THE FORMATION AND CLEAVAGE OF FRUCTOSE CATALYZED BY TRANSALDOLASE

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Transaldolase catalyzes the transfer of a dihydroxyacetone group from sedoheptulose-7-phosphate, or fructose-6-phosphate to acceptors such as D-glyceraldehyde-3-phosphate or D-erythrose-4-phosphate, but has been reported to be inactive with free glyceraldehyde (Horecker and Smyrniotis, 1955). In the present paper are described new results which demonstrate the following reversible reaction catalyzed by this enzyme:

Fructose-6-phosphate + D-glyceraldehyde $\rightleftarrows$
D-fructose + D-glyceraldehyde-3-phosphate.

Transaldolase [1] was purified from Torula yeast by a procedure which will be reported elsewhere [2]. These preparations are free of transketolase and possess only feeble phosoglucose isomerase activity. They are comparable in specific activity to preparations obtained by the older procedure (Horecker and Smyrniotis, 1955). When transaldolase is incubated with a mixture of F6P and D-glyceraldehyde, triose phosphate and D-fructose are formed. (Table I). Triose phosphate was determined by measurement of DPNH oxidation in the presence of glycerophosphate dehydrogenase and triose phosphate isomerase (Racker, 1947). Fructose was identified

[1] Abbreviations: TA, transaldolase; DPNH, reduced diphosphopyridine nucleotide; ATP, adenisine triphosphate; TPN, triphosphypyridine nucleotide; FDP, fructose-1, 6-diphosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate.

[2] Pontremoli, S., Bonsignore, A., Grazi, E. and Horecker, B.L. manuscript in preparation.

TABLE I

Fructose and Triose Phosphate Formation

Exper. No.	Initial concentrations			Final concentrations					K
	G-6-P	F-6-P	D-glycer-aldehyde	G-6-P	F-6-P	D-fructose	Triose phosphate	D-glycer-aldehyde	
	µmoles per ml	µmoles per ml	µmoles per ml	µmoles per ml	µmoles per ml	µmoles per ml	µmoles per ml	µmoles per ml	
1	1.38	6.98	4.50	3.71	1.59	2.93	3.00	1.57	0.28
2	0.71	3.41	3.00	1.60	0.70	1.81	1.80	1.18	0.25

The reaction mixture (2 ml.) containing 0.06 mg of TA (specific 32 units/mg) was incubated at 30° C. in 0.02 M triethanolamine buffer pH 7.6 containing 0.005 M ethylenediaminetetraacetate. At zero time and at different intervals aliquots were removed, treated with 10 percent TCA, and analyzed. The final concentration of D-glyceraldehyde was calculated by difference. The data reported in the table refer to aliquots removed after 3 hours at which time equilibrium was reached. The values are the average of 6 experiments. The initial amount of G-6-P is due to impurity in the F-6-P.

$$K = \frac{(\text{Fructose-6-phosphate}) \cdot (\text{D-glyceraldehyde})}{(\text{D-fructose}) \cdot (\text{Triose-P})}$$

and determined with an assay mixture containing ATP, hexokinase, glucose phosphate isomerase, glucose-6-phosphate dehydrogenase and TPN. In this system the reduction of TPN is a measure of the quantity of D-fructose. Controls lacking the isomerase were included to correct for D-glucose.

The equilibrium constant calculated from the concentration of total triose phosphate was about 0.30. Assuming complete equilibration of D-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate the true equilibrium constant is about 6.0; the reaction would thus appear to favor the formation of F6P and free glyceraldehyde.

Direct evidence for the reversibility of the reaction, namely the cleavage of free fructose and the formation of fructose-6-phosphate, was obtained with the following reaction mixture; 10 μmoles of D-fructose, 0.76 μmoles of FDP, 0.1 mg of glucose phosphate isomerase, 0.005 ml of aldolase, 0.45 mg of glucose-6-phosphate dehydrogenase, 0.024 mg of TA (specific activity = 32 unit/mg.), 10 μmoles of ethylenediaminetetraacetate in 1.1 ml of 0.04 M triethanolamine buffer, pH 7.6. Under these conditions the rate of TPN reduction was 4.0 mμmoles per minute, compared with a rate of 0.4 mμmoles per minute in a control lacking fructose. No F6P was formed in controls lacking either TA or FDP.

The reaction velocity increases with increasing concentration of D-glyceraldehyde up to about 0.015 M. K_m for D-glyceraldehyde calculated from the reciprocal plot (Lineweaver and Burk, 1934) is about 7×10^{-3} moles per liter; the affinity of TA for D-glyceraldehyde is thus about 30 times less that for D-glyceraldehyde-3-phosphate (Horecker and Smyrniotis, 1955).

The physiological significance of this reaction is not clear. D-glyceraldehyde is formed from fructose-1-phosphate in liver (Hers and Kusaka, 1953; Leuthardt and Wolf, 1954). It is possible that this substance re-enters the metabolic

pathways following its conversion to fructose, which is in turn phosphorylated.

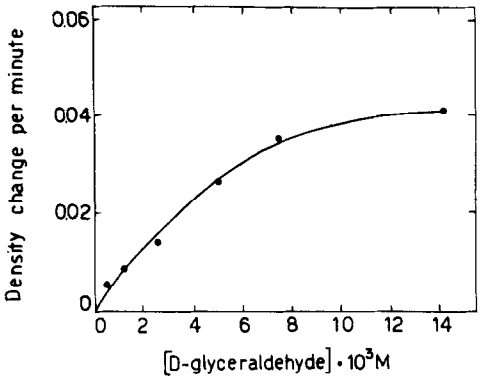

Fig. 1 - Effect of D-glyceraldehyde concentration on the velocity of the transaldolase reaction. The assay system (1.1 ml) contained 1.0 μ mole of F-6-P, 0.08 μ mole of DPNH, 0.01 ml. of glycerophosphate dehydrogenase containing triose phosphate isomerase, 0.0125 mg of TA (specific activity 32 units/mg), 10 μ moles of ethylenediaminetetraacetate in 0.04 M triethanolamine buffer pH 7.6. Transaldolase was added to start the reaction and readings were taken at 1 minute intervals at 340 mμ. The temperature was 20°.

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