Mechanism of Action of Transketolase

I. PROPERTIES OF THE CRYSTALLINE YEAST ENZYME*

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For detailed studies on the mode of action of an enzyme a readily available source and a convenient method of preparation are of distinct advantage. Accessible sources such as bakers' yeast (1) and spinach leaves (2) have, therefore, been used for large-scale preparations of transketolase in spite of the fact that extracts of Escherichia coli, in which the cleavage of ribose-5-P to triose-P was first observed (3), or Torula yeast (4) are 3 to 5 times as active. With the development of cellulose derivatives for protein fractionation (5), the procurement of transketolase in sufficient quantities (4) for isolation of enzyme-substrate intermediates has become feasible (6, 7). It is the purpose of this paper to report on properties of the crystalline yeast enzyme that have not been recorded previously.

EXPERIMENTAL PROCEDURE

Methods

Substrates—An equilibrium mixture of the n-isomers of ribose-5-P, ribulose-5-P, and xylulose-5-P was prepared as described previously (1) except that purified ribose-5-P isomerase and xylulose-5-P epimerase (8) were used instead of the crude yeast preparation. The equilibrium mixture was also prepared with spleen preparations by the method of Ashwell and Hickman (9). n-Deoxyribulose-3-P, n-ribose-5-P, and thiamine pyrophosphate were obtained from Schwarz Laboratories, Inc. The dimethyl acetal of glyceraldehyde-3-P was kindly donated by Dr. C. Ballou. L-Glyceraldehyde-3-P was prepared from n-n-glyceraldehyde-3-P according to Venkataraman et al. (10). n-Arabinose-5-P was prepared as described by Levin and Racker (11). Glycolaldehyde was obtained from Aldrich Chemical Company, Inc., and lithium hydroxypropionate was prepared by the method of Dickens and Williamson (12). Other chemicals were prepared as described previously (4, 8, 13).

Enzymes In all experiments described in this paper, crystalline transketolase prepared from bakers' yeast (4) was used. Transaldolase (4), ribose-5-P isomerase (8), xylulose-5-P epimerase (8), phosphofructokinase (14), and glucose-6-P isomerase (15) were prepared as described. Glyceraldehyde-3-P dehydrogenase, glucose-6-P dehydrogenase, alcohol dehydrogenase, and mixed crystals of α-glycerophosphate dehydrogenase and triose phosphate isomerase were purchased from Boehringer and Sons, Mannheim, Germany.

Definition of Unit—One unit of enzyme is defined as the amount of enzyme which catalyzes the turnover of 1 μmole of substrate per minute. Specific activity is defined as units of enzyme per milligram of protein.

Assays of Enzymes and Substrates—Activity measurements of transketolase and quantitative determination of the pentose-P cycle intermediates were carried out as described by Cooper et al. (13). In the transketolase assay, 20 μg of mixed crystals of α-glycerophosphate dehydrogenase and triose phosphate isomerase were used instead of the crude rabbit muscle fraction.

Assay of Ribose-5-P—Ribose-5-P was assayed with transketolase in the presence of an excess of xylulose-5-P as follows:

\[
\text{Ribose-5-P + xylulose-5-P} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{of Enzymes}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{of Substrates—Activity measurements of}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{transketolase and quantitative determination of the pentose-P}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{cycle intermediates were carried out as described by Cooper et al. (13). In the transketolase assay, 20 μg of mixed crystals of α-glycerophosphate dehydrogenase and triose phosphate isomerase were used instead of the crude rabbit muscle fraction.}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{Assay of Glycolaldehyde—Glycolaldehyde was assayed by measur-}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{ing the decrease in optical density at 340 nm in the presence of}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{DPNH and alcohol dehydrogenase. The reaction mixture con-}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{tained in a final volume of 1.0 ml: 25 μmoles of glycylglycine buffer, pH 7.6, 0.47 μmole of xylulose-5-P, a sample of ribose-5-P not exceeding 0.05 μmole, 0.6 μmole of DPN, 0.2 μmole of thiamine pyrophosphate, 2 μmole of MgCl₂, 4.5 μmole of arsenate, 100 μg of glyceraldehyde-3-P dehydrogenase, and 0.5 unit of transketolase (10 units/mg). The check cells contained all reagents including the unknown sample except DPN. The total change in optical density was stoichiometric to the amount of ribose-5-P, assuming 1 μmole of DPNH had an optical density of 0.62.}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{Assay of Erythulose—Erythulose was assayed in the same system measuring glycolaldehyde released by transketolase from erythulose in the presence of ribose-5-P as acceptor aldehyde. The reaction mixture contained the following reagents in a final}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{Assay of Glycolaldehyde—Glycolaldehyde was assayed by measur-}
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\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{ing the decrease in optical density at 340 nm in the presence of}
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\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{DPNH and alcohol dehydrogenase. The reaction mixture con-}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{tained in a final volume of 1.0 ml: 25 μmoles of glycylglycine buffer, pH 7.6, 0.12 μmole of DPNH, 600 μg of alcohol dehydro-}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{genase, and limiting amounts of the glycolaldehyde sample (0.02 to 0.05 μmole). The check cell contained all reagents, including the sample, except DPNH. Another control cell that contained all reagents except glycolaldehyde was used to correct for traces of DPNH oxidase present in some preparations of alcohol dehydrogenase. Optical density readings were taken before and after the addition of alcohol dehydrogenase and the reaction was followed until there was no further change in the optical density.}
\]

\[
\text{Ribose-5-P + DPNH} \xrightarrow{\text{transketolase}} \text{G-3-P dehydrogenase} \quad \text{Assay of Erythulose—Erythulose was assayed in the same system measuring glycolaldehyde released by transketolase from erythulose in the presence of ribose-5-P as acceptor aldehyde. The reaction mixture contained the following reagents in a final}
\]
of ribose-5-P, 2.0 µmole of MgCl₂, 0.12 µmole of DPNH, 1.61 units of transketolase, and 600 µg of crystalline alcohol dehydrogenase. The check cells contained all the reagents except DPNH. The control for DPNH oxidase contained all reagents except the erythulose sample.

Assay of Sedoheptulose-7-P—Sedoheptulose-7-P was measured colorimetrically according to Dische (16), or by the enzymic method (13).

Assay of Octulose-8-P—Glucose-6-P released by transketolase from octulose-8-P in the presence of glyceraldehyde-3-P as acceptor aldehyde was determined with glucose-6-P dehydrogenase. In a final volume of 1.0 ml, the following reagents were added: 25 µmoles of glycyglycine buffer, pH 7.6, limiting amount of octulose-8-P (0.02 to 0.05 µmole), 0.63 µmole of glyceraldehyde-3-P, 0.2 µmole of thiamine pyro-P, 2.0 µmoles of MgCl₂, 0.27 µmole of 1'PN, 0.1 unit of glucose-6-P dehydrogenase, and 1.0 unit of transketolase. Optical density readings were taken before and after the addition of transketolase and the reaction was followed until there was no further change in the optical density.

Assay of Hydroxypyruvate—Hydroxypyruvate was assayed at 340 nm in the presence of DPNH and 15 µg of lactic dehydrogenase.

RESULTS

Resolution of Crystalline Preparations of Transketolase—Two different kinds of preparation of transketolase were used which are dependent on thiamine pyro-P for activity. One preparation was obtained by dialysis of the enzyme against 1000 volumes of 1.6 m ammonium sulfate at pH 7.8 for 48 hours. This procedure resulted in a soluble transketolase preparation which was almost completely resolved but was considerably less stable than the crystalline holoenzyme. Simpson (17) observed a similar lower stability of pork liver transketolase in the absence of thiamine pyro-P.

A second type of preparation of resolved transketolase was observed accidentally. Crystalline suspensions of transketolase that had been stored in 2 m ammonium sulfate for 8 to 12 months in the cold room were found to be quite insoluble in water or dilute buffer. Such enzyme suspensions decreased the transmission of light as shown in Fig. 1. In these experiments, 340 nm was selected for convenience since activity measurements were also carried out at this wave length.

Addition of Mg⁺⁺ or thiamine pyro P had no effect on the light scattering but when both cofactors were added, rapid solubilization and increased transmission of light took place. As can be seen from Fig. 1, the order of addition of the two cofactors did not seem to affect the rate of solubilization. Such insoluble preparations could be washed with water thus removing contaminating proteins such as traces of xylulose-5-P 3'-epimerase. Since this useful procedure of purification was not applicable to freshly prepared crystals of transketolase, various attempts were made to accelerate the "aging process" that resulted in decreased solubility. Neither heating nor repeated fractionations of the enzyme with ethanol were successful. Since thiamine pyro-P and Mg⁺⁺ were needed for solubilization, transketolase resolved by dialysis against 1.6 m ammonium sulfate was repeatedly precipitated with ammonium sulfate. However, the enzyme remained soluble. The process of aging for 8 to 10 months was successfully repeated three times in the course of this work.

Effect of Order of Addition of Cofactors to Resolved Transketolase—In contrast to the above procedure of solubilization, activity measurements of resolved enzyme by addition of thiamine pyro-P and Mg⁺⁺ were markedly dependent on the order of addition. When thiamine pyro-P was added first and then Mg⁺⁺, the enzyme activity, as measured by the oxidation of DPNH, was markedly depressed as shown in Fig. 2. It appears from these experiments that thiamine pyro-P interacted with the enzyme without added Mg⁺⁺, resulting in a union which not only was catalytically inactive but which interfered with the proper alignment of thiamine pyro-P after Mg⁺⁺ addition.

Effect of pH on Transketolase Activity—In order to avoid secondary effects of pH on other enzymes used in the spectrophotometric test, transketolase activity was determined colorimetrically by the rate of sedoheptulose-7-P formation. Fig. 3 shows the effect of pH on the formation of sedoheptulose-7-P from pentose phosphates. A pH optimum at about 7.6 was observed.

Substrate Specificity of Yeast Transketolase—In the course of experiments on the formation of an active glycolaldehyde enzyme
intermediate (18) it was noted that with fructose-6-P as substrate considerably more erythrose-4-P was formed than the stoichiometric equivalent of the transketolase added. Formation of erythrose-4-P was measured in the presence of glyceraldehyde-3-P dehydrogenase by reduction of DPN (19, 20). To eliminate possible side reactions in the spectrophotometric test, fructose-6-P disappearance was determined as shown in Table I. Since neither glycolaldehyde nor erythrulose accumulated in amounts equivalent to fructose-6-P disappearance, it was considered likely that fructose-6-P contained or gave rise to an acceptor aldehyde such as glucose-6-P. Examination of several commercial preparations of fructose 6-P revealed glucose-6-P contamination. Traces of glucose-6-P isomerase activity were found in the transketolase preparations. That transketolase, indeed, acts on glucose-6-P was reexamined with relatively large amounts of the enzyme. It is already known that several keto sugars can act as donors and several aldehydes can serve as acceptors for transketolase (cf. 21). A convenient assay for the efficiency of various acceptor aldehydes was developed which depends on the rate of disappearance of hydroxypyruvate in the presence of transketolase-catalyzed reaction as shown in Table II. Relatively high concentrations of enzyme were used in these experiments except with n-glyceraldehyde-3-P as acceptor. In addition to the known acceptor aldehydes mentioned above, arabinose-5-P and glucose-6-P were found to serve as acceptors under these conditions. However, little or no reactivity with L-glyceraldehyde-3-P was observed. n-Glyceraldehyde-3-P was included in the table for comparative purposes; however only 1/50 of the enzyme concentration was used. With glucose-6-P and arabinose-3-P as acceptors the product was also analyzed with Dische's cysteine-sulfuric acid reagent (16). With arabinose-5-P, a color with a maximum at 510 mp was observed indicating the formation of a heptulose. With glucose-6-P as acceptor, a color with an absorption maximum at 480 mp was obtained suggesting the production of an octulose. In view of the possible metabolic significance of glucose-6-P as an acceptor, the product of this reaction was isolated and characterized.

**Preparation and Characterization of Octulose-8-P—Octulose-8-P was prepared by incubating 80.0 pmoles of glucose-6-P at 34° in a final volume of 4.5 ml with the following reagents: 250 pmoles of glycylglycine buffer, pH 7.6, 120 pmoles of lithium hydroxy-pyruvate, 10 pmoles of thiamine pyro-P, 100 pmoles of MgCl₂, and 386 units of transketolase (10 units/mg). When 50 pmoles (62%) of the glucose-6-P disappeared (2½ hours), the reaction was stopped and deproteinized by adding 0.5 ml of 50% trichloroacetic acid solution was added to stop the reaction. Sedoheptulose-7-P was assayed according to the method of Dische (16) with 0.1 ml of the centrifuged solution.

**TABLE I**

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>n-Glucose-6-P</th>
<th>n-Glyceraldehyde-3-P</th>
<th>n-Arabinose-5-P</th>
<th>n-Glucose-6-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr pmoles</td>
<td>0.04</td>
<td>0.51</td>
<td>0.28</td>
<td>0.53</td>
</tr>
<tr>
<td>2 hr pmoles</td>
<td>0.12</td>
<td>0.53</td>
<td>0.39</td>
<td>0.574</td>
</tr>
</tbody>
</table>

* All values were corrected for disappearance without added acceptor aldehyde.
Dowex I-formate column (22 cm × 1.3 sq cm) with gradient elution with 150 ml of water in the mixing chamber and 150 ml of buffer (0.5 M ammonium formate + 0.2 M formic acid) in the upper reservoir. Eluate samples of 2.5 ml each were collected and tested for sugar with anthrone (22) and cysteine-sulfuric acid (16). Octulose-8-P was eluted between 100 and 115 ml. This fraction was free of glucose-6-P as tested with glucose-6-P dehydrogenase and TPN. With Dische’s cysteine-sulfuric acid reagent (16) it gave a maximal absorption band at 480 mua indicating the presence of an octulose. Barium acetate (300 μmoles) was added to this fraction and the pH of the solution was adjusted to 7.8. Four volumes of ethanol were then added and the mixture was kept in the refrigerator overnight. The barium salt was collected by centrifugation, washed once with ethanol and once with ether, and dried in a vacuum desiccator; 35 mg of barium salt were obtained. Ten milligrams of this barium salt were converted to the sodium salt in a final volume of 2 ml and assayed for octulose-8-P as described under "Experimental Procedure." The enzymatic assay and total phosphorus gave values of 3.8 μmoles/ml and 4.2 μmoles/ml, respectively. Periodate oxidation of this material was measured according to Marinetti and Rouser (23). Octulose-8-P utilized 5.98 μmoles of periodate per μmole of substrate (as measured by total phosphorus) and 5.49 μmoles per μmole (as measured by enzymic analysis). The theoretical expected value is 6 μmoles. Octulose-8-P (1.5 μmoles) was dephosphorylated with 0.45 unit (μmoles of P₄ hydrolyzed per minute) of potato acid phosphatase,1 deionized, and chromatographed. The material gave the characteristic color changes for octulose in the orcinol spray and traveled with the same Rf as synthetic n-glycero-n-ido-octulose in two solvent systems which readily separate this octulose from the octuloses of D-glycero-D-manno, D-glycero-D-gulo, and D-glycero-D-altro configuration.

Table III

Comparison of components of transketolase-catalyzed reaction at equilibrium

In a final volume of 2.0 ml, the following reagents were incubated at 25°: 50 μmoles of glycglycine buffer, pH 7.6, 1.55 μmoles of xylulose-5-P, 1.75 μmoles of ribose-5-P, 0.2 μmole of thiamine pyro-P, 2.0 μmoles of MgCl₂, and 0.8 unit of transketolase. Small aliquots were removed at 10-minute intervals and glyceraldehyde-3-P production was measured. When the glyceraldehyde-3-P value became constant (usually within 40 minutes under this condition) 0.2 ml of 50% trichloroacetic acid was added and the mixture was centrifuged. The supernatant solution was neutralized and analyzed for the products. Values are expressed as total μmoles.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>0 time</th>
<th>40 min</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylulose-5-P</td>
<td>1.47</td>
<td>0.649</td>
<td>0.821</td>
</tr>
<tr>
<td>Ribose-5-P</td>
<td>1.83</td>
<td>1.09</td>
<td>0.74</td>
</tr>
<tr>
<td>Sedoheptulose-7-P</td>
<td>0.189</td>
<td>0.948</td>
<td>0.759</td>
</tr>
<tr>
<td>Glyceraldehyde-3-P</td>
<td>0.133</td>
<td>0.883</td>
<td>0.750</td>
</tr>
</tbody>
</table>

Table IV

Equilibrium constants with different substrates

System 1a. In a final volume of 2.0 ml, 0.82 unit of transketolase (8 units/mg) was incubated in quadruplicate at 25° with the following reagents: 50 μmoles of glycglycine buffer, pH 7.6, 1.55 μmoles of xylulose-5-P, 1.58 μmoles of ribose-5-P, 0.2 μmole of thiamine pyro-P, and 2.0 μmoles of MgCl₂. After incubating for various intervals of time (0, 10, 20, and 40 minutes), 0.2 ml of 50% trichloroacetic acid was added and the mixtures were centrifuged. The supernatant solutions were adjusted to pH 6.8 and analyzed as described under "Experimental Procedure." System 1b. In a final volume of 2.0 ml, 1.7 units of transketolase (11 units/mg) were incubated at 25° with the following reagents: 50 μmoles of glycglycine buffer, pH 7.6, 2.62 μmoles of sedoheptulose-7-P, 2.12 μmoles of glyceraldehyde-3-P, 0.2 μmole of thiamine pyro-P, and 2.0 μmoles of MgCl₂. The rest of the procedure was the same as for system 1a.

System 2. In a final volume of 2.0 ml, 1.6 units of transketolase (10 units/mg) were incubated with 2.02 μmoles of xylulose-5-P and 1.93 μmoles of erythrose-4-P, 0.2 μmole of thiamine pyro-P, and 2.0 μmoles of MgCl₂. Since trichloroacetic acid inhibits glucose-6-P dehydrogenase, metaphosphoric acid was used for deproteinization.

System 3. In a final volume of 2.0 ml, 2.0 units of transketolase (9 units/mg) were incubated at 25° with 2.0 μmoles of fructose-6-P, 2.0 μmoles of glycolaldehyde, 0.2 μmole of thiamine pyro-P, and 2.0 μmoles of MgCl₂. The rest of the procedure was the same as for system 2.

<table>
<thead>
<tr>
<th>System</th>
<th>Reaction</th>
<th>Equilibrium constants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 min</td>
</tr>
<tr>
<td>1a</td>
<td>X₅-P + R-5-P =&gt; S-7-P + G-3-P</td>
<td>1.28</td>
</tr>
<tr>
<td>1b</td>
<td>S-7-P + G-3-P =&gt; X₅-P + R-5-P</td>
<td>0.922</td>
</tr>
<tr>
<td>2</td>
<td>X₅-P + E-4-P =&gt; F-6-P + G-3-P</td>
<td>8.7</td>
</tr>
<tr>
<td>3</td>
<td>F-6-P + glycolaldehyde =&gt; erythrose + E-4-P</td>
<td>0.0152</td>
</tr>
</tbody>
</table>

1 A. Kornberg, unpublished procedure.
2 We wish to thank Dr. H. H. Sephton for carrying out these chromatographic experiments.
3 G. de la Haba and A. G. Datta, unpublished data.
progressive inhibition with time was observed (Fig. 5, Curve 2) indicating a slow displacement of enzyme-bound thiamine pyro-
P. There was very little inactivation after 4 hours at room temperature in the control containing holoenzyme alone (Curve 1). Attempts to displace oxythiamine pyro-P attached to the enzyme with excess thiamine pyro-P were less successful. Although approximately 1500-fold excess of thiamine pyro-P over oxythiamine pyro-P was used, only 20% of the original activity was restored in 3 hours (Curve 3). In contrast to these findings, the inhibition of wheat germ pyruvic decarboxylase by oxythiamine pyro-P (0.388 μM) is reversed by about 30% with the addition of thiamine pyro-P (0.195 μM) 20 minutes after the addition of oxythiamine pyro-P (24).

In the course of assays of thiamine pyro-P in transketolase preparations, it was observed that on boiling of a resolved transketolase preparation, a compound was released which was strongly inhibitory to the enzyme. Similar to oxythiamine pyro-P, the inhibitor was most effective when added to resolved enzyme before the addition of thiamine pyro-P. Thiamine monophosphate did not inhibit under these conditions.

**DISCUSSION**

In early stages of investigations, enzymes are often considered to be rather specific for a given substrate, usually a metabolic intermediate. Later on when the enzyme becomes available in larger quantities, additional substrates are found to be utilized by the enzyme, although frequently at a much lower rate. In the

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Table V

<table>
<thead>
<tr>
<th>System</th>
<th>Substrate</th>
<th>Cosubstrate</th>
<th>K_m</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xylose-5-P</td>
<td>Ribose-5-P (0.005 m)</td>
<td>2.1 x 10^{-4}</td>
</tr>
<tr>
<td>2</td>
<td>Fructose-6-P</td>
<td>Ribose-5-P (0.01 m)</td>
<td>1.8 x 10^{-3}</td>
</tr>
<tr>
<td>3</td>
<td>Erythrose-4-P</td>
<td>Glyceraldehyde-3-P (0.001 m)</td>
<td>4.0 x 10^{-4}</td>
</tr>
<tr>
<td>4</td>
<td>Ribose-5-P</td>
<td>Xylose-5-P (0.0019 m)</td>
<td>4.0 x 10^{-4}</td>
</tr>
</tbody>
</table>

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pyro-P has been found to be a potent inhibitor of resolved transketolase. As shown in Fig. 4, oxythiamine pyro-P in a final concentration of 3.6 x 10^{-4} M and 7.2 x 10^{-3} M (Curves 4 and 5) inhibited the transketolase approximately 60 and 80%. Addition of 0.5 μmole of thiamine pyro-P 4 minutes later (arrow) did not restore the activity. Oxythiamine pyro-P added 2 minutes after addition of thiamine pyro-P had no effect on the initial rate of transketolase (Curve 2). Simultaneous addition of oxythiamine pyro-P and a 100-fold excess of thiamine pyro-P still resulted in partial inhibition (Curve 3). These findings suggested that oxythiamine pyro-P had a considerably greater affinity for the enzyme than thiamine pyro-P. It could therefore be expected that in the course of time, oxythiamine pyro-P should displace thiamine pyro-P from the holoenzyme. When 0.013 unit of holoenzyme was incubated with 0.012 μmole of oxythiamine pyro-P and 25.0 μmoles of glycylglycine buffer, pH 7.6, a

4 We wish to thank Dr. W. Bartley for a generous gift of oxythiamine pyro-P.
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Interaction of Thiamine Pyro-P and Oxythiamine Pyro-P with Transketolase

Oxythiamine pyrophosphate is a potent inhibitor of transketolase (24) but the affinity of the natural coenzyme to the apoenzyme is greater than that of the inhibitor. In the case of transketolase, the inhibitor has an affinity of several orders greater than thiamine pyro-P. This suggests the possible use of oxythiamine pyro-P as a specific metabolic inhibitor in an analysis of transketolase participation in multienzyme systems in cell-free preparations.

That thiamine pyro-P may become attached to transketolase in more than one way is indicated by the experiments revealing an inhibitory effect of the thiamine pyro-P when added to the enzyme before Mg++. In order to obtain a fully active catalytic site, Mg++ may have to act as a ligand between the enzyme and the coenzyme.

Multiple attachments of thiamine pyro-P are suggested by experiments in which transketolase was treated with large amounts of the coenzyme and reprecipitated several times to remove the excess. Direct analysis indicated as many as 9 moles of thiamine pyro-P per mole of enzyme. The role of thiamine pyro-P as a carrier of active glycolaldehyde will be discussed in the accompanying paper.

SUMMARY

1. Transketolase from yeast becomes insoluble in water after storage for several months at 0° as a crystalline suspension in 2 M ammonium sulfate. The enzyme dissolves readily on addition of Mg++ and thiamine pyrophosphate.

2. Arabinose 5-phosphate and glucose 6-phosphate serve as acceptor aldehydes for yeast transketolase. With the latter, an octulose 8-phosphate accumulated which was isolated as a barium salt.

3. Equilibrium constants and Ke, values with different substrate pairs are reported.

4. Oxythiamine pyrophosphate is a potent inhibitor of transketolase.

G. R. Bartlett, personal communication.

N. K. Richtmyer, personal communication.

M. Tabachnick, unpublished observation.

G. de la Haba, unpublished observation.
ketolase. Its affinity to the apoenzyme is several orders of magnitude greater than that of thiamine pyrophosphate.

5. The significance of glucose 6-phosphate as a substrate for transketolase, the formation of octulose 8-phosphates, and the interaction between transketolase and thiamine pyrophosphate are discussed.

Acknowledgments—The authors wish to thank Mrs. E. Schroeder and Mr. D. Couri for their valuable technical assistance.

REFERENCES