THE NUMBER OF ACTIVE SITES IN A MOLECULE OF TRANSKETOLASE

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SUMMARY

It has been demonstrated that the previously described changes in the optical properties of apotransketolase interacting with thyamine pyrophosphate are associated only with the catalytically active molecules of coenzyme being bound. Titration of apoenzyme by TPP has shown a molecule of transketolase to have two active centres.

Transketolase from baker's yeast has a molecular weight of 140.000 /1/ and consists of two, presumably identical, subunits /2,3/. A molecule of the enzyme may attach 2 to 9 molecules of TPP* /4/. The first two in the presence of calcium have sufficiently high values of association constants ($3x10^7M^{-1}$ and $4x10^6M^{-1}$ respectively), whereas the affinity of other molecules of the coenzyme to apoTK is considerably lower /5/. No clear-cut correlation between the amount of bound coenzyme and the value of enzymatic activity was obtained. That is why the question about the quantity of active centres in a molecule of TK has remained open up until now (one could only say with certainly that there should be at least two of them /5/). The present work deals with this question.

MATERIALS AND METHODS

Transketolase /EC 2.2.1.1/ was isolated from baker's

* Abbreviation used: TPP - thiamine pyrophosphate

yeast as described in /6/; then the preparations were fractionated twice or thrice with a saturated solution of ammonium sulphate pH 7.6. The enzyme was proved to be homogenous by disc electrophoresis in polyacrylamide gel and had a specific activity of 12 U/mg. Transketolase activity was determined spectrophotometrically by the quantity of reduced NAD formed /7/. Apotransketolase was prepared as described previously /8/. Protein was determined by the optical density of transketolase solution at 280 nm, which for 1% enzyme solution is 14.5 /9/. Prior to being investigated, the enzyme preparations were passed through Sephadex G-50 equilibrated with 5 mM glycyl-glycine buffer, pH 7.6. The same buffer was used for elution. The binding between TPP and apotransketolase was followed by an optical method based on the previously established fact that the coenzyme-apoenzyme interaction involved formation of a charge-transfer complex. This is manifested by a negative Cotton effect with a maximum at 320 nm in the circular dichroism spectrum /10/ and by a wide band with a maximum at the same wavelength in the absorbtion spectrum /11/. The measurement were conducted according to a double-wave scheme, at 320 and 380 nm with the of a Hitachi 356 spectrophotometre.

Experimentation. The cuvette of the spectrophotometre was fitted with 3 ml of apotransketolase solution in 5 mM glycyl-glycine buffer, pH 7.6 containing 2.3 mM $CaCl_2$ (on titration of apoenzyme by coenzyme, the initial volume of the sample was 2.5 ml). The optical density of the mixture was measured, after which TPP solution was gradually added in 0.01 ml aliquots. Each TPP addition was done only after optical density changes could be observed no longer; where necessary, samples for enzymatic activity measurements were taken. Changes in the volumes of the samples as a result of adding TPP and taking samples for activity measurements were taken into consideration when the experimental data were treated. Several concentrations of apotransketolase were used for each experiment; with all the concentrations the results were identical.

RESULTS AND DISCUSSION

Fig. 1 shows the results of the experiments in which the enzymatic activity and changes in the optical density of apotransketolase on addition of different amounts of TPP were measured. The two sets of values are associated with a strict straight line dependence. This should be interpreted as meaning that catalytic activity is only inherent in the molecules of coenzyme whose interaction with the apoenzyme is accompanied by characteristic changes in the spectrum in the wavelength region mentioned above. In other words, the change in the optical density at 320 nm is indicative of formation

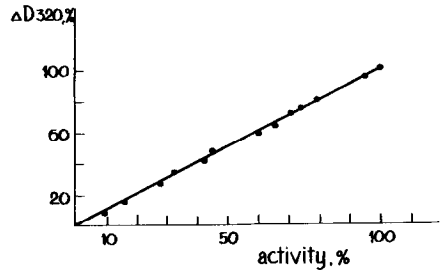

Fig. 1. Plot of transketolase activity v.s. optical density on thiamine pyrophosphate apotransketolase binding. On ordinate and abscissa, respectively, changes in the optical density at 320 nm and value of transketolase activity in per cent of the maximal values measured at saturating concentrations of TPP. Concentration of apotransketolase - 7.14×10^{-6} M.

of catalytically active holotransketolase, i.e. of interaction of TPP with the active centre(s) of the enzyme. It should be noted that in the case of freshly isolated preparations of the enzyme, the changes in the optical density at 320 nm as a result addition of saturating concentration of TPP was always the same and was equal to 6.44×10^{3} per 1 M protein solution.

Fig. 2 shows a titration curve of apotransketolase by TPP. With sufficiently low concentrations of coenzyme, the dependence between the optical density changes at 320 nm and the amount of added TPP is linear. Taking into consideration that, with different concentrations of apotransketolase addition of a certain sufficiently low concentration of TPP entails formation of the same quantity of holoenzyme, this should mean that in the above conditions apotransketolase binds the coenzyme almost entirely. With a mole of TPP binding a mole of protein, the change in the optical density at 320 nm is 50% of the maximal. It may be concluded therefrom that a molecule of transketolase has two active centres,

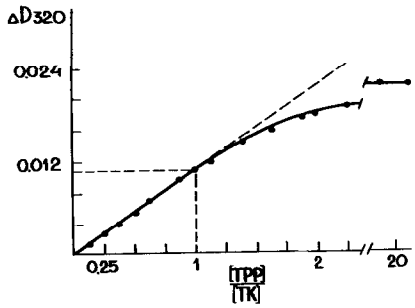

Fig. 2. Titration of apotransketolase of thiamine pyrophosphate
Ordinate - optical density at 320 nm;
Abscissa - quantity of moles of TPP per mole of apoenzyme added to the sample.
Concentration of apotransketolase - 3.57×10^{-6} M.

which is in accord with the statement that the molecule of the enzyme consists of two identical subunits /2,3/.

It follows from the data of Fig. 2 that with sufficiently high concentrations of TPP, when a mole of protein binds more that I mole of coenzyme the optical density v.s. the amount of TPP curve is no longer linear. This agrees with the data that the affinity of coenzyme to the binding sites on the apoenzyme is different - the association constant for the first site is by one order of magnitude higher than for the second site /5/.

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