THE FORWARD AND REVERSE REACTIONS OF TRANSALDOLASE

T. WOOD
Department of Biochemistry, McGill University, Montreal, Quebec, Canada

Received 2 June 1972

1. Introduction

Transaldolase and transketolase catalyse important reactions of the pentose phosphate pathway, and studies of the relative amounts of the enzymes of the pathway in animal tissues indicate that either, or both of these enzymes may control the rate *in vivo* of the non-oxidative part of the pathway [1–3]. Transaldolase catalyses the following reaction:

$$\text{S-7-P} + \text{GAP} \rightleftharpoons \text{F-6-P} + \text{Er-4-P}$$

The level of transaldolase in a tissue has usually been measured by determining the maximal rate at which an extract or homogenate of the tissue can form GAP from F-6-P and Er-4-P, in the reverse reaction as written above [1, 2, 4]. The data obtained is often used to estimate the overall rate of hexose monophosphate formation or the flow of glucose through the pathway. Consequently, it is important to know the relative maximal rates of the transaldolase reaction in both directions so that the maximal rate of the forward direction may be calculated from the experimentally determined maximal rate in the reverse direction.

The equilibrium constant of the reaction and data on these relative rates have been published by Venkataraman and Racker [5] who showed that the rate of the forward reaction depended markedly on the methods used to supply the substrates and follow the reaction. This letter describes experiments in which the forward reaction was carried out in several different ways and its rate compared with that of the reverse reaction.

Abbreviations: S-7-P, D-sedoheptulose 7-phosphate; GAP, D-glyceraldehyde 3-phosphate; F-6-P, D-fructose 6-phosphate; Er-4-P, erythrose 4-phosphate; FDP, D-fructose 1,6-diphosphate; R-5-P, D-ribose 5-phosphate; Xu-5-P, D-xylulose 5-phosphate; TPP, thiamine pyrophosphate.

2. Materials and methods

Crystalline transaldolase (26 units/mg) and other enzymes were purchased from Sigma. Transketolase was from *Candida utilis* [6]. Erythrose 4-phosphate was prepared by oxidation of glucose 6-phosphate [7] and sedoheptulose 7-phosphate was prepared as described previously [8] or purchased from Sigma. Hexose monophosphate was determined in the presence of 0.25 mM NADP by the addition of 0.3 unit of glucose 6-phosphate dehydrogenase and 2.4 units phosphoglucose isomerase using a Beckman DB spectrophotometer and recorder, and also fluorometrically [9] using a Turner 450 spectrofluorometer attached to a recorder.

3. Results and discussion

The conditions used and the rates obtained are given in table 1. The essential difficulty in attaining the maximal rate of the forward reaction lay in providing a saturating concentration of GAP, the K_m of which is reported as 0.22 mM [10]. This substrate was supplied directly or generated from FDP, or generated together with S-7-P from pentose phosphates and transketolase. The K_m for S-7-P is 0.17 mM [5, 10] and doubling the concentration from

Table 1
Rates of transaldolase in the forward and reverse reactions.

Conditions	Method of following reaction	Rate (μmoles/min/ml)	Reverse reaction / Forward reaction
Reverse reaction			
5 mM F-6-P, 0.15 mM Er-4-P, 0.13 mM NADH, 0.26 unit glycerol 1-phosphate dehydrogenase, 1.6 units triose phosphate isomerase	I	13.9 ± 2.0 [6]	
Forward reaction			
4 mM FDP, 1 mM S-7-P, 0.25 mM NADP, 0.2 unit aldolase	II	1.8	7.7
4 mM FDP, 1 mM S-7-P, 0.25 mM NADP, 0.2 unit aldolase, 0.3 unit glucose 6-phosphate dehydrogenase, 2.4 units phosphoglucoisomerase	III	2.4	5.8
1.7 mM GAP, 1 mM S-7-P, 0.25 mM NADP, 0.3 unit glucose 6-phosphate dehydrogenase, 2.4 units phosphoglucose isomerase	III	2.3	6.0
3.4 mM GAP, 1 mM S-7-P	V	3.3	4.2
4 mM FDP, 1 mM S-7-P, 0.2 unit aldolase	IV	4.1	3.4
1.8 mM Xu-5-P, 3 mM R-5-P, 0.1 mM TPP, 2.5 mM magnesium chloride, 0.2 unit transketolase	V	4.5	3.1
4 mM FDP, 1 mM S-7-P, 0.2 unit aldolase	V	4.5 ± 0.9 [5]	3.1

Buffer was 50 mM triethanolamine-chloride, pH 7.4, and temperature 37° in all experiments. Methods of following reaction: I, NADH oxidation followed; II, incubated for 20 min, then 0.3 unit glucose 6-phosphate dehydrogenase and 2.4 units phosphoglucose isomerase added; III, NADP reduction followed; IV, ultrafiltered after 20 min and hexose monophosphate measured; V, incubated for 20 min, then heated 3 min at 100°, cooled, and hexose monophosphate measured.

1 to 2 mM had no detectable effect on the rate, nor did doubling the FDP concentration from 4 mM to 8 mM, and it was concluded that at these concentrations, which were greater than those employed by Venkataraman and Racker [5], the transaldolase was saturated. Care was taken to include controls containing all components except transaldolase in each experiment. The total amount of hexose monophosphate present at the end of the incubation was never more than 10–15% of the equilibrium value based upon a K_{eq} of 1.9 calculated from the published equilibrium concentrations of Venkataraman and Racker [5]. (It should be noted that the K_{eq} value of 0.95 at the head of table IV of these authors does not correspond to the concentrations in the table.) The maximal amount of ammonium sulphate introduced into the reaction system with the transaldolase and auxiliary enzymes was 7.5 mM, a concentration that was found to have no significant inhibitory effect on the transaldolase reaction in either direction.

The method used to follow the forward reaction presented further problems. F-6-P was measured in the presence of phosphoglucose isomerase by conversion to glucose 6-phosphate which was then oxidised by NADP in the presence of glucose 6-phosphate dehydrogenase. Phosphoglucose isomerase, however, is strongly inhibited by S-7-P [5], and even more so by Er-4-P [5, 11]. I have confirmed that the latter compound was destroyed, without affecting the F-6-P, when the reaction was stopped by heating for 3 min at 100° [5]. Even in the presence of Er-4-P, when NADP reduction was followed in the spectrophotometer, 2.4 units of phosphoglucose isomerase was sufficient to give rates of about 50% of the maximum obtained and to permit the complete oxidation of 0.05 μmole of added F-6-P in 8 min.

The measurement of the maximal rate of the reverse reaction presented no difficulties. The K_m for F-6-P lies between 0.32 and 0.53 mM [5, 12, 13] and doubling the concentration from 5 mM to 10 mM did not affect the rate. Er-4-P has a K_m of 0.02 mM [12, 13] and at concentrations above 0.1 mM it in-

hibits the reaction [12]. In the system used here, the rate reached a maximum at 0.15 mM Er-4-P.

Venkataraman and Racker [5] reported that 10 mM cysteine, mercaptoethanol, and glutathione, were able to inhibit the reverse reaction and speed up the forward reaction, apparently by complexing Er-4-P. In common with Srivastava and Hubscher [14], I have been unable to confirm these findings. However, a non-enzymic reaction was observed between 10 mM cysteine and NADH, leading to a steady fall in absorption at 340 nm, the rate of which was accelerated by the addition of F-6-P.

The rate of the forward reaction, carried out in several different ways, varied by a factor of 2.5, compared to a factor of 15 in Venkataraman and Racker's experiments [5]. The smallest ratio obtained of the rates of the reverse and forward reactions was about 3 compared to a ratio of 1 reported by Venkataraman and Racker [5]. However, their enzyme was only half-saturated with F-6-P when the reverse reaction was studied and at saturation their rate ratio would have been 2. Furthermore, it is not clear from the description of their procedure whether the further reaction of Er-4-P with xylulose-5-phosphate to form F-6-P, in the presence of transketolase, was taken into account when calculating the rate of transaldolase in their 'assay IV'. In my experiments, the observed rate of F-6-P formation was halved to correct for this further reaction. A ratio of 3 may also be calculated from the data of Srivastava and Hubscher [14] for the transaldolase in intestinal mucosa. But, these workers also used a sub-optimal concentration of F-6-P and may not have used optimal conditions for the forward reaction.

Triose phosphate isomerase is present in crystalline transaldolase from brewer's yeast [10, 15] and 21 units/mg was found in the crystalline enzyme from *Candida utilis* used here. Consequently, the concentrations of glyceraldehyde phosphate may have been less than calculated, due to its conversion to dihydroxyacetone phosphate. The same objection applies also to the baker's yeast enzyme used by Venkataraman and Racker since they did not show that the isomerase was absent. The highest rate of the forward reaction reported here may not represent the maximal rate obtainable under laboratory conditions with an enzyme completely free of triose phosphate isomerase. Nonetheless, it probably does represent the maximal rate generally attainable both *in vivo* and *in vitro* in the presence of triose phosphate isomerase, a ubiquitous enzyme likely to be present in all tissues and extracts containing transaldolase. It may be said that, for all practical purposes, the maximal forward rate of transaldolase will be about one-third of the maximal reverse rate as measured at a saturating concentration of fructose 6-phosphate and an optimal concentration of erythrose 4-phosphate.

References

[1] F. Novello and P. McLean, Biochem. J. 107 (1968) 775.
[2] E.L. Tan and T. Wood, Compar. Biochem. Physiol. 31 (1969) 635.
[3] K. Brand, K. Deckner and J. Musil, Z. Physiol. Chem. 351 (1970) 213.
[4] F.C. Kauffman, J. Neurochem. 19 (1972) 1.
[5] R. Venkataraman and E. Racker, J. Biol. Chem. 236 (1961) 1876.
[6] M.E. Kieley, E.L. Tan and T. Wood, Can. J. Biochem. 47 (1969) 455.
[7] A.S. Sieben, A.S. Perlin and F.J. Simpson, Can. J. Biochem. 44 (1966) 663.
[8] T. Wood and W.M. Poon, Arch. Biochem. Biophys. 141 (1970) 440.
[9] F.C. Kauffman, J.G. Brown, J.V. Passoneau and O.H. Lowry, J. Biol. Chem. 244 (1969) 3647.
[10] B.L. Horecker and P.Z. Smyrniotis, J. Biol. Chem. 212 (1955) 811.
[11] E. Grazi, A. De Flora and S. Pontremoli, Biochem. Biophys. Res. Commun. 2 (1960) 121.
[12] S. Pontremoli, A. Bonsignore, E. Grazi and B.L. Horecker, J. Biol. Chem. 235 (1960) 1881.
[13] O. Tchola and B.L. Horecker, in: Methods in Enzymology, Vol. IX, ed. W.A. Wood (Academic Press Inc., New York, 1966) p. 499.
[14] L.M. Srivastava and G. Hubscher, Biochem. J. 101 (1966) 48.
[15] B.L. Horecker and P.Z. Smyrniotis, in: Methods in Enzymology, Vol. I, eds. S.P. Colowick and N.O. Kaplan (Academic Press Inc., New York, 1955) p. 381.