THE QUANTITY OF THE COENZYME-BINDING SITES IN THE MOLECULE OF TRANSKETOLASE

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SUMMARY

In the course of incubation of apotransketolase with thiamine pyrophosphate a distinct correlation has been revealed between the quantity of bound coenzyme and the value of activity measured. The activity is maximal when two moles of thiamine pyrophosphate is bound to a mole of the enzyme. A conclusion is made that in the molecule of transketolase there are two active centres.

TPP* is the coenzyme of TK /EC 2.2.I.I/ (I,2). The amount of TPP in native holoTK was not determined. In the reconstructed holoenzyme there were found 2-9 moles of the coenzyme per mole of the enzyme (3). It was not clear, however, if all the quantity of the coenzyme is involved in the catalytic activity. So, the question about the number of active centres in the TK molecule was open to discussion. The present paper is a description of the investigation carried out along this line.

MATERIALS AND METHODS

TK was isolated from baker's yeast essentially as described by Racker et al. (4). The enzyme was proved homogeneous in polyacrylamide disc electrophoresis and had a specific

* Abbreviations used: TPP - thiamine pyrophosphate; TK - transketolase.

activity of 13.5 U/mg protein. It was stored in 40% saturated ammonium sulphate solution. Before being used the enzyme was passed through Sephadex G-50 equilibrated with 0.02 M phosphate buffer, pH 7.6. The pentose-5-phosphate equilibrium mixture, which was used as substrate in the transketolase assay, was prepared according to Racker et al. (5). Transketolase activity was determined at 20°C spectrophotometrically, by the rate of NAD oxidation (6). Composition of the reaction mixture: glycyl-glycine - 0.05 M; NAD - 3.7×10^{-4}M; glyceraldehydephosphate dehydrogenase from rabbit muscle - 3 U; cystein - 3.2×10^{-3} M; arsenate - 1.1×10^{-3} M; magnesium chloride - 3×10^{-3} M; TPP - 2.3×10^{-4} M; substrate - 1×10^{-2}M; transketolase. Total volume - 2 ml; pH 7.6.

Protein was determined by the method of Lowry et al.(7); TPP - by enzymatic method (3).

Conditions of reconstruction of holoTK: 120-200 mg apoTK was incubated at room temperature with TPP (final concentration 3.5×10^{-7} - 2.3×10^{-4}M) or TPP and magnesium chloride (final concentration of magnesium chloride - 4×10^{-3}M) in 1.3 - 1.5 ml of 0.02 M phosphate buffer, pH 7.6 for 15-180 minutes (the time required for the maximum amount of holoTK to be formed at a given concentration of TPP*). When the reconstruction was over the samples were passed through Sephadex G-50 equilibrated with 0.02 M phosphate buffer, pH 7.6 to remove free cofactors. Aliquots were taken for determining protein, TPP and enzymatic activity as is described above. Activity was also measured when no cofactors had been added - to characterize the quantity of the holoenzyme for-

* As was demonstrated earlier (8), interaction of apoTK and the coenzyme proceeds in time. It is the greater, the lower is TPP concentration in the incubated sample.

med in the course of reconstruction*. The initial apoenzyme preincubated without the cofactors served as control.

RESULTS AND DISCUSSION

As follows from the data shown in Fig.1 (curve 1) a good correlation was observed between the value of enzymatic activity and the quantity of the bound coenzyme (the activity measured with both cofactors was similar in all cases and was equal to that of the initial enzyme). When two moles of TPP get bound with one mole of protein the activity is the maximal and it does not change if the concentration of TPP in the preincubated sample is raised.

If preincubation was carried out in the presence of magnesium (curve 2 in Fig.1) the quantity of the bound coenzyme does not change. It is only the value of the activity that increased, and only when less than two moles of TPP got bound with the molecule of the enzyme.

We failed to isolate TK with 100 per cent of the holoenzyme as the latter partially breaks off in the course of isolation of TK, when it is being treated with ammonium sulphate (and almost completely when the enzyme is stored in ammonium sulphate solution). In freshly isolated preparation of the TK there was 0.86 moles of TPP per mole of the enzyme. The activity measured without TPP was 55 per cent of the maximal activity (that measured with TPP and magnesium). The ratio of the quantitative content of TPP and the value of measurable activity is in good agreement with the results of reconstruction (see Fig.1).

Thus, the experimental data reported above should be interpreted to mean that one molecule of TK binds two mole-

*The holoenzyme is stable and no TPP cleaved off it in the course of the experiment.

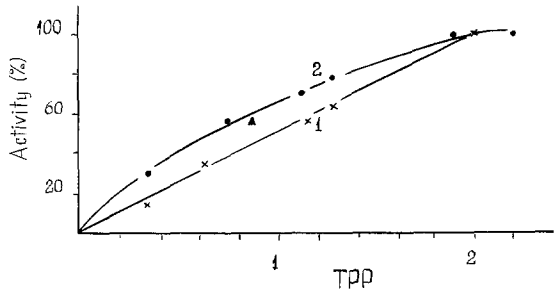

Fig.I. Reconstruction of holotransketolase
I - apoenzyme + TPP
2 - apoenzyme + TPP + magnesium
Abscissa - content of TPP, moles per mole of enzyme / TK was assumed to have a molecular weight of I40.000 (3)/.
Ordinate - activity of reconstructed enzyme /measured without adding cofactors/ in per cent of the maximal activity /that measured with TPP and magnesium/.
▲ - enzymatic activity of the freshly isolated TK preparation and content of TPP in it.

cules of the coenzyme performing catalytic function. In other words, the enzyme has two active centres.

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