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CHARACTERIZATION AND PROPERTIES OF PIG LIVER TRANSKETOLASE

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Summary

Some properties of homogeneous transketolase (sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate glycolaldehydetransferase, EC 2.2.1.1) from pig liver were studied. It was shown that the pH optimum of the transketolase reaction lies within the range of 7.8—8.2; the isoelectric point is at pH 7.6—7.8. The molecular weight of transketolase is 138 000 ± 3000 as determined by the sedimentation equilibrium method. The enzyme molecule is a tetramer of the $\alpha_2\beta_2$ type. The molecular weights of the α- and β-subunits determined by polyacrylamide gel in the presence of sodium dodecyl sulphate are 52 000—56 000 and 27 000—29 000, respectively. Transketolase contains about 2 mol of thiamine pyrophosphate per mol of protein and does not require metal ions for its catalytic activity.

Introduction

Transketolase (sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate glycolaldehydetransferase, EC 2.2.1.1) has been isolated from several sources [1—5]; the enzyme from baker's yeast is better studied than others, whereas almost nothing is known about the transketolase from pig liver. Simpson [6] was the first to isolate transketolase from pig liver in a partially purified form; he also showed that this enzyme, unlike transketolase from baker's yeast, does not require thiamine pyrophosphate for its catalytic activity to be displayed. The present work is concerned with the isolation and the properties of homogeneous transketolase from pig liver.

Methods

Preparation of transketolase from pig liver. Pig liver was homogenized for 20 min in a 4-fold vol. of acetone cooled to —20°C; the homogenate was filtered in

a Büchner's funnel and the precipitate was subjected to a similar treatment. The resultant mass was homogenized and dried at room temperature in vacuo until the smell of acetone disappeared. (The dry sifted powder can be stored at −20°C for several months.) The acetone powder (15—20 g) was extracted at room temperature with a 10-fold vol. of 15 mM Na_2Co_3 [6]. The extract was centrifuged for 15 min at 20 000 × *g* (this and the next steps were carried out in cold), the sediment was discarded and the supernatant was supplemented with dry protamine sulphate (1 mg per 20 mg of protein), stirred for 5 min and centrifuged (38 000 × *g* for 15 min). The supernatant obtained was supplemented in portions (600 mg/ml of enzyme solution) with a dense suspension of DEAE-cellulose preequilibrated with 2 mM potassium phosphate buffer (pH 7.6); after addition of each portion of the ion-exchanger, the mixture was stirred for 10 min and centrifuged (20 000 × *g* for 5 min). When the concentration of protein in the enzyme solution was as low as 6—8 mg/ml, no more DEAE-cellulose supension was added, and the supernatant was supplemented by stirring with dry ammonium sulphate to 50% saturation. The precipitate was removed by centrifugation (38 000 × *g* for 15 min); the supernatant was supplemented with dry ammonium sulphate to 75% saturation; the sediment was collected by centrifugation (38 000 × *g* for 20 min) and dissolved in 20 ml of 50 mM Tris-HCl buffer (pH 8.5). The solution was applied onto a DEAE-Sephadex A-25 column (6 × 25 cm) through which 5 l of 0.1 M Tris-HCl buffer (pH 9.2), 15 l of 20 mM Tris-HCl buffer (pH 9.2) and 1.1 l of 20 mM KCl solution were passed. 0.1 M KCl solution served as an eluent. The flow rate was 1 ml/min, the volume of the fractions to be collected was 6 ml. Active fractions of the eluate were joined and applied onto a 1.5 × 5.0 cm column packed with hydroxyapatite preequilibrated with 10 mM potassium phosphate buffer (pH 6.5). The protein was eluted by a 0.01—0.50 M gradient of potassium phosphate buffer (pH 6.5) (flow rate 0.5 ml/min; 2-ml fractions).

The specific activity of the transketolase from pig liver preparations may vary from 0.3 to 0.9 U/mg. The enzyme is stored at −20°C for several days with no decrease in catalytic activity.

Preparation of other enzymes. Glyceraldehydephosphate dehydrogenase, a mixture of glycerolphosphate dehydrogenase and triosophosphate isomerase were obtained from rabbit muscles [8,9]. The preparations were stored in cold in an ammonium sulphate solution [8,9] which was removed on a Sephadex G-50 ('crude') column.

As a transketolase substrate, use was made of a mixture of phosphopentoses (ribose 5-phosphate and xylulose 5-phosphate) [10] which was stored as the barium salt and before use converted into the potassium salt on a Dowex 50-X10 column (1 × 5 cm).

Transketolase activity was assayed in the following reaction mixture: 50 mM glycylglycine buffer (pH 7.8), 3.7 mM NAD, 32 mM cystein (pH 7.8), 1.1 M arsenate, 3 units of glyceraldehyde-phosphate dehydrogenase, 10 mM mixture of phosphopentoses, transketolase from pig liver, total volume 2 ml. For assay of transketolase activity in acetone powder extracts, a different reaction mixture was used in some cases, namely 50 mM glycylglycine buffer (pH 7.8), 20 mM NADH, a mixture of glycerolphosphate dehydrogenase and triosophosphate isomerase (2 mg per sample), 10 mM mixture of phosphopentose, trans-

ketolase from pig liver, total volume 2 ml. In both cases the reaction was carried out at 20°C in quartz cuvettes with a 10-mm optical pathway. (The above methods are essentially described in Ref. 9.) On studying the pH dependence of transketolase activity, the reaction mixture (buffer with a required pH, 20 mM phosphopentose mixture, 100 μg of transketolase from pig liver; total volume 0.8 ml) was incubated at 30°C. At certain time intervals (0, 5, 10 and 15 min) the samples were withdrawn into glass tubes containing concentrated H_2SO_4 and water (6 : 1, v/v), the tubes were then placed into a boiling water bath for 4 min, quickly cooled and after stirring, the mixture was supplemented with either 0.05 ml of 3% cysteine or 0.05 ml of 6.3% thioglycollic acid. After 18 h incubation at room temperature in the first case or 30 min incubation at 50°C in the second case, the content of sedoheptulose 7-phosphate was determined in the samples by the differences in absorbance at 510 and 540 nm, with sedoheptulose anhydride as standard [12,13].

Amount of protein during purification of transketolase from pig liver. Protein was determined with the help of a nomogram [9] by the absorbance of the enzyme solution at 280 and 260 nm; in the homogeneous transketolase from pig liver preparations by the absorbance at 280 nm, which for 0.1% transketolase solution (50 mM Tris-HCl buffer, pH 8.5) is 1.24 [7]. In some cases the method of Lowry et al. [16] and the biuret method [17] were employed.

Metal content of transketolase from pig liver. This was determined on a Hitachi (Japan) atomic absorption spectrophotometer. Before analysis, transketolase from pig liver dissolved in 2.5 mM potassium phosphate buffer (pH 7.6) was passed through a 1.3 × 30 cm Sephadex G-50 column, for elution the same buffer was used. All the solutions were prepared with deionized water, the specific resistance value being above 1 MΩ/cm.

Amount of thiamine pyrophosphate. Thiamine pyrophosphate was determined by the thiochrom method as described [14,15].

Transketolase circular dichroism spectra. These were taken in a Rousset-Jouan (France) dichrographin, a cuvette with a 50 mm optical pathway. The samples contained 5 mM Tris-HCl buffer (pH 8.5), 0.9 mg/ml of transketolase from pig liver, with or without 0.34 mg/ml of hydroxypyruvate; total volume 5 ml.

Isoelectrofocusing of transketolase from pig liver. This was carried out in an LKB (Sweden) device. An LKB 8101 column (volume 110 ml) was used, isoelectrofocusing was performed over a pH range of 6—10 in 26% ampholine solution. The time of separation was 64 h; the initial voltage was 500 V, the current 2.4 mA at the end of the experiment 1000 V and 1 mA, respectively. The composition of the buffer solutions and the conditions of column packing were as recommended by LKB. Before transketolase activity assay, the eluent fractions were passed through a 1 × 20 cm Sephadex G-50 (fine) column equilibrated with 20 mM Tris-HCl buffer (pH 8.0).

Sedimentation equilibrium experiments. Experiments were performed as described by Yphantis [18] in a Beckman (USA) 'Spinco E' analytical ultracentrifuge with interference optics; an An-I analytical rotor with a 12-mm six-channel cell with sapphire windows was used. The interferential picture was photographed on Kodak plates, type II-G. Three transketolase preparations with initial protein concentrations of 0.26, 0.30, 0.56 mg/ml (Tris-HCl buffer,

pH 8.0) were used. The experiment lasted 29 h at 20° C, at 16 200 rev./min. The final experiments were made only with buffer in the same cells and at the same rate for the basal line to be corrected. Calculation and treatment of the data were performed as described [19]. The specific partial volume of transketolase was assumed to be 0.744 ml/g [19].

Sodium dodecyl sulphate (SDS)-electrophoresis of transketolase from pig liver. Electrophoresis was carried out as described [27]. Before the electrophoresis, the enzyme (concentration 1 mg/ml) was incubated for 10 min, at 100°C in 20 mM Tris-HCl buffer (pH 8.0) containing 1% SDS and 0.1% 2-mercaptoethanol. Then the enzyme was dialyzed overnight against 10 mM sodium phosphate buffer (pH 7.1) containing 0.1% SDS and 0.1% 2-mercaptoethanol (when the molecular weight of the subunits was determined), the marker protein was treated in the same way as transketolase from pig liver; use was made of 10% gel and of electrode buffer (0.1 M sodium phosphate buffer, pH 7.1) containing 0.1% SDS and 0.1% 2-mercaptoethanol. Each tube (7 × 80 mm) contained about 50 μg of protein, electrophoresis lasted 6 h at room temperature at a current of 8 mA per tube. The protein was identified after being stained with Coomassie blue [27] or by the opalescence of the protein areas [28].

Gel filtration of transketolase subunits in the presence of SDS. Filtration was carried out on a 0.7 × 55 cm Sephadex G-100 column preequilibrated with 0.1 M NaCl and 0.1% SDS (the same solution served as an eluent), about 1 mg of protein was treated in the same way as before electrophoresis, the flow rate was 1.2 ml/h and the volume of the fraction was 1.2 ml. Before rechromatography, excess SDS was removed by addition of 0.1 M KCl solution at 40°C. On determination of the molecular weight by gel filtration, the marker protein was treated in the same way as transketolase from pig liver.

Reassociation of transketolase subunits. Reassociation was carried out using a preparation which was incubated in 1% SDS for 6 h at 25°C, dialyzed against 0.1 M sodium phosphate buffer (pH 7.1) that contained 0.05% SDS for 12 h at room temperature and subjected to electrophoresis in polyacrylamide gel [26, 27]. Each subunit was eluted from gel (opalescent areas of complexes of subunits with SDS). SDS was removed by adding 0.1 M KCl to the eluate at 40°C which was then centrifuged (5 min at 6000 × *g*). The supernatant was dialyzed against 9 mM Tris-HCl buffer, pH 8.0, for 4 h. Then subunit preparations were mixed in equimolar quantities and reassociated in the presence or absence of thiamine pyrophosphate (12 h incubation in 2 mM Tris-HCl buffer, pH 8.0 at room temperature). The incubation was followed by an electrophoretic analysis [26] of the mixture.

Reagents. DEAE-cellulose was purchased from the Olainsky factory of chemical reagents (U.S.S.R.); DEAE-Sephadex, Sephadexes G-50, G-100, G-200 from Pharmacia (Sweden); NAD, NADH, tryptophan, glycyltryptophan, glycylglycine, cysteine, trypsin, chymotrypsin, pyruvate kinase from rabbit muscle, glutamate dehydrogenase from horse liver and reagents for polyacrylamide gel electrophoresis from Reanal (Hungary); phenazine methosulphate and nitroblue tetrazolium from Schuchardt F.R.G.); protamine sulphate, guanidine chloride and thiamine pyrophosphate from Merck (F.R.G.); TEAE-cellulose, Dowex 50-X10 from Serva (F.R.G.); sedoheptulose anhydride and 2-mercaptoethanol from Sigma (U.S.A.); dextran blue, Coomassie blue R-250 and SDS

from Ferak (F.R.G.); cytochrome *c*, bovine serum albumin and ovalbumin from Biomed (Poland) and yeast hexokinase from Fluka (Switzerland).

Results

Homogeneity of purified transketolase from pig liver preparations

When purified by the method including preparation of acetone powder, extraction with Na_2CO_3 solution, treatment with protamine sulphate and DEAE-cellulose, fractionation with ammonium sulphate, chromatography on DEAE-Sephadex and hydroxyapatite columns (Table I) and subjected to disc electrophoresis in polyacrylamide gel [20,21], at six different pH values (3.8, 6.0, 7.8, 8.0, 8.9 and 9.4), transketolase gives a single protein band. Then the transketolase activity band, when determined in gel [22], proves to have the same position as the protein band. Homogeneity of the purified transketolase preparations was also confirmed by gel filtration on Sephadex G-200 (a single symmetrical protein peak coinciding with that of enzymatic activity), isoelectrofocusing (a single peak of protein possessing enzymatic activity), high speed ultracentrifugation (the absence of minor components) and sedimentation equilibrium (linearity of the $\ln C$ vs. r^2 curve (see below).

pH optimum of the activity and the stability of transketolase from pig liver

The pH optimum of the transketolase activity in Tris-HCl and glycylglycine buffers is in the range of 7.8—8.2. The activity is almost completely retained after 20 min incubation at pH 6.5—8.7 (20°C), whereas at pH values lower than 6.5 and higher than 8.7 the enzyme is less stable. The enzyme retains its activity after 1 h incubation at 40°C (pH 8.0), but after 10 min of incubation at 50° C it is inactivated by 50%. In the presence of 4 mM thiamine pyrophosphate, the transketolase activity does not decrease even after 1 h incubation at 50°C.

TABLE I

PURIFICATION OF TRANSKETOLASE FROM PIG LIVER

Stage of purification	Volume of enzyme solution (ml)	Total amount of protein (mg)	Total activity (IU/mg)	Specific activity (IU/mg)	Purification (-fold)	Yield (%)
1. Extraction	150	6820	9.1	0.0013	—	100.0
2. Treatment with protamine sulphate	145	2889	8.7	0.003	2.3	95.6
3. Treatment with DEAE-cellulose	102	816	8.2	0.01	7.6	90.1
4. Fractionation with ammonium sulphate	22	630	7.5	0.011	8.5	82.4
5. Chromatography on DEAE-Sephadex A-25	45	25	6.3 *	0.25	192.0	69.2
6. Chromatography on hydroxyapatite	20	2.4	2.1 *	0.88	677.0	23.1

* Activity was determined after removal of excess KCl (step 5) or phosphate (step 6) which, at the concentrations used, inhibited the enzyme about 2-fold.

Metal content of transketolase from pig liver

Determination of the content of some metals in two different freshly isolated transketolase preparations (Table II) showed that there were appreciable amounts of zinc and calcium (1.01 and 0.49 gatom of metal per mol protein, respectively) in one of these preparations and of copper and zinc (0.56 and 0.47 gatom/mol) in the other. The amounts of other metals were stoichiometrically insignificant. After 24—48 h dialysis against 1% EDTA solution (pH 8.2), the total amount of remaining metals in both preparations became stoichiometrically smaller, although the enzymatic activity remained unaltered.

Content of thiamine pyrophosphate in transketolase from pig liver

The fluorescence method allows one to determine in the transketolase preparations treated with ferricyanide alkaline solution up to 1 mol of thiamine pyrophosphate per mol enzyme [7]. After proteolysis hydrolysis (trypsin plus α-chymotrypsin in a 10-fold (w/w) excess over transketolase from pig liver, for 12 h at 37°C pH 8.2) of such transketolase preparations, in some cases up to 2 mol thiamine pyrophosphate per mol protein can be found.

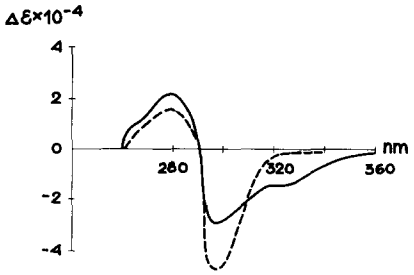
Thiamine pyrophosphate remains bound to the enzyme even after 15 min boiling, treatment with 1.7 M $HClO_4$, 30 min boiling in 1—2 N HCl and 15 min boiling in 1% SDS [7]. The transketolase circular dichroism spectrum (Fig. 1) contains a negative band at 320 nm which is also present in the spectrum of holotransketolase from baker's yeast, but absent in yeast apotransketolase [24,25]. Both in transketolase from pig liver and in yeast holotransketolase the

TABLE II

METAL CONTENT OF TRANSKETOLASE FROM PIG LIVER

Traces of Mn^{2+}, Fe^{2+} and Co^{2+} were present in all metal preparations. The molecular weight of transketolase was assumed to be 138 000.

Pig liver transketolase preparation	Metal	Amount of metal	
		μg/mg protein	gatom/mol protein
(A)			
Before dialysis (protein concentration, 0.88 mg/ml)	Mg^{2+}	0.034	0.20
	Ca^{2+}	0.14	0.49
	Zn^{2+}	0.47	1.01
	Cu^{2+}	0.11	0.25
After dialysis against 1% EDTA (concentration of protein in the samples 0.82 mg/ml)	Mg^{2+}	0.006	0.03
	Ca^{2+}	0.02	0.07
	Zn^{2+}	0.04	0.09
	Cu^{2+}	0.02	0.05
(B)			
Before dialysis (concentration of protein in the samples 1.7 mg/ml)	Mg^{2+}	0.029	0.17
	Ca^{2+}	0.02	0.14
	Zn^{2+}	0.25	0.56
	Cu^{2+}		
After dialysis against 1% EDTA (concentration of protein in the samples 1.3 mg/ml)	Mg^{2+}	0.075	0.09
	Ca^{2+}	0.04	0.12
	Zn^{2+}	0.01	0.03
	Cu^{2+}	0.01	0.02



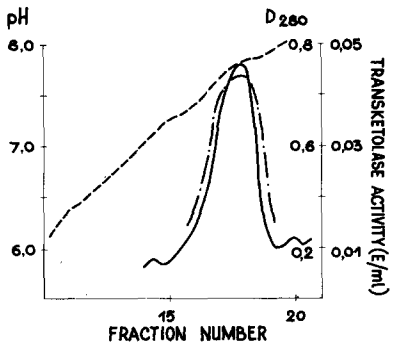


Fig. 1. CD spectrum of transketolase from pig liver. Transketolase from pig liver (———); transketolase from pig liver hydroxypyruvate (- - - - -).

Fig. 2. Isoelectric focusing of transketolase from pig liver. Protein (———); transketolase from pig liver activity (· — · — ·); pH (———).

intensity of this band sharply decreases when the enzyme solution is supplemented with a substrate (hydroxypyruvate).

Isoelectric point of transketolase from pig liver

On isoelectric focusing of transketolase in the pH range 6—10, a single protein peak is obtained which coincides with that of the transketolase activity. The isoelectric point of the enzyme is in the pH range from 7.6 to 7.8 (Fig. 2).

The sedimentation coefficient and the molecular weight of transketolase from pig liver

Determination of the sedimentation coefficient for transketolase preparations with the concentration of the protein in the samples of 0.26 mg/ml gave the value of $s_{20,w} = 6.64$. The molecular weight of transketolase determined by the sedimentation equilibrium method is 138 000 ± 3000. The ln$C$ vs. r^2 plot for one of the enzyme preparations is shown in Fig. 3.

Transketolase subunits

As stated above, on electrophoresis in polyacrylamide gel, native transketolase from pig liver gives a single protein band. After treatment with 1% SDS solution in the presence of 2-mercaptoethanol, the enzyme gives two protein bands (Fig. 4). (The electrophoretic profile does not change if transketolase is isolated in the presence of the protease inhibitor, phenylmethylsulphonyl fluoride.) Treatment of the native transketolase from pig liver before SDS electrophoresis with 6 M guanidine-hydrochloride (pH 2.0), 6 M urea and 0.1% SDS in the presence of 0.1% 2-mercaptoethanol [26] gives the same picture as in Fig. 4. After the higher molecular weight component is eluted from the gel and the treatment is repeated, it no longer breaks down. When the electrophoretic mobilities of the marker protein treated under the same conditions as the transketolase were compared, the molecular weights of the transketolase subunits were found to be, 52 000—56 000 (α) and 27 000—29 000 (β), respectively (Fig. 5).

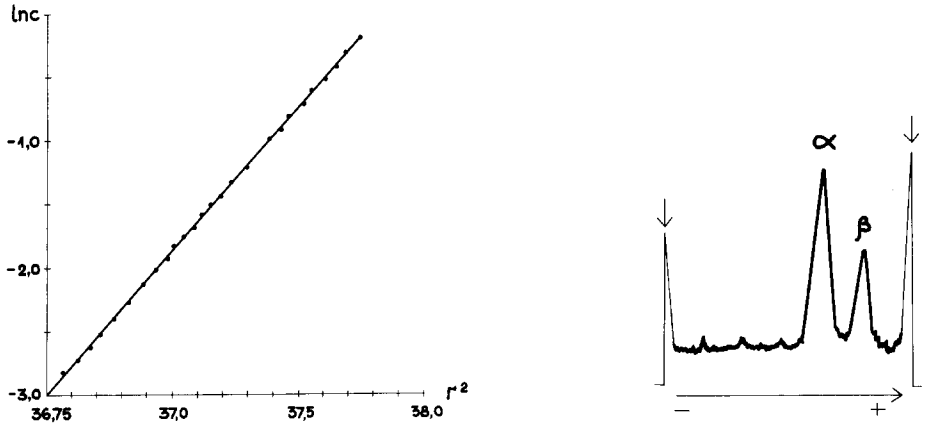


Fig. 3. Log of transketolase from pig liver concentration in the rotor cell (at a distance of r from its axis) vs. r^2.

Fig. 4. SDS-electrophoresis of transketolase from pig liver (densitogram of gel). Arrows indicate borders of gel.

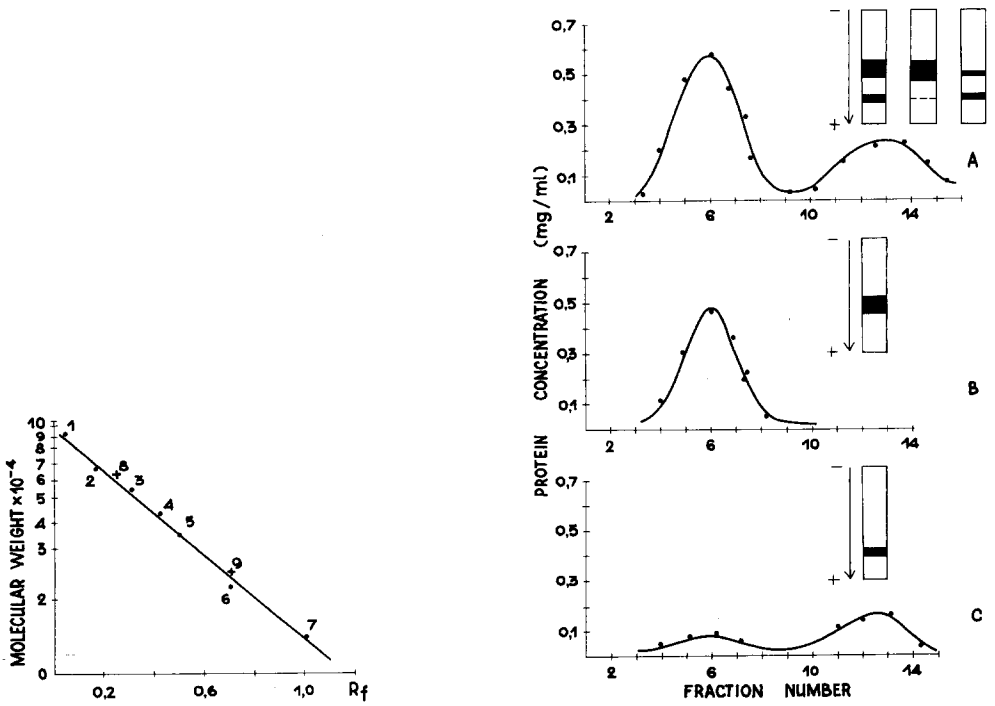


Fig. 5. Determination of the molecular weight of transketolase subunits in SDS electrophoresis. Designations used (in brackets the molecular weight of a monomer is indicated): 1, phosphorylase *b* from rabbit muscle (92 500); 2, bovine serum albumin (68 000); 3, glutamate dehydrogenase from horse liver (53 000); 4, ovalbumin (43 000); 5, glyceraldehydephosphate dehydrogenase from rabbit muscle (36 000); 6 and 7, respectively, the dimer of cytochrome *c* (24 000) and its monomer (12 000); 8 and 9, transketolase subunits. Relative mobility of protein areas (R_F) was measured with respect to the monomer of cytochrome *c*.

Fig. 6. Chromatography (A) and rechromatography of α-subunit (B) and β-subunit (C) of transketolase from pig liver on Sephadex G-100.

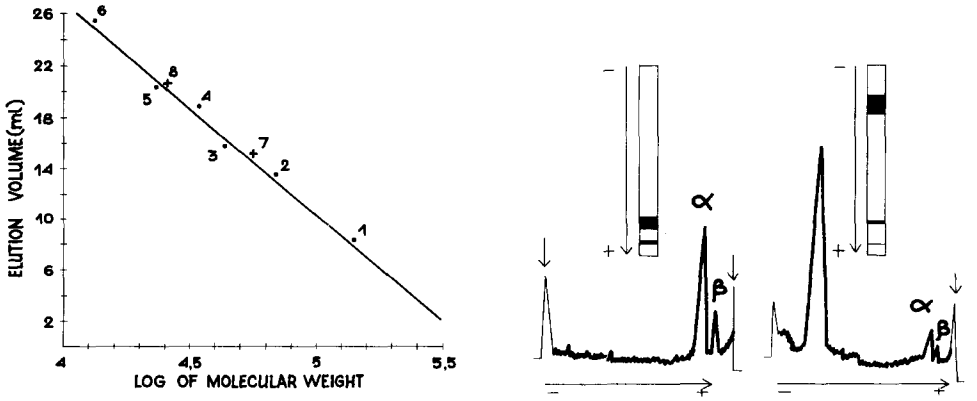


Fig. 7. Determination of the molecular weight of transketolase from pig liver subunits by gel filtration on Sephadex G-100. Designation: 1, dimer (136 000) and 2, monomer (68 000) of bovine serum albumin; 3 and 4, respectively, monomers of ovalbumin (43 000) and glyceraldehydephosphate dehydrogenase from rabbit muscle (36 000); 5, dimer (24 000) and 6, monomer (12 000) of cytochrome *c*; 7 and 8, transketolase from pig liver subunits.

Fig. 8. Electrophoretograms and densitograms of transketolase from pig liver subunit mixture (A) before and (B) after incubation in the presence of thiamine pyrophosphate. Arrows indicate borders of gel.

On the gel filtration on Sephadex G-100 in the presence of 0.1% SDS, transketolase from pig liver pretreated with 1% SDS, yielded two protein peaks (Fig. 6A). Determination of the molecular weights of these proteins by gel filtration gave the values of 30 000 and 54 000, close to those of the molecular weight of the subunits obtained on SDS electrophoresis. As can be seen in Fig. 6A, each subunit, after the first gel filtration, contains an admixture of the other; rechromatography allows individual subunits to be obtained (Fig. 6B and C).

Reassociation of transketolase subunits

Comparison of electrophoretograms of the transketolase subunit mixture before (Fig. 8A) and after (Fig. 8B) 12 h incubation in the presence of 0.22 mM thiamine pyrophosphate (2 mM Tris-HCl buffer, pH 8.0) demonstrates that a new component is formed as a result of the incubation, with a molecular weight higher than that of the starting subunits; the amount of the latter decreases. Judging by its electrophoretic behaviour, the new component is similar if not identical, to non-dissociated transketolase from pig liver. However, this component fails to display catalytic activity. If preincubation of the subunit mixture is carried out in the absence of thiamine pyrophosphate, electrophoretograms reveal, besides the basic component and the starting subunits, minor bands lying between α-subunit and non-dissociated transketolase from pig liver.

Discussion

The method described in the given paper permits isolation of transketolase from pig liver which is homogeneous according to the data of disc electrophoresis in polyacrylamide gel, gel filtration, isoelectrofocusing and sedimenta-

tion analysis. The pH optima of the activity of transketolase from various sources are similar, i.e. for transketolase from pig liver it is from 7.8 to 8.2, for transketolases from baker's and brewer's yeast, *Candida utilis* and spinach leaves from 7.5 to 7.6, and for human erythrocyte enzymes 7.75 [1—6]. There are significant differences between the values of isoelectric points of transketolases from *C. utilis* 4.8 [3], baker's yeast 6.5 [30], human erythrocytes 8.5 [4] and pig liver 7.6—7.8.

It has been previously shown that baker's yeast transketolase contains calcium which is readily cleaved off protein; this and other divalent cations stimulate the enzyme activity [31]. After dialysis against 1% EDTA (pH 8.2), transketolase from pig liver fully retains its catalytic activity, although metals are present in these enzyme preparations in stoichiometrically insignificant quantitites. Hence it may concluded that transketolase from pig liver is not a metallo-enzyme.

The moelcular weight of transketolase from pig liver, determined previously by gel filtration on Sephadex G-200, is 152 000 [7]. The molecular weight of the enzyme, according to the sedimentation equilibrium data, which are assumed to be more reliable, is 138 000 ± 300. For comparison, we shall give molecular weights known for transketolases from other sources: *C. utilis* 163 000 [3]; baker's yeast 158 000—159 000 [20,32]; brewer's yeast [2] and spinach leaves [3] 100 000 and human erythrocytes 136 000 [4].

The molecular structure of transketolases from brewer's [2] and baker's [32,33] yeast and *C. utilis* is assumed to be dimeric with identical monomers; transketolase from spinach leaves is a monomer [5]. Under the action of SDS transketolase from pig liver dissociates into subunits of two types which are capable of reassociation, with the initial oligomer being formed. Comparison between the molecular weights of the transketolase subunits (α-subunit 52 000—59 000; β-subunit 27 000—30 000) and the native enzyme (about 140 000) allows one to suggest the following structures of the transketolase from pig liver molecule: $\alpha_2\beta_2$, $\alpha_2\beta$ and $\alpha\beta_3$. Since the weight ratio of the subunits α and β after their separation (Fig. 6) is 1.7—2.2, the most plausible structure is $\alpha_2\beta_2$.

Unlike transketolases from baker's and brewer's yeast [2,10], spinach leaves [5,11] and rat liver [11], transketolase from pig liver does not require exogenous thiamine pyrophosphate for its catalytic activity to be displayed [6]. Determination by the thiochrome method gives approx. 1 mol coenzyme per mol transketolase [7]. After proteolytic hydrolysis, in some enzyme preparations up to 2 mol coenzyme per mol protein can be found. Thus it may be suggested that the transketolase molecule contains 2 coenzyme molecules. This conclusion should, however, be regarded as preliminary, since on determination by the thiochrome method of the amount of coenzyme bound with protein (or a protein molecule fragment), free thiamine pyrophosphate was used as standard. It is, however, not ruled out that the bound coenzyme differs in its reactivity (when treated with ferricyanide) from free coenzyme and that the fluorescent forms of bound and free coenzymes differ by the intensity of their fluorescence.

It should be emphasized that a strong linkage exists between coenzyme and protein in the transketolase molecule, and although no covalent binding has

been previously revealed in thiamine enzymes, such a possibility cannot be ruled out in the case of transketolase from pig liver.

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