Birns, M. (1961), *Exp. Cell Res. 22*, 317.
Castellino, F. J., and Barker, R. (1968), *Biochemistry 7*, 2207.
Changeux, J.-P. (1961), *Cold Spring Harbor Symp. Quant. Biol. 26*, 313.
Davis, B. J. (1964), *Ann. N. Y. Acad. Sci. 121*, 404.
Gerhart, J. C., and Pardee, A. B. (1962), *J. Biol. Chem. 237*, 891.
Iwatsuki, N., and Okazaki, R. (1967), *J. Mol. Biol. 29*, 155.
Jovin, T., Chrambach, A., and Naughton, M. A. (1964), *Anal. Biochem. 9*, 351.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Martin, R. G. (1963), *J. Biol. Chem. 238*, 257.
Martin, R. G., and Ames, B. N. (1961), *J. Biol. Chem. 236*, 1372.
Massey, V., Curti, B., and Ganther, H. (1966), *J. Biol. Chem. 241*, 2347.
Orengo, A. (1966), *Exp. Cell Res. 41*, 338.
Orengo, A. (1969), *J. Biol. Chem. 244*, 2204.
Ornstein, L. (1964), *Ann. N. Y. Acad. Sci. 121*, 321.
Patte, J., LeBras, G., Loving, T., and Cohen, G. N. (1963), *Biochim. Biophys. Acta 67*, 16.
Saunders, G. F., and Campbell, L. L. (1966), *J. Bacteriol. 91*, 332.
Saunders, P. P., Wilson, B. A., and Saunders, G. F. (1969), *J. Biol. Chem. 244*, 3691.
Vallee, B. L., and Hoch, F. L. (1955), *Proc. Nat. Acad. Sci. U. S. 41*, 327.

Purification and Properties of Transaldolase from Bovine Mammary Gland†

Ekkehard Kuhn and Karl Brand*

ABSTRACT: Transaldolase (EC 2.2.1.2) was purified from bovine mammary gland to a specific activity of 4 IU/mg by a combination of ammonium sulfate precipitation and chromatography on DEAE-Sephadex A-50, Sephadex G-100, and DEAE-cellulose. This purification procedure was found to be reproducible within ±10%. The enzyme appears to be homogeneous as judged from sedimentation equilibrium and sedimentation velocity experiments in the ultracentrifuge. An aqueous and neutral solution of this transaldolase preparation is stable at 2° for at least 8 weeks. No evidence was obtained for the requirement of a cofactor. The molecular weight (M) determined by equilibrium sedimentation and thin-layer gel chromatography was found to be 65,000 ± 2%. From sedimentation velocity experiments a sedimentation coefficient $s_{20,w}$ of 3.67 S was obtained. The diffusion constant and the frictional ratio were calculated from M and $s_{20,w}$ to be 5.5×10^{-7} cm^2 sec^{-1} and 1.37 S, respectively. Apparent K_m values for the substrates fructose 6-phosphate and erythrose 4-phosphate were determined to be 2×10^{-4} and 7×10^{-6} M. The enzyme is stable between pH 4 and 9 and has an optimum activity at pH 8.2. In contrast to transaldolase from *Candida utilis* transaldolase from bovine mammary gland was completely inactivated by treatment with sodium borohydride at pH 6.0 and 2° in the absence of substrate fructose 6-phosphate. The rate of inactivation, however, was strongly enhanced by the addition of substrate. No radioactively labeled amino acid derivative could be detected in the acid hydrolysate with two different methods, when the enzyme was treated with borohydride in the presence of excess U-^{14}C-labeled fructose 6-phosphate. The results are discussed and compared to the structural and catalytic properties reported from the yeast enzyme.

Transaldolase (D-sedoheptulose 7-phosphate:D-glyceraldehyde 3-phosphate dihydroxyacetone transferase, EC 2.2.1.2) catalyzes fructose-6-P + erythrose-4-P ⇌ sedoheptulose-7-P + glyceraldehyde-3-P.

When transaldolase isolated from *Candida utilis* is incubated with fructose 6-phosphate in the absence of the acceptor, erythrose-4-P, a stable enzymatically active intermediate accumulates (Horecker *et al.*, 1961; Venkataraman and Racker, 1961). This intermediate has been identified as a Schiff base containing dihydroxyacetone linked to the ε-amino group of a lysine residue of the enzyme (Horecker *et al*, 1963). Further evidence for the azomethine structure of the intermediate was presented by demonstrating the addition of HCN to the isolated transaldolase–dihydroxyacetone complex (Brand and Horecker, 1968). Histidine residues in transaldolase from yeast were reported to have a specific function in removing the proton from the C-4 hydroxyl group of fructose 6-phosphate and thus promoting the aldol-cleavage reaction (Brand *et al.*, 1969).

It is known that transaldolase is widely distributed in microorganisms, plants, and animal tissues. So far, however, the enzyme was purified only from various types of yeast cells (Pontremoli *et al.*, 1961, Venkataraman and Racker, 1961; Tsolas and Horecker, 1970). No isolation from animal tissues is reported in the literature.

In the present work a purification procedure of transaldolase from bovine mammary gland is described. Furthermore results obtained from studies on the molecular and kinetic properties of the enzyme and its modification by sodium borohydride are presented.

† From the Max-Planck-Institut für Ernährungsphysiologie, 46 Dortmund, Rheinlanddamm 201 Germany. *Received November 5, 1971.* Dedicated to Professor A. E. Braunstein, Moscow, on the occasion of his 70th anniversary.

* To whom correspondence should be sent.

TABLE I: Transaldolase Activity in Different Bovine Tissues.

Tissue	Act. (IU × g of Tissue^{-1})	Sp Act. (IU × mg of Extract Protein^{-1})
Pancreas	1.53	0.010
Testis	0.82	0.007
Mammary gland	1.38	0.020
Kidney	1.61	0.011
Adrenal gland	0.74	0.005
Thymus	0.32	0.002

Materials

Bovine mammary gland and the other tissues were freshly supplied by the local slaughterhouse. D-Erythrose 4-phosphate, fructose 6-phosphate, NADH, NADPH, ATP, α-glycerophosphate dehydrogenase, triosephosphate isomerase, hexokinase, and the protein calibration kit for the molecular weight determination by thin-layer chromatography were purchased from Boehringer Mannheim, Germany. Sodium borohydride was obtained from Fluka AG, Buchs, Switzerland, and Coomassie Brilliant Blue R 250 from Serva Entwicklungslabor, Heidelberg. Sephadex G-100, G-100 Superfine, G-50, and A-50 were products of Pharmacia Fine Chemicals Inc., Frankfurt. DEAE-cellulose was purchased from W. and R. Balston Ltd., Maidstone, England. The gels and ion exchangers were used according to the recommendation of the producers. [^{14}C]Fructose 6-phosphate was prepared from [^{14}C]fructose with adenosine triphosphate and hexokinase (specific radioactivity 124,000 dpm/μmole of fructose 6-phosphate). Uniformly labeled [^{14}C]fructose was purchased from Nuclear Research Chemicals Inc., Orlando, Fla. Transaldolase (type III) was purified and crystallized from *C. utilis* according to the procedure described by Tsolas and Horecker (1970).Davies buffer was prepared according to Davies (1959).

Methods

Protein concentrations were determined by the Biuret method (Layne, 1957). Transaldolase activity was assayed as described by Tchola and Horecker (1966). The concentration of fructose 6-phosphate and erythrose 4-phosphate in the assay mixtures were 2.65×10^{-3} and 1.89×10^{-4} M respectively. Enzyme activity is expressed in International Units. Fructose 6-phosphate and erythrose 4-phosphate were measured enzymatically using transaldolase, triosephosphate isomerase, and α-glycerophosphate dehydrogenase.

Radioactivity was measured in a Tracerlab Model LSC 40 liquid scintillation counter, using the solvent of Bray. Quenching corrections were made with an external standard and the calculations from counts per minute to disintegrations per minute were carried out with an Olivetti Computer Programma 101.

Amino acid analyses were performed according to the method of Spackman *et al.* (1958) with a Spinco Model 120B automatic amino acid analyzer, equipped with a high-sensitivity cuvet (6-mm light path) and a recorder for the 4- to 5-mV range. The accuracy of the analyses was 5% at the 5-nmole range. Acid hydrolysis was carried out at 110° for 24 hr with 5.7 N HCl (constant boiling) in a sealed evacuated Pyrex tube. For comparison amino acid analyses were performed under the same conditions with acid hydrolysates of yeast enzyme.

High-voltage paper electrophoreses of acid hydrolysates were carried out on Schleicher & Schüll 2043B paper in 1.5 M formic acid (pH 1.8) at 4000 V (75 mA) for 35 min. Reduction with sodium borohydride was accomplished at pH 6 and 2°. pH and temperature were carefully controlled throughout the experiment. A 0.5 M aqueous solution of sodium borohydride and simultaneously about the same volume of 0.25 M acetic acid was added continuously by means of a Technicon standard AutoAnalyzer pump to maintain the pH at 6.0.

Molecular Weight determination. The molecular weight of the enzyme was determined by thin-layer chromatography on Sephadex G-100 and by sedimentation equilibrium experiments in the ultracentrifuge. The first method was carried out with a commercial apparatus of Boehringer Mannheim using Sephadex G-100 superfine and the following standard proteins of known molecular weight: cytochrome *c*, chymotrypsinogen, ovalbumin, and albumin. Proteins were stained by the procedure of Radola (1968) with Coomassie Brilliant Blue R 250.

Sedimentation equilibrium experiments were carried out according to van Holde (1967) with a Beckman Model E analytical ultracentrifuge equipped with uv optics and a photoelectric scanning system, using a six-channel centerpiece. Measurements were made at 280 nm. The light path was 12 mm. The rotor speed was kept constant within ±0.15% by an electronic speed control unit. The solution columns were underlayed with 0.01 ml of an inert oil, Fluorocarbon 43, to give sharp bottom lines. The enzyme was dialyzed against 100 mM sodium phosphate buffer before use. The same buffer was used in the reference channels.

The sedimentation coefficient was determined from sedimentation velocity experiments carried out at 25° and 44,000 rpm. Double-sector charcoal-filled Epon centerpieces were used. A multiplexer accessory allowed the use of multicell rotors.

Results

Purification of Transaldolase. Unless otherwise stated, all operations were carried out at 0–5°. pH and conductivity values refer to this temperature too. In the chromatography steps, fractions were collected automatically using a LKB 7000 Ultrorac fraction collector. The effluent was monitored for protein at 280 nm, pH, and conductivity and the fractions were assayed for enzyme activity.

In order to find a suitable source for purifying transaldolase several bovine tissues were tested for transaldolase activity. As shown on Table I lactating mammary gland, kidney and pancreas contained the highest transaldolase activity. Comparing specific activities in cell extracts from different bovine tissues mammary gland was found to be the most suitable source to isolate transaldolase.

STEP 1. EXTRACTION. Six 100-g portions of frozen tissue were sawed into small pieces and homogenized with six 400-ml portions of Davies buffer (pH 4.0) in a blender at room temperature for 3 min. The homogenate was centrifuged for 20 min at 20,000*g* and 20°. The supernatant was sucked off through a paper filter (Schleicher & Schüll no. 5891) and the volume adjusted to 3000 ml with Davies buffer (pH 4.0) (fraction A*n*/1 of Table II).

STEP 2. AMMONIUM SULFATE PRECIPITATION. To fraction A*n*/1 ammonium sulfate (873 g) was added under stirring at 20°. The mixture was allowed to stand for 10 min and then

TABLE II: Purification Procedure of Transaldolase from Bovine Mammary Gland.

Fraction	No.	Vol (ml)	Act. (IU/ml)	Act. × Vol (IU)	Concn (mg/ml)	Sp Act. (IU/mg)	Yield (%)	Purifcn
Extract	A1/1	3000	0.112	336	3.49	0.032		
	A2/1	3000	0.117	351	2.98	0.039		
	A3/1	3000	0.115	345	2.77	0.041	100	1.00
	A4/1	3000	0.126	378	2.79	0.045		
	A5/1	3000	0.133	399	3.11	0.042		
Ammonium sulfate fraction	A1/2	60	1.90	114	11.30	0.168		
	A2/2	60	2.47	148	12.10	0.204		
	A3/2	60	2.64	158	11.60	0.227	43.8	5.27
	A4/2	60	3.12	187	13.50	0.231		
	A5/2	60	3.10	186	14.10	0.219		
Combined ammonium sulfate fractions	B/1	300	2.55	765	12.40	0.205	42.2	5.15
Sephadex G-50	B/2	1130	0.68	768	4.12	0.165	42.4	4.14
DEAE-Sephadex A-50	C	1000	0.73	730	0.73	1.000	40.3	25.10
Concentrated DEAE-Sephadex A-50	D/1	25	28.50	712	12.40	2.290	39.3	57.50
Sephadex G-100	D/2	122	4.91	599	1.62	3.030	33.1	76.10
DEAE-cellulose DE-52	E	10	40.80	408	10.61	3.840	22.5	96.40

heated to 35° in a bath within 40–50 min. When a temperature of 35° was reached, the pH was adjusted to 3.6 with 2 N HCl and the mixture was stirred for an additional 10 min. After centrifugation at 20,000*g* and 2° for 20 min, the supernatant was treated with 697 g of ammonium sulfate at 2° under continuous stirring for another 10 min. The precipitate was then collected by centrifugation for 20 min at 20,000*g* and 2°, and suspended in 35 ml of Davies buffer. After 10-min stirring the turbidity was removed by centrifugation for 10 min at 35,000*g* and 2°. The supernatant was adjusted to pH 7.6 with 2 N NaOH and the volume was brought to 60 ml with Davies buffer (pH 7.6) (fractions A1/2 to A5/2 of Table II). At this state the material can be stored at −40° for several weeks without loss of activity. The procedure described was repeated four times yielding fractions A1/2 to A5/2. Purification and yield in this step do strongly depend on pH. Therefore the pH should be strictly maintained in the range of 3.5–3.7.

STEP 3. FILTRATION THROUGH SEPHADEX G-50. Fractions A1/2 to A5/2 were thawed and combined (fraction B/1 of Table II). Sephadex G-50 was packed into a 85-mm column to a height of 585 mm and equilibrated with 0.02 M Tris buffer (pH 7.6). Fraction B/1 (300 ml) was applied to this column and eluted with 0.02 M Tris buffer (pH 7.6). Fractions (200) of 15 ml each were collected at a flow rate of 16 ml/min. The protein containing fractions were identified by uv absorption and combined (fraction B/2 of Table II).

STEP 4. CHROMATOGRAPHY ON DEAE-SEPHADEX A-50 (FIGURE 1). A 40-mm column was packed to a height of 355 mm with DEAE-Sephadex A-50 equilibrated with 0.02 M Tris buffer (pH 7.6). Fraction B/2 (*ca.* 1100 ml) was applied to this column and the first effluent (*ca.* 1100 ml) was discarded. Elution was carried out with a linear Tris gradient, from 0.02 M Tris-HCl buffer pH 7.6 to 0.42 M. The reservoir and mixing bottle each contained 2 l. of the appropriate solution. The head of pressure was 50 cm. Fractions (200; 200 drops/fraction) were collected and assayed for enzyme activity and protein at 280 nm. The enzymatically active fractions were combined and brought to a volume of 1000 ml with 0.02 M Tris-HCl buffer (pH 7.6; fraction C of Table II).

STEP 5. FILTRATION THROUGH SEPHADEX G-100. Fraction C (1000 ml) was concentrated by precipitation with 561 g of ammonium sulfate under continuous stirring. After 10 min the precipitate was collected by centrifugation at 35,000*g* and 2° for 10 min. The precipitate was suspended in 15 ml of 0.004 M triethylamine–0.001 M EDTA buffer (pH 6.5), turbidity was removed by centrifugation, and the final volume was brought to 25 ml (fraction D/1 of Table II). A 2.5-cm column was packed to a height of 100 cm with Sephadex G-100 medium and equilibrated with 0.004 M triethylamine–0.001 M EDTA buffer (pH 6.5). Fraction D/1 was applied to the column and eluted with the same buffer. Fractions containing transaldolase activity were pooled (fraction D/2 of Table II).

STEP 6. CHROMATOGRAPHY ON DEAE-CELLULOSE. A 17-mm column was packed to a height of 300 mm with DEAE-cellulose (Whatman DE-52) and equilibrated with the buffer used in step 5. Fraction D/2 was applied to the column and eluted with the same buffer. Fractions (120; 200 drops/fraction) were collected and assayed for protein and enzyme activity.

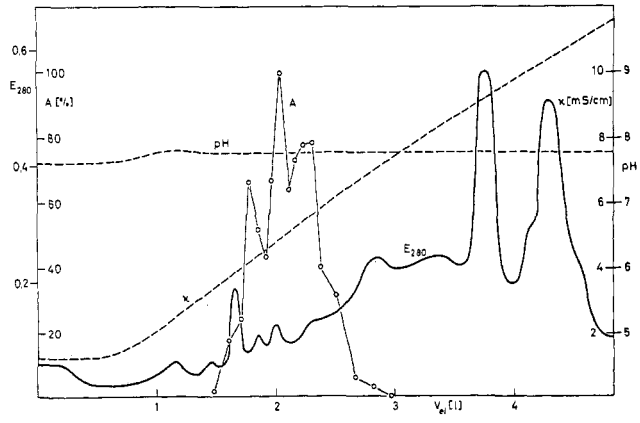

FIGURE 1: Chromatography of transaldolase preparation on DEAE-Sephadex A-50. For details, see purification procedure (step 4). Transaldolase activity (A) was measured in each fraction spectrophotometrically as described in Methods (E_{280} = absorbance at 280 nm; χ = conductivity; V_{el} = effluent volume).

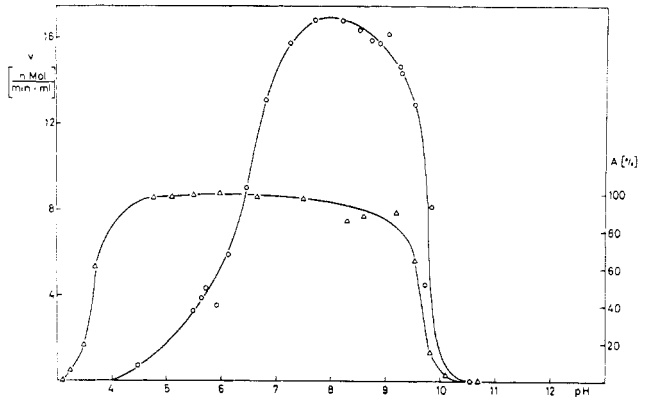

FIGURE 2: Effect of pH on transaldolase. To determine the effect of pH on the stability of transaldolase (△), the enzyme was exposed for 10 min to the pH values given and the activity was tested at pH 7.6. The effect of pH on the reaction velocity (○) was tested by measuring the activity at the pH values given.

The active fractions were pooled and concentrated *in vacuo* at 30°. The turbidity was removed by centrifugation at 35,000*g* and 2° for 10 min. The supernatant was brought to a volume of 10 ml with distilled water (fraction E of Table II).

This final fraction can be stored for several months at −40° without loss of enzymatic activity. The aqueous neutral solution of the enzyme was found to be stable for at least 8 weeks when kept at 2°. The procedure resulted in a 100-fold purification with a recovery of 22%. The transaldolase preparation was found to be homogeneous in ultracentrifugation analyses and free of the following enzyme activities: aldolase, glycerin-3-phosphate dehydrogenase, lactate dehydrogenase, phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase.

Effect of pH on Transaldolase. As shown on Figure 2, the enzyme is stable in the range between pH 4 and 9. From the pH–activity profile it appears that the pH optimum of transaldolase lies between 7 and 9 falling sharply on either side.

Kinetic Constants. Apparent Michaelis constants were determined in 0.1 M triethylamine–0.01 M EDTA buffer (pH 8.0) at 25°. Under these conditions the apparent K_m for fructose 6-phosphate was 2×10^{-4} M and for erythrose 4-phosphate 7×10^{-6} M.

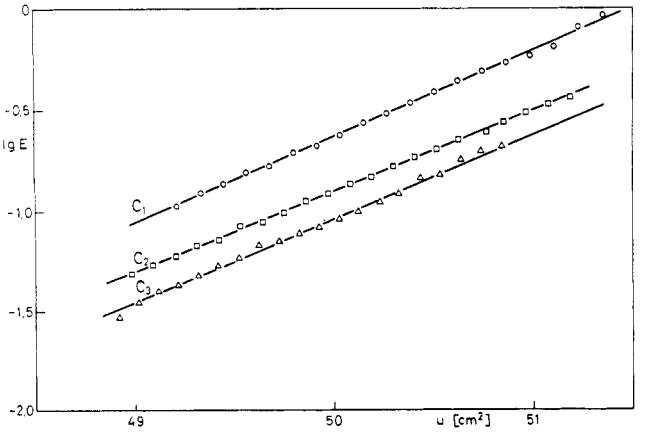

FIGURE 3: Data from equilibrium sedimentation of transaldolase obtained with three different protein concentrations (C_1, C_2, and C_3). In order to calculate the correct values for the square of the distance from the rotation center (r^2) the following equations have to be used: curve C_1, $r^2 = u$; curve C_2, $r^2 = u + 6.9$ cm²; curve C_3, $r^2 = u + 13.3$ cm². For experimental details, see the Methods section.

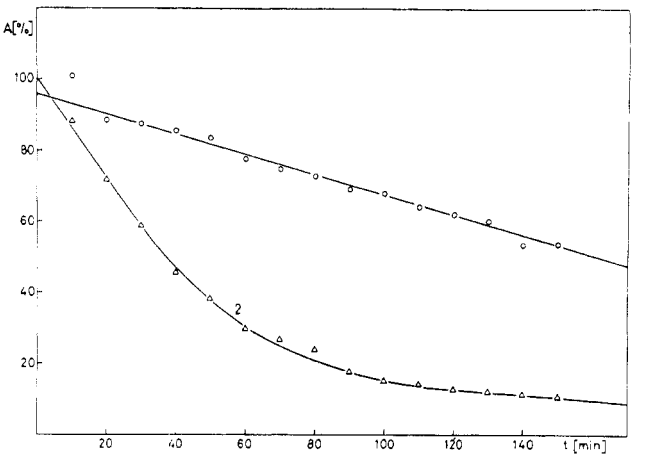

FIGURE 4: Inactivation of transaldolase from bovine mammary gland by reduction with sodium borohydride at pH 6 and 2° (curve 1, without fructose 6-phosphate; curve 2, with fructose 6-phosphate; A = enzymatic activity). Experimental conditions: 1 mg of transaldolase preparation (specific activity 4 IU/mg) was dissolved in 20 ml of 0.02 M triethylamine buffer (pH 6.0). The reaction mixture was cooled to 2°. A 0.5 M solution of sodium borohydride and simultaneously a 0.25 M acetic acid were continuously added to the mixture at a rate of 15 and 7.5 μmoles per min, respectively. In intervals of 10 min, aliquots were taken and assayed for transaldolase activity. The final volume was 28 ml. The values for the residual activity were corrected for dilution. Each curve represents the mean values from three experiments.

Molecular Weight. The molecular weight of the enzyme was determined by thin-layer gel chromatography and sedimentation equilibrium centrifugation (Figure 3). In the second method a partial specific volume of the enzyme protein of 0.746 cm³/g was assumed as reported for yeast transaldolase type III (Tsolas and Horecker, 1970). Both methods yielded a value of 65,000 ± 2%. The linear shape of the curves in Figure 3 indicates the molecular homogeneity of the enzyme preparation.

The sedimentation coefficient determined by sedimentation velocity experiments in the ultracentrifuge was found to be 3.67 S ± 3%. From the molecular weight and the sedimentation coefficient the diffusion constant and the frictional ratio were calculated to be 5.5×10^{-7} cm²/sec and 1.37, respectively, which is typical for globular proteins.

Reduction with Sodium Borohydride. As demonstrated on Figure 4 treatment of the enzyme with sodium borohydride caused a rapid loss of enzyme activity in the presence of substrate fructose 6-phosphate. In the absence of substrate the inactivation proceeded at a slow rate. In both cases, however, prolonged exposure to borohydride eventually caused a complete loss of activity. Under the experimental conditions no inactivation was observed when borohydride was omitted. In parallel experiments with higher protein concentrations similar inactivation reactions were observed.

In order to study the mechanism of inactivation by borohydride, about 5 mg of animal transaldolase and the same amount of yeast transaldolase were treated in parallel experiments with borohydride in the presence of excess of uniformly ^{14}C-labeled fructose 6-phosphate. The inactivated enzyme was hydrolyzed with 5.7 N HCl. The acid hydrolysates were used for amino acid analyses and high-voltage paper electrophoreses to examine whether ^{14}C-labeled amino acid derivatives were formed during borohydride reduction. Figure

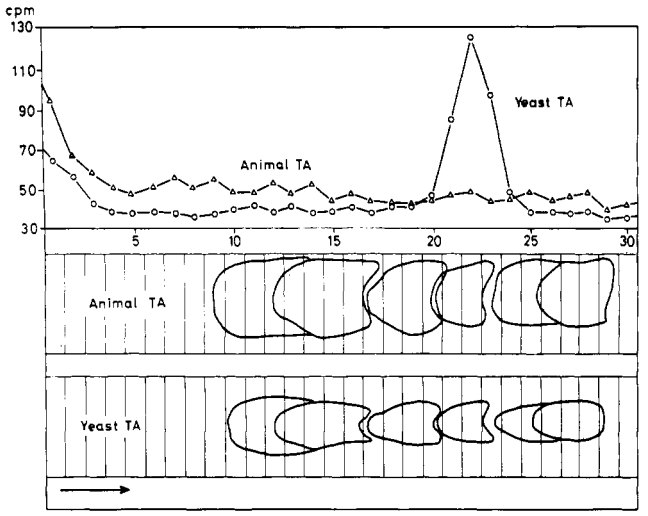

FIGURE 5: High-voltage electrophoresis of acid hydrolysates of yeast transaldolase and animal transaldolase after reduction with borohydride in the presence of excess [U-^{14}C]fructose 6-phosphate. Treatment with borohydride was performed as described in the legend of Figure 4. Protein concentrations were 5 mg/ml. 80 μl of the acid hydrolysate of animal transaldolase, correspondng to 0.8 mg of protein, and 20 μl of the acid hydrolysate of the yeast transaldolase, corresponding to 0.3 mg of protein, were applied to high-voltage electrophoresis. The amino acids were located by spraying with ninhydrin. The chromatograms were cut into 1-cm sections for counting radioactivity. For experimental details, see the Methods section.

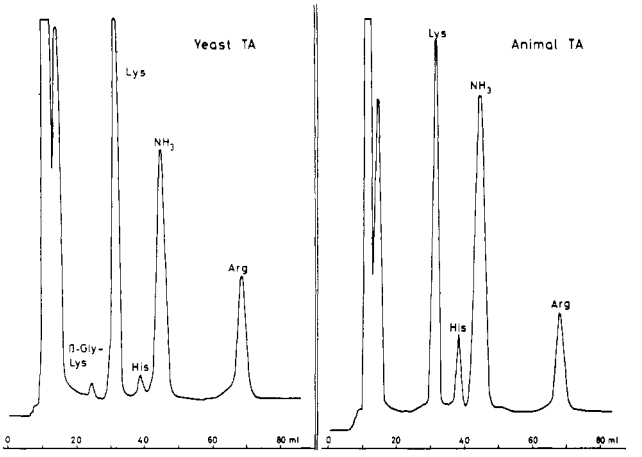

FIGURE 6: Amino acid analyses of acid hydrolysates of yeast transaldolase (left) and animal transaldolase (right). The same acid hydrolysates as described in the legend of Figure 5 were used. In this figure only the analyses of the basic amino acids separated on the short column of the Beckman amino acid analyzer are shown. For details, see the Methods section.

5 shows high-voltage electrophoresis of acid hydrolysates of yeast and of animal transaldolase. Radioactivity measurements shown on the upper part revealed that no radioactivity was incorporated into the separated amino acids in case of animal transaldolase, while in yeast enzyme the usual radioactive peak corresponding to ^{14}C-labeled β-glyceryllysine was found.

Another part of each acid hydrolysate was applied to a short column of the amino acid analyzer in order to separate the basic amino acids. As can be seen on Figure 6 a small peak before the lysine peak appeared in the case of yeast enzyme. From earlier experiments (Lai *et al.*, 1967) it is known that this peak corresponds to β-glyceryllysine. In the case of animal transaldolase, however, no β-glyceryllysine could be detected.

In order to study whether ^{14}C-labeled substrate is covalently bound to the enzyme after treatment with sodium borohydride the inactivated complex was repeatedly passed through a Sephadex G-50 and the radioactivity was incorporated into the protein fraction measured. After the first filtration about 0.8 equiv of fructose 6-phosphate was found to be incorporated into the protein fraction while after the second passage only 0.02 equiv of fructose 6-phosphate remained attached to the protein. From this result it appears that in contrast to the yeast enzyme borohydride reduction does not yield a covalently linked enzyme–substrate intermediate.

Discussion

Since until now transaldolase has been isolated only from various types of yeast cells (Pontremoli *et al.*, 1961; Venkataraman and Racker, 1961; Tsolas and Horecker, 1970), it was of interest to purify this enzyme from mammalian tissue. A comparison of transaldolase from various sources appears to be relevant since in the case of fructose 1,6-diphosphate aldolase two different classes are known: class I aldolase being a Schiff base enzyme and class II aldolase obtained from yeast and microorganisms being a metal enzyme. From the various mammalian tissues tested (Table I) mammary gland was found to be the most suitable source for the isolation of transaldolase. It should be emphasized, however, that the cellular concentration of transaldolase and also that of the other enzymes of the pentose phosphate pathway in all the mammalian tissues tested is extremely low. Because of this low concentration, the lack of information in the literature, and the special properties of the tissue used, a new purification procedure had to be worked out, based mainly on modern chromatographic techniques. Starting from a specific activity of the tissue extract in the range of 0.01–0.04 IU/mg it was possible to purify the enzyme up to a specific activity of 4 IU/mg corresponding to a 100- to 300-fold purification. The final preparation was found to be stable at 4° and pH 7.6 in aqueous solution for at least 8 weeks indicating that no proteolytic enzymes are present. The preparation was also found to be free of a variety of enzymes of the glycolytic and the pentose phosphate pathway. The pattern of the ion-exchange chromatography, however, revealed usually more than one peak with transaldolase activity, possibly due to the presence of isoenzymes. In spite of the high purification all attempts to crystallize the enzyme failed. The values for the apparent Michaelis constants for fructose 6-phosphate and erythrose 4-phosphate were by a factor of 3 lower than those reported from yeast enzyme (Tsolas, 1967). The pH profile is similar to that of the yeast enzyme with a pH optimum between 7 and 9.

The determination of the molecular weight by thin-layer gel chromatography and sedimentation equilibrium centrifugation yielded identical values of 65,000 ± 2%. Comparing the hydrodynamic parameters of yeast (Tsolas *et al.*, 1970) and animal transaldolase it appears that both enzymes are very similar in size and shape (Table III). The data obtained from ultracentrifugation studies correspond to those of a small globular protein.

Striking differences, however, between transaldolase from mammary gland and yeast have been observed with respect to the reaction mechanism. While in case of yeast transaldolase the occurrence of a Schiff base intermediate during the

TABLE III

Parameter	Animal Transaldolase	Yeast Transaldolase
M	65,000	65,900
$s^0_{20,w}$ (S)	3.67	4.24
$D^0_{20,w}$ (cm²/sec)	5.50×10^{-7}	6.40×10^{-7}
f/f_k	1.37	1.29

aldol-cleavage reaction of fructose 6-phosphate has been well established (Horecker *et al.*, 1961, 1963) no covalent binding of a triose to the enzyme protein could be detected in the case of the animal enzyme as judged from studies using the borohydride technique with ^{14}C-labeled fructose 6-phosphate. In parallel experiments carried out under equal conditions with both transaldolases no β-glyceryllysine derivative could be detected in amino acid analyses of acid hydrolysate from animal enzyme and no radioactive amino acid derivative was found when the hydrolysate was subjected to high-voltage electrophoreses. In control experiments with yeast enzyme, however, the presence of ^{14}C-labeled β-glyceryllysine with both methods could be demonstrated. Borohydride reduction in the presence of fructose 6-phosphate was accompanied by a complete loss of enzymatic activity in both transaldolases. In contrast to the yeast enzyme, however, transaldolase from bovine mammary gland was slowly but completely inactivated by borohydride also in the absence of substrate. pH 6 has been found to be the optimum value for reduction with borohydride in the cases of fructose 1,6-diphosphate aldolase and yeast transaldolase (Horecker *et al.*, 1963) and therefore this pH was used also in our experiments.

The possibility exists that pH 6 is not the optimum value for the formation or reduction of the Schiff base intermediate in case of mammalian enzyme. If, however, both transaldolases follow the same mechanism at least part of the enzyme should have been found to be labeled under the conditions employed. Since only 0.02 equiv of substrate remained bound to the enzyme after repeated gel filtration, it is very unlikely that a variation of pH would result in a stoichiometric fixation of substrate due to the formation of a Schiff base intermediate. A tight binding of substrate molecules to the animal enzyme throughout the purification procedure could also result in a temporary blockage of a possible lysine amino group at the active site. In case of erythrose 4-phosphate this would not affect our results since an excess of labeled fructose 6-phosphate was used. Only an unusual strong binding of fructose 6-phosphate or sedoheptulose 7-phosphate, which is stable throughout the various purification steps including heating to 40° at pH 3.6 could prevent labeling with radioactive substrate.

The chemical nature of the inactivation of animal enzyme by borohydride both in the absence as well as in the presence of substrate fructose 6-phosphate is not yet clear.

The accelerated inactivation by reduction in the presence of fructose 6-phosphate might be due to a substrate-mediated change of conformation making certain functional groups more accessible to the reducing agent. Evidences for substrate induced conformational changes have been reported by Brand (1970) in case of the yeast enzyme.

Since no covalent linkage of the substrate moiety to animal transaldolase could be detected, a different mechanism has to be assumed, which does not involve formation of a Schiff base intermediate during aldol cleavage of fructose 6-phosphate. The results reported here do not allow to propose an alternative mechanism of action. Further investigations, mainly on the basis of kinetic studies, should provide additional informations about the different reaction mechanisms of transaldolases.

References

Brand, K. (1970), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 7*, 235.

Brand, K., and Horecker, B. L. (1968), *Arch. Biochem. Biophys. 123*, 312.

Brand, K., Tsolas, O., and Horecker, B. L. (1969), *Arch. Biochem. Biophys. 130*, 521.

Davies, M. T. (1959), *Analyst 84*, 284.

Horecker, B. L., Pontremoli, S., Ricci, C., and Cheng, T. (1961), *Proc. Nat. Acad. Sci. U. S. 47*, 1949.

Horecker, B. L., Rowley, P. T., Grazi, S., Cheng, T., and Tchola, O. (1963), *Biochem. Z. 338*, 36.

Lai, C. Y., Chen, C., and Tsolas, O. (1967), *Arch. Biochem. Biophys. 121*, 790.

Layne, S. (1957), *Methods Enzymol. 3*, 447.

Pontremoli, S., Prandini, B. A., Bonsignore, A., and Horecker, B. L. (1961), *Proc. Nat. Acad. Sci. U. S. 47*, 1942.

Radola, B. J. (1968), *J. Chromatogr. 38*, 61.

Spackman, D. H., Stein, W. H., and Moore, S. (1958), *Anal. Chem. 30*, 1190.

Tsolas, O. (1967), Ph.D. Dissertation, Albert Einstein College of Medicine, Bronx, N. Y.

Tchola, O., and Horecker, B. L. (1966), *Methods Enzymol. 9*, 499.

Tsolas, O., and Horecker, B. L. (1970), *Arch. Biochem. Biophys. 136*, 287.

Tsolas, O., Horecker, B. L., and Sia, C. L. (1970), *Arch. Biochem. Biophys. 136*, 303.

van Holde, K. E. (1967), Fractions 1, Beckman Instruments Inc., Palo Alto, Calif.

Venkataraman, R., and Racker, E. (1961), *J. Biol. Chem. 236*, 1883.