KINETICS OF RECONSTRUCTION OF HOLO-TRANSKETOLASE

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The study of interaction between thiamine pyrophosphate (TPP) and transketolase (TK) from baker's yeast is the object of numerous papers, but only in some of them [1–3] are there data on a pre-stationary phase of interaction between co-enzyme and apo-enzyme. Since it remained obscure how holo-TK was formed from apo-TK and TPP, via one or more stages, the aim of present work was to elucidate this question.

TK was isolated as described in [4], after which the preparations obtained were fractionated 2–3 times with a saturated solution of ammonium sulphate (pH 7.6), spec. act. of the preparations was about 10 U (25°C). Transketolase activity was measured as described earlier [5]. The amount of protein was determined by the value of the absorbance of TK solutions at 280 nm, which was 14.5 for 1% enzyme solution [6]. Composition of samples in reconstruction of holo-TK: 50 mM Tris–HCl buffer (pH 7.6), 0.35 μM TK (50 μg/ml), 2.3 mM $CaCl_2$ or $MgCl_2$, TPP; 5.0–40.0 μM in the presence of Ca^{2+} and 13.3–100 μM in the presence of Mg^{2+}. The process of reconstruction of holo-TK was followed by the change in protein fluorescence at 350 nm; the wavelength of excitation was 285 nm [2]. Fluorescence was measured in an 'Aminco-Bowman' (USA) spectrofluorometer with a thermostatted cell holder at 25°C, the solution in the 1-cm square quartz cell being constantly stirred. The solution of apo-TK placed into a cell was kept in the cell holder for 5–10 min until the temperature equilibrium was achieved, then by adding the TPP solution (2.5% of the total vol of the sample) to the apo-TK solution reconstruction of holo-TK was initiated. In further calculations, a relatively small change observed in fluorescence of enzyme solutions in the absence of TPP was taken into account.

Under the conditions of $[TPP]_o \gg [apo\text{-}TK]_o$ observed in our experiment, the dependence of the holo-TK concentration on time from the moment of addition of the coenzyme is described by the following equation:

$$[holo\text{-}TK]_t = [holo\text{-}TK]_\infty \cdot \left|1 - 1^{-k_{eff} \cdot t}\right.$$

where $[holo\text{-}TK]_t$ and $[holo\text{-}TK]_\infty$ are concentrations of holo-enzyme at time t and at $t \to \infty$, respectively, and k_{eff} is an effective rate constant of the pseudo-first-order, whose value depends on the TTP concentration. In principle, values of k_{eff} at every given concentration of coenzyme can be derived from the dependence of $\log\left(1 - \frac{[holo\text{-}TK]_t}{[holo\text{-}TK]_\infty}\right)$ vs t. But since in the present work not holo-enzyme concentration, but correlating change of intensity of fluorescence I was measured, the values of k_{eff} were derived from the dependence of $\log\left(1 - \frac{[\Delta I]_t}{[\Delta I]_\infty}\right)$ vs t. Moreover, it turned out that the dependence of k_{eff} on $[TPP]_o$ may be described by a Michaelis type equation:

$$k_{eff} \frac{k\,[TPP]_o}{K + [TPP]_o}$$

which, generally speaking, indicates that the holo-enzyme formation is a two-step process:

$$apo\text{-}TK + TPP \overset{K}{\rightleftarrows} (holo\text{-}TK)_1 \overset{k}{\rightarrow} (holo\text{-}TK)_2, \quad (1)$$

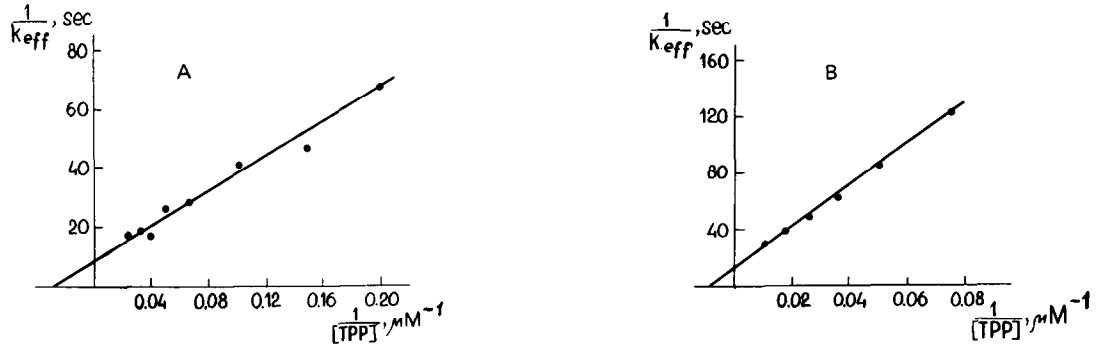

Fig.1. Dependence of effective rate constant of pseudo first-order on TPP concentration in the presence of Ca^{2+} (A) and Mg^{2+} (B). For experimental conditions see 'Methods'. Straight lines are drawn by the least squares method.

where K is the dissociation constant of the $(\text{holo-TK})_1$ and k is a monomolecular rate constant of the second stage. The values of constants K and k are derived from the graphs presented in fig.1: K is equal to 25 and 107 μM, and k is equal to 0.103 and 0.075 sec^{-1} in the presence of Ca^{2+} or Mg^{2+}, respectively.

It should be noted that the two step mechanism (1) is not unequivocally established from the present studies. Under some conditions of ratios of rate constants the alternative scheme (2) could result in similar kinetic behaviour:

$$\begin{aligned} &\text{apo-TK} + \text{TPP} \overset{K}{\rightleftarrows} (\text{holo-TK})_1, \\ &\text{apo-TK} + \text{TPP} \overset{k}{\rightarrow} (\text{holo-TK})_2, \end{aligned} \qquad (2)$$

where the $(\text{apo-TK})_1$ complex is catalytically inactive, and the $(\text{apo-TK})_2$ complex is an active holo-enzyme.

But since it is known [7] that TPP interacts with apo-TK in at least three sites, a single step binding of the coenzyme with the enzyme scheme (2) is hardly probable.

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