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Effect of Orthophosphate on the Transaldolase Reaction*

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In previous studies[1] with a dialyzed supernatant fraction from rat liver it was observed that the formation of heptulose phosphate from fructose 6-phosphate was significantly inhibited by inorganic phosphate. It has since been shown (1, 2) that the activity of the dialyzed liver fraction can be related to its content of transketolase and transaldolase and it became of interest to determine whether the inhibition of the over-all process by phosphate was due to its effect on one or both of the enzymes involved in this process.

Preliminary experiments indicated that both transketolase and transaldolase were inhibited by inorganic phosphate when the enzymes were tested in the usual assay procedures. The effect of phosphate on transaldolase is reported in the present paper.

EXPERIMENTAL PROCEDURE

Materials and Methods

Transaldolase and 6-phosphogluconic dehydrogenase were purified from yeast as described elsewhere (2, 3). The phosphogluconic dehydrogenase preparation is a concentrated, active source of glucose 6-phosphate dehydrogenase. Glycerophosphate dehydrogenase containing triosephosphate isomerase was prepared according to Racker (4). The ammonium sulfate fraction was stored as a paste and dissolved as needed (400 mg of wet paste per ml of water). This fraction also contained a very active hexosephosphate isomerase. Other enzymes were commercial crystalline preparations. Sedoheptulose 7-phosphate was prepared as previously described (5) by one of us; the other substrates were commercial preparations. Fructose 1,6-diphosphate was purified (6) to remove contaminating glucose 6-phosphate and fructose 6-phosphate.

RESULTS

The following reactions (6, 7) catalyzed by transaldolase were studied:

$$\text{Fructose 6-phosphate} + \text{D-glyceraldehyde} \rightleftharpoons \text{D-glyceraldehyde 3-phosphate} + \text{D-fructose} \quad (1)$$

$$\text{Sedoheptulose 7-phosphate} + \text{D-glyceraldehyde 3-phosphate} \rightleftharpoons \text{D-fructose 6-phosphate} + \text{D-erythrose 4-phosphate} \quad (2)$$

In these reactions, fructose 6-phosphate and sedoheptulose 7-phosphate act as dihydroxyacetone donors and D-glyceraldehyde and D-glyceraldehyde 3-phosphate as acceptors.

Reaction 1 was followed spectrophotometrically at 340 mμ with triose phosphate formation measured by DPNH oxidation. The assay system (1.0 ml) contained 0.08 μmole of DPNH, 0.013 or 0.004 mg of transaldolase (specific activity, 32 units per mg), 0.01 ml of glycerophosphate dehydrogenase containing triosephosphate isomerase, 2 μmoles of ethylenediaminetetraacetate, and 40 μmoles of triethanolamine buffer, pH 7.6. The temperature was 20°. Transaldolase was added to start the reaction and readings were taken at 1-minute intervals. Phosphate, D-glyceraldehyde, and fructose 6-phosphate were added in the concentrations indicated.

The inhibition of transaldolase by phosphate is competitive with fructose 6-phosphate (Fig. 1). K_s for fructose 6-phosphate was calculated to be 2×10^{-4} M. K_i for inorganic phosphate was found to be 5×10^{-2} M. On the other hand, inhibition by phosphate is noncompetitive with glyceraldehyde (Fig. 2). Under the conditions of the experiment the extent of inhibition did not vary significantly over a 5-fold range of glyceraldehyde concentration. K_s for glyceraldehyde is about 7×10^{-3} M; this is sufficiently smaller than the K_i for inorganic phosphate so that competitive inhibition could have been detected.

Reaction 2 was followed spectrophotometrically at 340 mμ with hexose monophosphate formation measured by TPN reduction. The assay system (1.0 ml) contained: 0.07 μmole of TPN, 0.30 mg of phosphoglucose isomerase, 0.45 mg of 6-phosphogluconic dehydrogenase as the source of glucose 6-phosphate dehydrogenase, 0.005 ml of crystalline aldolase, 0.013 mg of transaldolase (specific activity 32 units per mg), 2 μmoles of ethylenediaminetetraacetate, and 40 μmoles of triethanolamine buffer, pH 7.6. The temperature was 20°. Transaldolase was added to start the reaction and readings were taken at 1-minute intervals. The other components were added as indicated.

Inhibition of transaldolase by phosphate was found to be noncompetitive with respect to either sedoheptulose 7-phosphate (Fig. 3) or glyceraldehyde 3-phosphate (Fig. 4). In the six experiments shown, K_i for inorganic phosphate was calculated to lie between 5.1 and 7.4×10^{-2} M, with a mean value of 6.6×10^{-2}, which compares favorably with the value of 5.0×10^{-2} M derived from the result in Fig. 1. Thus, whether inhibition be competitive or noncompetitive, inorganic phosphate is bound with the same affinity, indicating that we are dealing with a single binding site for this substance.

The reaction catalyzed by transaldolase can be considered to occur in two steps:

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[1] A. Bonsignore, S. Pontremoli, and E. Grazi, unpublished observations.

Donor + transaldolase ⇌
transaldolase-dihydroxyacetone complex (*a*)

Transaldolase-dihydroxyacetone complex + acceptor ⇌
transaldolase + product (*b*)

Since neither of the acceptors tested, glyceraldehyde or glyceraldehyde 3-phosphate, competes with inorganic phosphate, it can be concluded that the second step, transfer of the bound dihydroxyacetone fragment to the acceptor, is not affected by inorganic phosphate. Phosphate must therefore inhibit formation of the transaldolase-dihydroxyacetone complex. The inorganic phosphate so bound can be displaced by fructose 6-phosphate, but not by sedoheptulose 7-phosphate. It is unlikely, in view of this result, that the binding site for fructose 6-phosphate is

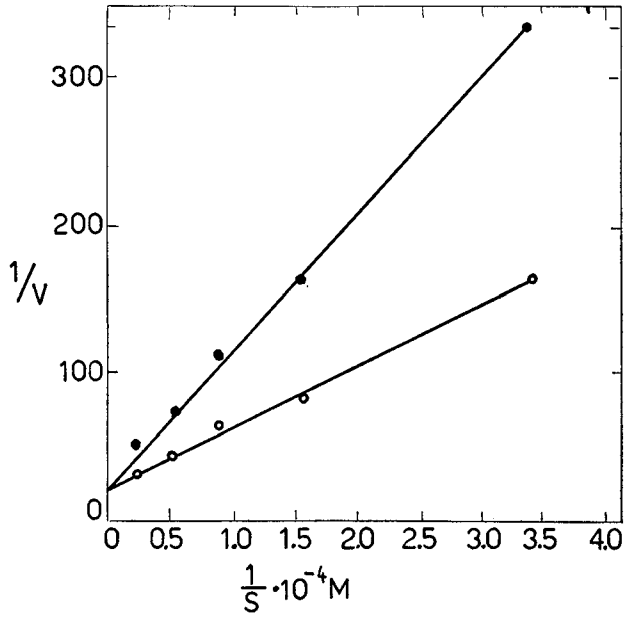

FIG. 1. Phosphate inhibition of Reaction 1 as a function of the concentration of fructose 6-phosphate. The reaction conditions are given in the text. In addition, the reaction mixtures contained 60 μmoles of phosphate, 3.0 μmoles of D-glyceraldehyde, and 0.03, 0.06, 0.12, 0.24, or 0.48 μmole of fructose 6-phosphate. The results are plotted according to Lineweaver and Burk (8). *Open circles*, without phosphate; *closed circles*, with phosphate. *V* is the density change per minute at 340 mμ.

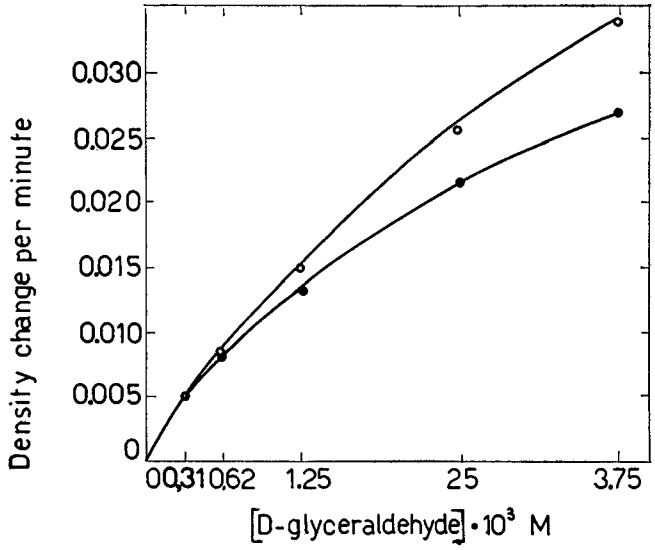

FIG. 2. Phosphate inhibition of Reaction 1 as a function of the concentration of D-glyceraldehyde. The reaction conditions were as described in the text, with 60 μmoles of phosphate buffer, pH 7.6, and 0.9 μmole of fructose 6-phosphate. D-Glyceraldehyde was employed at concentrations indicated. *Open circles*, without phosphate; *closed circles*, with phosphate.

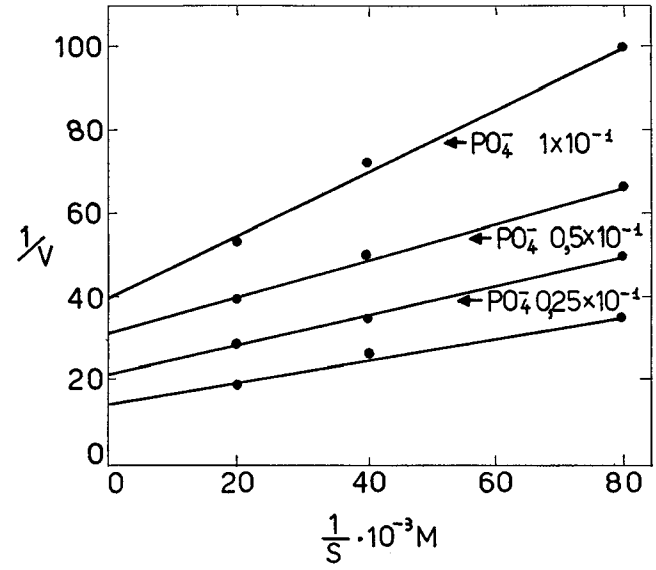

FIG. 3. Phosphate inhibition of Reaction 2 as a function of the concentration of sedoheptulose 7-phosphate. The reaction conditions were as described in the text, with 0.25 μmole of fructose diphosphate, and phosphate and sedoheptulose 7-phosphate as indicated in the figure. The lowest curve was obtained in the absence of inorganic phosphate.

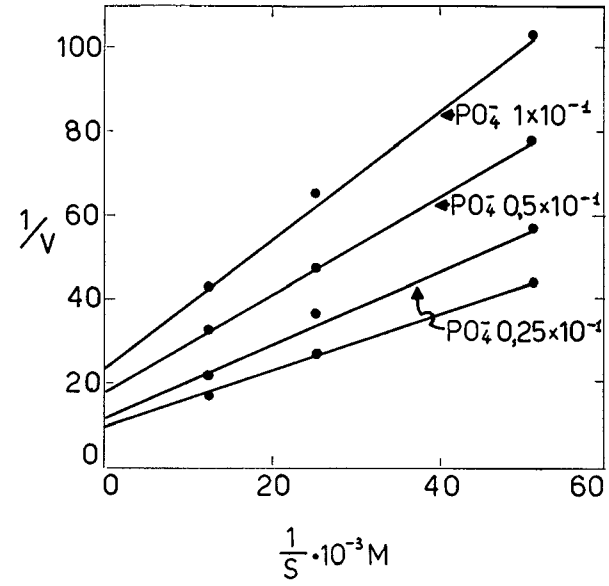

FIG. 4. Phosphate inhibition of Reaction 2 as a function of the concentration of fructose diphosphate. The reaction conditions were as described in the text, with 1.7×10^{-3} μmoles of sedoheptolose 7-phosphate and fructose diphosphate as indicated. The lowest curve was obtained in the absence of inorganic phosphate.

identical with that involved in the binding of sedoheptulose 7-phosphate.

A solution of this problem is essential to an understanding of the mechanism of action of transaldolase. Further evidence will be sought by direct measurement of substrate and inhibitor binding with stoichiometric quantities of enzyme.

SUMMARY

Orthophosphate inhibits the following reactions catalyzed by transaldolase:

D-Fructose 6-phosphate + D-glyceraldehyde ⇌ D-glyceraldehyde 3-phosphate + D-fructose (1)

Sedoheptulose 7-phosphate + D-glyceraldehyde 3-phosphate ⇌ D-fructose 6-phosphate + D-erythrose 4-phosphate (2)

The inhibition of transaldolase by inorganic phosphate appears to be competitive with fructose 6-phosphate and noncompetitive with sedoheptulose 7-phosphate, glyceraldehyde, or glyceraldehyde 3-phosphate. K_i for inorganic phosphate is approximately 5.0×10^{-7} M, as measured in either the competitive or noncompetitive test system. This suggests a single binding site for inorganic phosphate. The bearing of these observations on the reaction mechanism is discussed.

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