SUBUNIT SIZE OF TRANSKETOLASE FROM BAKER'S YEAST

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1. Introduction

It is well established that thiamine pyrophosphate (TPP) is essential as coenzyme for transketolase (EC 2.2.1.1.) [1, 2]. According to Racker [3], two equivalents of thiamine pyrophosphate were present per mole of enzyme. There are no data on the subunit structure of transketolase.

The present communication shows that transketolase from baker's yeast was found to be separable into two identical polypeptide chains with a molecular weight of 70,000.

2. Materials and methods

Transketolase was prepared from baker's yeast [4]. The pentose-5-phosphate equilibrium mixture, which was used as substrate in the transketolase assay, was prepared according to Ashwell and Hickman [5]. The transketolase activity was assayed spectrophotometrically by the rate of oxidation of NADH in the coupled reaction with triosephosphate isomerase and glycerophosphate dehydrogenase [6]. Protein was determined by the method of Lowry et al. [7]. The analytical acrylamide disk gel electrophoresis was performed according to the technique of Davis [8] with tris/glycine buffer with a running pH of 9.5. The upper gel was omitted. Bromophenol blue was used as tracking dye. Molecular weights were estimated by the general procedure of Shapiro et al. [9] and Weber and Osborn [10]. Denaturation and separation into subunits were achieved by treatment of the enzyme with 6.0 M urea, 1% sodium dodecyl sulfate (SDS) and 1 M β-mercaptoethanol in 0.1 M phosphate buffer, pH 7.2 for 3 hr at 37°. Protein concentration was 0.35 mg/ml. After incubation, the protein solution was dialyzed overnight at room temperature against 500 ml of 0.1 M sodium phosphate buffer, pH 7.2, containing 0.1% SDS, 0.1 M β-mercaptoethanol and 6.0 M urea. Samples were either run side by side with a mixture of reference proteins in adjacent slots in gel slabs (8 × 18 cm, 3 mm thickness, 3.5 hr at 60 mA) or from the same slot of a gel slab flanked by unmixed samples. The gels contained 5% acrylamide and 0.25% bisacrylamide. The same relative mobilities were found whether or not urea was present. Ultracentrifugation was carried out in a Spinco Model E ultracentrifuge. The sedimentation run was performed at 40,000 rpm, 6.2° in a band forming cell. The enzyme at a protein concentration of 1.6 mg/ml tris/HCl buffer, 0.01 M, pH 7.6 moved into 1.0 M tris/HCl, pH 7.6.

The determination of the *N*-terminal amino acid was carried out by use of the dansylation method [11]. 0.5 mg of transketolase was used for dansylation. Finger-printing was done by two-dimensional thin-layer silica gel chromatography. The chromatogram was developed in the first dimension with toluene–pyridine–acetic acid (150:30:3.5), in the second dimension with toluene–2-chloroethanol–25% ammonia (100:80:6.7). The plates were examined under UV light (365 nm).

3. Results and discussion

The transketolase preparation used had a specific activity of 16 units/mg at 10^{-4} M TPP and 3×10^{-3} M Mg^{2+} in 10 mM tris/HCl, pH 7.7 and 25°, and contained less than 5% of impurities as checked by analytical acrylamide electrophoresis (fig. 1) and analytical ultracentrifugation using the band forming technique.

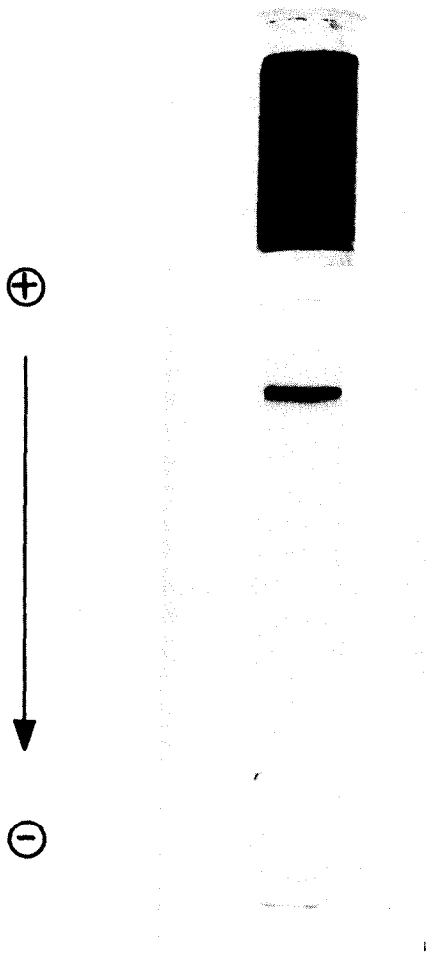

Fig. 1. Acrylamide gel (7.5%) electrophoresis of transketolase. Approximately 50 μg of protein was applied. Electrophoresis was carried out for 2 hr at 10° with a current of 2 mA per tube. The gels were stained with 1% amido black in 7% acetic acid.

Racker [12] obtained a value of 140,000 ± 1,000 as the molecular weight of transketolase by the Archibald method. We could confirm this value by sedimentation equilibrium runs. Denaturation in 6.0 M urea, β-mercaptoethanol and SDS results in the formation of subunits. In fig. 2, the electrophoretic mobilities for marker polypeptide chains are plotted against the log of their molecular weights. From the

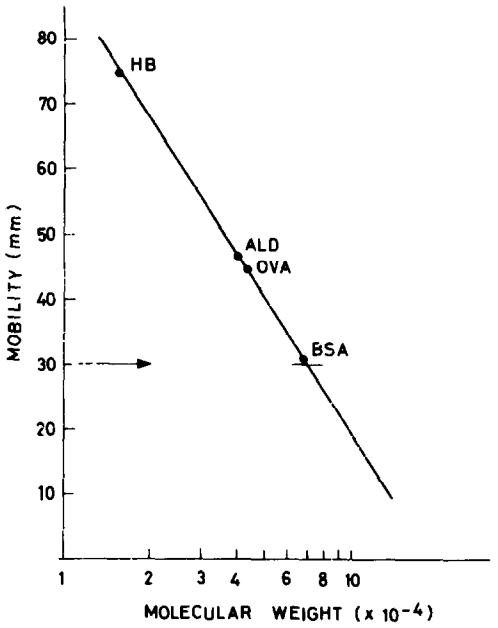

Fig. 2. Determination of the subunit size of transketolase by acrylamide gel (5%) electrophoresis in 0.1% SDS, 0.1 M β-mercaptoethanol and 6 M urea. The proteins used were hemoglobin (HB), aldolase (ALD), ovalbumin (OV), and bovine serum albumin (BSA).

Table 1
Transketolase activity in the presence of urea or β-mercaptoethanol, respectively.

Concentration (M)	Enzyme activity (%)
Urea	
0	100
0.1	83
1.0	78
2.0	45
4.0	0
6.0	0
β-Mercaptoethanol	
0.01	69
0.05	48
0.1	35
0.3	38
0.5	25
1.0	22

electrophoretic mobility of transketolase, which gave only one single band, a molecular weight of 70,000 is found. A 10% accuracy range is suggested for this method [10]. This means that transketolase consists of two subunits. The identity of the subunits could be demonstrated by end group determination. Two-dimensional thin-layer chromatography [11] of the acid hydrolyzed dansylated transketolase showed one spot under UV light (in addition to 1-dimethyl amino naphthalene 5-sulfonic acid), which had the same R_f value as dansyl-arginine. Therefore, arginine must be the *N*-terminal amino acid of transketolase.

Attempts to cleave the enzyme into active subunits by the addition of urea, β-mercaptoethanol and SDS alone or in combination were unsuccessful. Table 1 shows that the enzyme activity is inhibited at rather small concentrations of urea as well as β-mercapto-ethanol. Some reactivation after dialysis was found to occur in the sample treated with β-mercaptoethanol. SDS as well as urea caused an irreversible denaturation of transketolase.

Acknowledgements

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