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ACTIVE SUBUNITS OF TRANSKETOLASE FROM BAKER'S YEAST

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SUMMARY

Transketolase from baker's yeast was covalently bound to Sepharose via one subunit. Storage in glycine buffer pH 11 entailled loss of half of the protein and a 50% decrease in the activity of the immobilized enzyme. Addition to the system of free subunits of transketolase doubled the amount of the protein attached to the matrix and restored the catalytic activity to the initial level. Thermoinactivation of the initial immobilized dimer of transketolase and the renatured dimer formed on reassociation of the immobilized subunits with the free ones was the same and considerably differed from the thermoinactivation of the immobilized subunits. The conclusion is made that the individual subunits of transketolase are catalytically active.

Baker's yeast transketolase consists of two identical or similar subunits (1,2) and has two active centres (3). If the concentration of the enzyme in solution is low it reversibly dissociates into subunits, as a result of which an equilibrium of a dimer $\rightleftharpoons$ 2 monomers is established (4).

It was shown in sedimentation experiments that the dimeric form of transketolase is catalytically active (5). It could not be stated, however, that in solution the monomeric form was present as well, because the studied system had been supplemented with cofactors (thiamine pyrophosphate and magnesium), which are known to shift the above equilibrium, to a great extent or completely, towards the dimer. Therefore the question about the catalytic activity of transketolase subunits had not been solved. We have filled the gap by pre-

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paring the subunits immobilized on an insoluble matrix. The paper describes the results of these experiments.

MATERIALS AND METHODS

Transketolase (EC 2.2.1.1.) was isolated from baker's yeast essentially as described by Racker et al. (6). The enzyme was homogenous as shown by disc electrophoresis, and had a specific activity of 11 U/mg protein. The apoenzyme was obtained by keeping transketolase (0.9 mg/ml) in 1.6 M ammonium sulphate, pH 7.6, for 48 hours at 4°C (7). Before being used, it was passed through Sephadex G-50 equilibrated with 50 mM glycyl-glycine buffer pH 7.9.

Sepharose 4B was a product of Pharmacia Fine Chemicals Inc., β-mercaptoethanol of Sigma Chemical Company.

Transketolase was immobilized on Sepharose 4B activated by BrCN as described in (8). 5 mg of BrCN was used to activate 1 ml of the packed gel. Immobilization took 18 h at 4°. The technique was described in detail previously (9). The absence of the sorption of the enzyme on the support was established by washing the immobilized enzyme with a solution of 5 M K-phosphate buffer pH 7.6 and with a solution of 2 M Tris-HCl pH 7.6.

The enzymatic activity was assayed spectrophotometrically by the rate of reduction of NAD in a system containing glyceraldehyde-3-phosphate dehydrogenase (10). The technique of measurements of the activity of immobilized transketolase consisted in stirring the reaction mixture on a magnetic stirrer and placing the cuvette every minute into the spectrophotometer for a few seconds (11).

A mixture of phosphopentoses used as a substrate was prepared enzymatically from ribose-5-phosphate (12). Barium salt of phosphopentoses was converted into potassium salt in a Dowex-50 column (cathionic form).

The concentration of protein bound with Sepharose was determined by the modified method of Lowry et al. (13,14).

The matrix-bound subunits were obtained by incubating the immobilized transketolase in 50 mM glycine buffer pH 11 containing 10 mM β-mercaptoethanol for 12 h at room temperature with stirring. The dissociated subunits were removed by washing in the same buffer. Then the Sepharose suspension was placed into an ice bath and the pH of the suspension was adjusted to 7.6 by 1 M glycyl-glycine solution for 15 min.

Reassociation of the dimer from immobilized and free subunits was carried out in 50 mM glycyl-glycine buffer pH 7.9 containing 10 mM β-mercaptoethanol and 10 mM $CaCl_2$. The immobilized subunits were supplemented with a 10-fold excess of the free apoenzyme in a concentration of 0.05 mg/ml and incubated for 30 min at room temperature; the unbound protein was removed with 50 mM glycyl-glycine buffer pH 7.9.

Thermoinactivation of immobilized transketolase was carried out at 50° in 50 mM K-phosphate buffer pH 7.6 with continuous stirring on a magnetic stirrer, the concentration of protein being 0.01 mg/ml. At certain time intervals, aliquots of the suspension were taken and placed into a spectrophotometric cuvette containing all the components required for the transketolase activity assay.

RESULTS AND DISCUSSION

Sepharose activated with low amounts of BrCN (5 mg/ml) was used to ensure the covalent binding of the transketolase molecule via one subunit. The specific activity of the enzyme immobilized in this way was about 70% of the initial activity.

It was demonstrated previously (15) that at high pH values, transketolase, even if its concentration in the solution was high, totally dissociates into subunits. In our work this method was used for converting the dimeric form of immobilized enzyme into the monomeric form. In immobilized transketolase kept in glycine buffer pH 11, the amount of protein bound to the matrix decreases by half (Table 1). This indicates that, firstly, the enzyme was primarily bound to Sepharose via one subunit and secondly there is dissociation of the dimer into monomer, as a result of which all the transketolase proved to be bound to Sepharose in a monomeric form.

The transketolase activity decreased to the same degree as the quantity of the matrix-bound protein. In other words, the subunits of transketolase are catalytically active and do not differ from the dimer in specific activity (Table 1).

After incubating the immobilized subunits with free transketolase (under the conditions when part of the enzyme is in the monomeric form), the amount of Sepharose-bound protein doubled up. Its catalytic activity also increased two-fold (Table 1). Thus, reassociation of immobilized and free subunits of transketolase took place, and formation of the renatured dimeric form of the immobilized enzyme was accompanied by restoration of the catalytic activity of the initial immobilized dimer.

Table 1

Activity and Protein Content in Immobilized Derivatives of Transketolase.

The data are calculated per ml of packed gel or per mg of bound protein

Derivative of transketolase	Protein content		Activity		Specific activity	
	mg/ml	%	U/ml	%	U/ml	%
Bound	0.3±0.01	100	2.2±0.03	100	7.3	100
Bound-subunit	0.15±0.005	50	1.1±0.02	50	7.3	100
Bound-renatured	0.3±0.01	100	2.2±0.03	100	7.3	100

Table 2

Inactivation of Immobilized Derivatives of Transketolase at 50°C.

Time of incubation 3 h.

Derivative of transketolase	Activity as percentage of the initial activity
Bound	25
Bound-subunit	67
Bound-renatured	25

Table 2 shows the results of thermoinactivation of matrix-bound derivatives of transketolase. It is obvious that the lability of the matrix-bound dimer and the matrix-bound re-

natured dimer is equal and differs from the lability of the matrix-bound subunit of transketolase*.

The above data should be interpreted to mean that catalytic activity is equally inherent in the dimeric and monomeric forms (calculated per mg protein).

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* The time of thermoinactivation in this case was 3 h. If the preparations of the immobilized enzyme were kept for a longer time, their catalytic activity did not decrease any further.