 0.5 unit of transaldolase and 12 μmoles of either glucose 6-phosphate or fructose 6-phosphate as indicated.

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![Table II](https://via.placeholder.com/150)

**Table II**

**Stoichiometry in the reaction**

The reaction mixture (2.5 ml) contained 10.2 μmoles of fructose 6-phosphate, 1.2 μmoles of thiamine pyrophosphate, 4 μmoles of MgCl₂, 1.1 units of transketolase, and 0.35 unit of transaldolase, in 0.05 M glycylglycine buffer. Aliquots (0.5 ml) were collected, treated with 0.1 ml of 60% perchloric acid, and centrifuged. The supernatant solutions were neutralized with 0.11 ml of 7 N KOH. Potassium perchlorate was allowed to crystallize at 0°C and suitable aliquots of the supernatant solution were assayed for the several components as described in the “Methods” section.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Minutes</th>
<th>Theory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Hexose phosphate utilized</td>
<td>1.80</td>
<td>2.57</td>
</tr>
<tr>
<td>Heptulose phosphate formed</td>
<td>1.36</td>
<td>2.11</td>
</tr>
<tr>
<td>Triose phosphate formed</td>
<td>0.20</td>
<td>0.52</td>
</tr>
<tr>
<td>Pentose phosphate formed</td>
<td>0.08</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* Predicted for 3.4 μmoles of hexose utilized.
hexose monophosphate utilized, 0.75 mole of heptulose phosphate was formed, accounting for 87% of the hexose carbon atoms. In addition, lesser quantities of triose phosphate and pentose phosphate accumulated, representing 7% and 3% of the hexose carbon atoms, respectively. No tetrose phosphate could be detected (see Reaction 2). The reaction stoichiometry is in good agreement with that expected from Reactions 1 and 2 and the further conversion of pentose phosphate:

\[
2 \text{Hexose monophosphate} \rightarrow \text{heptulose phosphate} + \text{pentose phosphate} \quad (1 + 2)
\]

Pentose phosphate

\[
0.5 \text{heptulose phosphate} + 0.5 \text{triose phosphate} \quad (3)
\]

Sum 1 + 2 + 3: 2 Hexose monophosphate

1.5 heptulose phosphate + 0.5 triose phosphate

The reaction reached equilibrium when one-third of the hexose monophosphate was utilized; at this point the molar ratio of hexose monophosphate to heptulose phosphate was approximately 2.3. To demonstrate that the reaction had indeed reached equilibrium and not come to rest because of enzyme inactivation, sedoheptulose 7-phosphate was added after the reaction had stopped (Table III). This addition resulted in an increase in hexose monophosphate and produced a final mixture containing essentially the same proportion of hexose and heptulose esters as was present before the reaction was reversed. One-half (0.7 \mu\text{mole}) of the sedoheptulose 7-phosphate added at 120 minutes was consumed, with the production of 1.0 \mu\text{mole} of hexose monophosphate. The ratio \( \Delta \text{heptulose to } \Delta \text{hexose} \) was 0.70, identical with the ratio calculated from the reaction in the reverse direction (Table II). These results indicate that the reaction had come to rest because a true equilibrium was reached.

**Incubation of Sedoheptulose 7-phosphate with Transketolase and Transaldolase**—Since the addition of sedoheptulose 7-phosphate to the reaction mixture at equilibrium produced an increase in hexose monophosphate, it was expected that a similar result might follow the addition of sedoheptulose 7-phosphate directly to a mixture of transketolase and transaldolase. However, little reaction was found to occur with sedoheptulose 7-phosphate added alone, or even when this was added together with catalytic quantities of triose phosphate or glucose 6-phosphate (Table IV). The ratio of hexose monophosphate to heptulose phosphate after 1 hour was 0.11 compared with 2.3 calculated in experiments with fructose 6-phosphate. Possible reasons for this difference in end point with sedoheptulose 7-phosphate and fructose 6-phosphate will be considered later.

### Primer Requirement

In the conversion of hexose monophosphosphate to heptulose phosphate, no evidence could be found for the need for priming quantities of an acceptor for either transketolase or transaldolase. The reaction was observed to begin at once on addition of fructose 6-phosphate (Fig. 2) and was not more rapid when significant quantities of triose phosphate, which can serve as acceptor for transketolase (Equation 1), had accumulated (cf. Table II). It was therefore of interest to consider whether any other component of the reaction mixture might serve as acceptor for transketolase or transaldolase. Unidentified impurities in the fructose 6-phosphate preparation might play such a role; an important possibility was glucose 6-phosphate itself, since it has been suggested (22) that octulose 8-phosphate is a substrate for transketolase. The following experiments were carried out to test these possibilities.

### Purification of Fructose 6-Phosphate

The commercial fructose 6-phosphate preparation employed in these studies was found to contain only 60% of the total organic phosphate as fructose 6-phosphate (by enzymatic assay). Approximately 10% was glucose 6-phosphate; the remainder could not be identified. The preparation was chromatographed on paper with amyl acetate-acetic acid-water (3:3:1) and eluted. After this treatment, glucose 6-phosphate could no longer be detected and fructose 6-phosphate accounted for more than 90% of the total phosphate. However, when the product was tested with a mixture of transketolase and transaldolase it was found that the rate of formation of heptulose phosphate was unchanged (Fig. 4). Reference has previously been made to the low affinity of the system for fructose 6-phosphate; \( K_m \) calculated from the data in Fig. 4 was 3 \times 10^{-3} M. The fact that the dissociation constant was unchanged by purification of the substrate supports the conclusion that this is indeed the \( K_m \) of fructose 6-phosphate which is being measured, and not that of a trace contaminant which limits the reaction rate. This result tends to exclude the possibility that a contaminant in the fructose 6-phosphate preparation serves as acceptor in the transketolase or transaldolase reactions.

### Possible Primer Function of Glucose 6-Phosphate and Role of

### Table III

**Reversal of the reaction by sedoheptulose 7-phosphate**

The reaction mixtures (1.3 ml) contained 4.7 \mu\text{moles} of fructose 6-phosphate, 0.1 \mu\text{mole} of thiamine pyrophosphate, 2.0 \mu\text{moles} of MgCl\(_2\), 0.54 unit of transketolase, and 0.21 unit of transaldolase, in 0.05 M glycylglycine buffer, pH 7.4. One mixture was treated at 120 minutes with 1.4 \mu\text{moles} of sedoheptulose 7-phosphate and incubated for 120 minutes longer. Each reaction was stopped at the time indicated by the addition of 0.05 ml of 60% perchloric acid, centrifuged, and the supernatant solution neutralized with 0.055 ml of KOH.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Hexose monophosphate (A)</th>
<th>Heptulose phosphate (B)</th>
<th>Ratio A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.70 \mu\text{moles}</td>
<td>0.00 \mu\text{moles}</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>3.11 \mu\text{moles}</td>
<td>1.32 \mu\text{moles}</td>
<td>2.35</td>
</tr>
<tr>
<td>120</td>
<td>(3.11)* \mu\text{moles}</td>
<td>(2.72)* \mu\text{moles}</td>
<td>2.05</td>
</tr>
<tr>
<td>240</td>
<td>4.10 \mu\text{moles}</td>
<td>2.00 \mu\text{moles}</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated to be present immediately after addition of sedoheptulose 7-phosphate at 120 minutes.

### Table IV

**Hexose monophosphate formation from sedoheptulose 7-phosphate**

The reaction mixtures were as described for Table III, except that 5.5 \mu\text{moles} of sedoheptulose 7-phosphate were added initially instead of fructose 6-phosphate. Incubation was for 60 minutes at 37°C.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Sedoheptulose utilized ( \mu\text{moles} )</th>
<th>Hexose monophosphate formed ( \mu\text{moles} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate (0.13 \mu\text{mole})</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Glucose 6-phosphate (0.15 \mu\text{mole})</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>
FRUCTOSE-6-P, M x 10^3 (C)

Fig. 4. Heptulose phosphate formation from commercial and chromatographed fructose 6-phosphate. The reaction mixture (1.0 ml) contained fructose 6-phosphate as indicated, 1.6 amoles of MgCl₂, 0.08 amole of thiamine pyrophosphate, 0.4 unit of transketolase, and 0.18 unit of transaldolase, in 0.05 M glycylglycine buffer, pH 7.4. Incubation was at 37°. At 10- and 20-minute intervals, aliquots were analyzed for heptulose phosphate. The rates shown in the figure were calculated from the mean values. In the upper portion of the figure the data is plotted according to Lineweaver and Burke (24).

Triose Phosphate—Since purification of fructose 6-phosphate did not affect its activity with respect to heptulose formation, it seemed unlikely that glucose 6-phosphate played a role in this process. In addition it could be shown that neither glucose 6-phosphate nor triose phosphate altered the rate of heptulose phosphate formation from chromatographed fructose 6-phosphate.

Enzyme-Substrate Affinity Constants

To assess the possible role of traces of acceptors in the reaction and to determine which of the two enzymes set the requirement for high concentrations of fructose 6-phosphate, enzyme-substrate affinity constants were determined.

Transaldolase with Erythrose 4-Phosphate and Fructose 6-Phosphate—The assay system described by Cooper et al. (23) was used for the assay of erythrose 4-phosphate and for determining the effect of substrate concentration on the activity of transaldolase (Reaction 2). The rate of formation of triose phosphate was measured with α-glycerophosphate dehydrogenase and DPNH (Fig. 5). In this assay system, with excess erythrose 4-phosphate and fructose 6-phosphate the rate of triose phosphate formation was proportional to the activity of transaldolase. The reaction was inhibited by high concentrations of erythrose 4-phosphate, but at low levels of this substrate the Lineweaver-Burk equation (24) was obeyed and $K_s$ at 25° was calculated to be $1.8 \times 10^{-5}$ M. For fructose 6-phosphate, $K_s$ at 25° was calculated to be $3.2 \times 10^{-4}$ M.

Transketolase and Fructose 6-Phosphate—When fructose 6-phosphate was employed as substrate the reaction could be followed by measuring the rate of erythrose 4-phosphate formation (Fig. 6). In the presence of excess transaldolase and with D,L-glyceraldehyde as acceptor for transketolase the rate of DPNH oxidation became proportional to the quantity of transketolase added. Maximal reaction velocity was not reached in the range of fructose 6-phosphate added; $K_s$ at 25° was calculated to be $3.2 \times 10^{-4}$ M. A similar $K_s$ for fructose 6-phosphate was derived from measurement of the rate of heptulose phosphate formation (Fig. 4).

FRUCTOSE-6-P, M x 10^3 (C)

FIG. 5. The effect of erythrose 4-phosphate and fructose 6-phosphate concentration on the reactivity of transaldolase. For erythrose 4-phosphate the reaction mixtures (1.5 ml) contained 1 amole of fructose 6-phosphate, 0.24 amole of DPNH, 0.03 ml of glyceraldehyde 3-phosphate dehydrogenase (with triose phosphate isomerase), 0.088 unit of transaldolase, and 0.03 M triethanolamine buffer, pH 7.4. For fructose 6-phosphate the reaction mixture was similar except that 0.2 amole of erythrose 4-phosphate was added in each case. The change in optical density at 340 mp was measured in a Cary spectrophotometer. The reaction temperature was 25°.

FRUCTOSE-6-P, M x 10^3 (C)

FIG. 6. The effect of fructose 6-phosphate concentration on the activity of transketolase. The reaction mixture was similar to that described in Table V except that 0.9 unit of transaldolase and 1.0 amole of D,L-glyceraldehyde were added. The temperature was 25°.
**Inability of glucose 6-phosphate to serve as acceptor for active glycolaldehyde**

<table>
<thead>
<tr>
<th>Fructose-6-P + acceptor</th>
<th>Tetrose phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transketolase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>+ fructose-6-P</th>
<th>Triose phosphate + sedoheptulose-7-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transaldolase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Addition</th>
<th>Triose phosphate formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.01*</td>
</tr>
<tr>
<td>Glucose 6-phosphate (5 μmoles)</td>
<td>0.01</td>
</tr>
<tr>
<td>D,L-Glyceraldehyde (5 μmoles)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

* In the absence of the triose phosphate assay system, which removes this product, the rate of triose phosphate formation would be about 0.06 μmole per 10 minutes (calculated from the data in Table II). The reaction mixtures (0.9 ml) contained 0.51 μmole of fructose 6-phosphate, 0.1 μmole of DPNH, 0.09 unit of transketolase, 0.21 unit of transaldolase, 0.003 ml of crystalline glycerophosphate dehydrogenase, 0.005 ml of triosephosphate isomerase (0.02 unit), 0.1 μmole of thiamine pyrophosphate, and 2.0 μmoles of MgCl₂ in 0.12 M glycylglycine buffer, pH 7.4.

**DISCUSSION**

It is now clear that the formation of heptulose phosphate from hexose monophosphate in liver extracts, as described by Boussigno and co-workers (8, 7) is due to the activity of transketolase and transaldolase in these extracts. The activity of the liver preparation can be precisely duplicated by equivalent mixtures of highly purified spinach transketolase and yeast transaldolase. We have shown that the true substrate for this reaction is fructose 6-phosphate, rather than glucose 6-phosphate. However, it remains to explain how the reaction can proceed in the absence of priming quantities of a suitable acceptor, since neither enzyme alone shows any activity with fructose 6-phosphate.

With this substrate the reaction catalyzed by the transketolase-transaldolase mixture begins at once with no evidence for a lag phase, and is not appreciably faster in the later stages when detectable quantities of triose phosphate have accumulated (Fig. 2 and Table II).

The possibility that contaminants in the fructose 6-phosphate preparation were fulfilling the role of acceptor was excluded by the fact that the activity was unchanged following purification of the substrate. High concentrations of fructose 6-phosphate are required, not to provide an essential component present in the commercial fructose 6-phosphate preparation, but because of the low affinity of transketolase for this substrate.

Since both transketolase and transaldolase were essential for the utilization of heptulose monophosphate it appears likely that each enzyme provides an acceptor for the other, as indicated in Equations 1 and 2. However, in this case the reaction would be expected to exhibit a definite lag, since the concentration of each enzyme limits the initial concentration of acceptor, and this can be shown by calculation to be well below the saturation level. Thus, fructose phosphate formed in quantities stoichiometric with those of transketolase could not exceed 10⁻⁴ M. The Kₜ for this substrate with transaldolase is 1000 times greater. The possibility remains that free thiamine pyrophosphate is the initial acceptor, although this function of the free coenzyme has not yet been demonstrated.

A coupled action of transketolase and transaldolase, without free diffusion of intermediates into the solution, remains to be considered. This possibility is supported by the unusual kinetics observed with mixtures of these enzymes. In terms of comparable units, much more transketolase is required to saturate the reaction than transaldolase. The behavior of the two-enzyme systems resembles that of the tryptophan synthetase system described by Crawford and Yanofsky (26), although direct evidence for a combination of these enzymes has not been obtained.
No sign of aggregation could be obtained by ultracentrifugal methods.

The heptulose ester formed in these experiments was presumed to be sedoheptulose 7-phosphate, although it has not been isolated as such in the present work. This product formed from hexose monophosphate with the liver preparation has been characterized by Bonsignore et al. (6), who have converted it to sedoheptulose tetrabenzoeate. The fact that sedoheptulose 7-phosphate reverses the reaction until equilibrium is re-established provides presumptive evidence that it is this ester which is produced in the forward reaction. The reaction differs from that reported by Sie et al. (27) in that it is not stimulated by triose phosphate and is most rapid with fructose 6-phosphate.

With hexose monophosphate as substrate, the reaction stops when the ratio of hexose monophosphate to heptulose phosphate is about 2.5:1. However, with sedoheptulose 7-phosphate as substrate no such equilibrium is reached. The reasons for this become apparent when the over-all reaction is considered. With hexose monophosphate, triose phosphate accumulates (Equations 1 + 2 + 5); with sedoheptulose 7-phosphate, it is consumed:

\[
\text{Sedoheptulose 7-phosphate} + \text{fructose 6-phosphate} \xrightarrow{\text{transaldolase}} \text{fructose 6-phosphate} + \text{tetrose phosphate}
\]

\[
\text{Sedoheptulose 7-phosphate} + \text{tetrose phosphate} \xrightarrow{\text{transketolase}} \text{fructose 6-phosphate} + \text{pentose phosphate}
\]

\[
\text{Pentose phosphate} \xrightarrow{\text{transketolase}} 0.5 \text{ sedoheptulose 7-phosphate} + 0.5 \text{ triose phosphate}
\]

Sum: 1.5 Sedoheptulose 7-phosphate + 0.5 triose phosphate = 2 fructose 6-phosphate

The reaction cannot be self-sustaining in the direction from heptulose to hexose, but can continue only so long as triose phosphate is provided.

The formation of heptulose phosphate from fructose 6-phosphate represents the first step in the synthesis of pentose phosphate by the nonoxidative pathway. It is significant that this occurs without a requirement for triose phosphate or tetrose phosphate. These reactive compounds do not accumulate in actively metabolizing cells and a system dependent upon such accumulation would be unlikely to function effectively under all conditions. The fact that the nonoxidative pathway is quantitatively the important mechanism for pentose synthesis becomes the more understandable in view of the great efficiency of transketolase and transaldolase in this process.

**SUMMARY**

The formation of heptulose phosphate from hexose monophosphate which is catalyzed by liver extracts can be attributed to the transketolase and transaldolase content of these extracts. The true substrate is fructose 6-phosphate. No requirement for triose phosphate can be detected. Other possible acceptors for transketolase and transaldolase have been excluded.

The reaction proceeds at maximal rates with very low levels of transaldolase, although both enzymes are required. A mechanism based on the coupled action of transketolase and transaldolase on two molecules of fructose 6-phosphate is discussed.

**REFERENCES**

A Coupled Reaction Catalyzed by the Enzymes Transketolase and Transaldolase

S. Pontremoli, A. Bonsignore, E. Grazi and B. L. Horecker