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The presence of a hydroxyl group at the C-1 atom of the transketolase substrate molecule is necessary for the enzyme to perform the transferase reaction

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Abstract Transketolase catalyzes the transfer of an aldehyde residue from keto sugars to aldo sugars. The intermediate product is dihydroxyethylthiamine pyrophosphate (DHETPP). In the absence of an acceptor substrate, the reaction is stopped at this stage and DHETPP does not undergo subsequent transformations. Pyruvate decarboxylase catalyses pyruvate decarboxylation to yield free aldehyde. The intermediate product is hydroxyethylthiamine pyrophosphate (HETPP). It differs from DHETPP only in that it has no hydroxyl at the C-2 atom of the aldehyde residue. We have shown that transketolase can bind HETPP and split the aldehyde residue from it. This fact suggests that the path of the reaction is determined by the absence (in HETPP) or presence (in DHETPP) of a hydroxyl group. In the former case the reaction will yield free aldehyde, in the latter the aldehyde residue will be transferred onto an acceptor substrate.

Key words: Transketolase; Pyruvate decarboxylase; Dihydroxyethylthiamine pyrophosphate; Hydroxyethylthiamine pyrophosphate

1. Introduction

Reactions catalyzed by thiamine enzymes yield a common intermediate, an aldehyde residue bound to the second carbon atom of the TPP thiazole ring. The transketolase reaction gives DHETPP, the pyruvate decarboxylase reaction produces HETPP (for review see [1,2]). HETPP differs from DHETPP only by the absence of a hydroxyl at the C-2 atom of the aldehyde residue. TK catalyzes the transferase reaction, i.e. glycolaldehyde residue transfer from donor substrate to acceptor substrate. If glycolaldehyde is used as an acceptor substrate, for example, the reaction will yield erythrulose. With no acceptor substrate the reaction is stopped at the stage of formation of the intermediate, DHETPP [3]. When pyruvate undergoes decarboxylation by PDC, the intermediate is HETPP and the reaction product is free aldehyde. With hydroxypyruvate as a substrate for PDC, the reaction will give erythrulose (with DHETPP as an intermediate). In experiments with glyceraldehyde as an acceptor substrate the reaction product is ketopentose. That is, in the last two cases we deal with a typical transferase reaction, a transfer of a glycolaldehyde residue onto an acceptor substrate [4].

In the present work we show that TK is capable of binding HETPP and of splitting the hydroxyethyl residue from it at a high rate.

Thus both for PDC and for TK the path the reaction will take depends on the presence (in DHETPP) or absence (in HETPP) of a hydroxyl group in the intermediate of the enzymatic reaction: either the aldehyde residue will be transferred onto an acceptor substrate or it will be split off and free aldehyde will form.

2. Materials and methods

The Li salt of hydroxypyruvate was from Sigma (USA), Sephadex G-50 (crude) was from Pharmacia (Sweden), TPP was obtained from Serva (Germany). All other reagents were reagent grade. HETPP was synthesized as described previously [5] and was stored at −20°C; by the thin-layer chromatography data it was free from other admixed compounds. Freshly prepared solutions of HETPP were used in the work. TPP and HETPP concentrations were assayed by absorption at the isobestic point (272.5 nm), pH 2.7, according to $A_{272.5}$ equal to 7400 $M^{-1} \cdot cm^{-1}$ [6]. TK was obtained from baker's yeast by the method of Racker et al. [7] with some modifications [8] and was stored in crystals in ammonium sulfate solution. ApoTK was obtained by incubating the enzyme (2 mg/ml) in 1.6 M ammonium sulfate, pH 8.2, for 48 h; prior to using, it was passed through a Sephadex G-50 column equilibrated with 50 mM glycyl-glycine buffer, pH 7.6. The enzyme was homogeneous according to SDS-PAGE. The specific activity was 13 U/mg. TK concentration was determined spectrophotometrically, using $A^{1\%}_{1cm}$ equal to 14.5 at 280 nm [9]. Transketolase activity was measured spectrophotometrically by the rate of NAD reduction using glyceraldehydephosphate dehydrogenase as an auxiliary enzyme [8]. The reaction mixture contained: 50 mM glycyl-glycine, 0.37 mM NAD, 0.5 U of glyceraldehydephosphate dehydrogenase, 3.2 mM cysteine, 2.5 mM $MgCl_2$, 0.1 mM TPP, 10 mM phosphopentose mixture, transketolase. The volume of samples was 2 ml, pH 7.6. The measurements were made with a Hitachi-557 spectrophotometer (Japan). CD spectra were recorded on a Mark-III, Jobin Ivon dichrograph (France) in cuvettes with the optical pathway 0.5 or 1 cm at a dichroic optical density of $2 \cdot 10^{-6}$ units per 1 mm scale.

ApoTK was titrated with HETPP by subsequent addition of HETPP aliquots to 2 ml of the apoTK solution in 50 mM glycyl-glycine buffer, pH 7.6, containing 1.2 mM $CaCl_2$. Upon each HETPP addition, the mixture was incubated for 6 min after which the change in the amplitude of the negative CD peak at 320 nm was registered. The amount of HETPP bound in the active center of TK was calculated from the titration data [10].

3. Results and discussion

As was shown previously [10], binding of the coenzyme (TPP) to apoTK resulted in a negative band (a maximum at 320

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Abbreviations: TK, transketolase; PDC, pyruvate decarboxylase; TPP, thiamine pyrophosphate; DHETPP, dihydroxyethylthiamine pyrophosphate; HETPP, hydroxyethylthiamine pyrophosphate; CD, circular dichroism.

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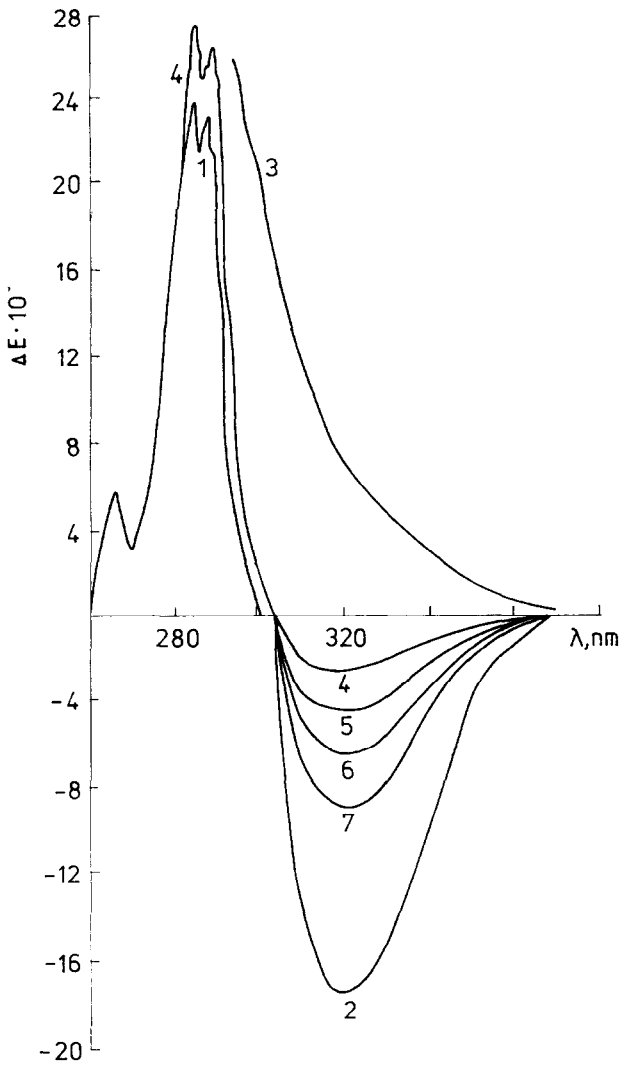

Fig. 1. CD spectra of the transketolase. 1, apoTK; 2, apoTK + TPP (0.1 mM); 3, like 2 + hydroxypyruvate (2.2 mM); 4–7, apoTK + HETPP, respectively 2.5, 5.0, 7.5 and 10 μM. TK, 8.3 μM; $CaCl_2$ (in samples 4–7), 1.2 mM; glycyl-glycine buffer, 50 mM, pH 7.6. The CD spectra in the range of 260–360 nm are given only for apoTK and apoTK + 2.5 μM HETPP; in all other cases the optical density of the samples was too high to measure the spectra in the shortwave region.

nm) in the CD spectrum which was absent from the CD spectra of the initial components (cf. 1 and 2 in Fig. 1); at the same time the optical activity of protein aromatic chromophores in the UV region of the spectrum was increased (not shown). These changes of the CD spectrum reflected the formation of TK active center [12]. On addition of a donor substrate to holoTK (without an acceptor substrate the reaction was stopped at the stage of formation of DHETPP bound with apoTK) inversion of the negative band of the CD spectrum was observed (3 in Fig. 1). This band is superimposed on the CD spectrum resulting from the optical activity of protein aromatic chromophores [11]. Changes in the CD spectrum observed on addition of HETPP to apoTK are similar to those observed on addition of TPP to apoTK (cf. 4–7 and 2 in Fig. 1). This is evidence that when HETPP binds with apoTK the hydroxyethyl residue is split off and holoTK is formed.

When titrating apoTK with HETPP, a clear-cut relationship was observed between the added amount of HETPP and the change in the amplitude of the peak with a maximum at 320 nm (4–7 in Fig. 1). Given the same concentration of HETPP but different concentrations of apoTK, the value of ΔE_{320} was the same. With the apoTK and HETPP concentrations used in experiments shown in Fig. 1, the amount of the resulting holoenzyme was equal to the amount of HETPP added to the sample. Consequently TK can bind both enantiomeres of HETPP (used in the work was chemically synthesized HETPP) and perform their catalytic transformation, with the result that acetaldehyde is split out and holoTK is formed. We did not observe spontaneous (non-enzymatic) splitting of acetaldehyde from HETPP under our experimental conditions.

The formation of holoTK by the interaction of HETPP with apoTK is also confirmed in experiments which have shown that HETPP can act as a coenzyme in the transketolase reaction. At saturating concentrations of HETPP and TPP the transketolase reaction proceeds at the same rate in both cases (Fig. 2).

Thus, HETPP is converting into enzyme-bound TPP and reaction product under the action of TK. This fact suggests that the absence (in HETPP) or presence (in DHETPP) of the HO-

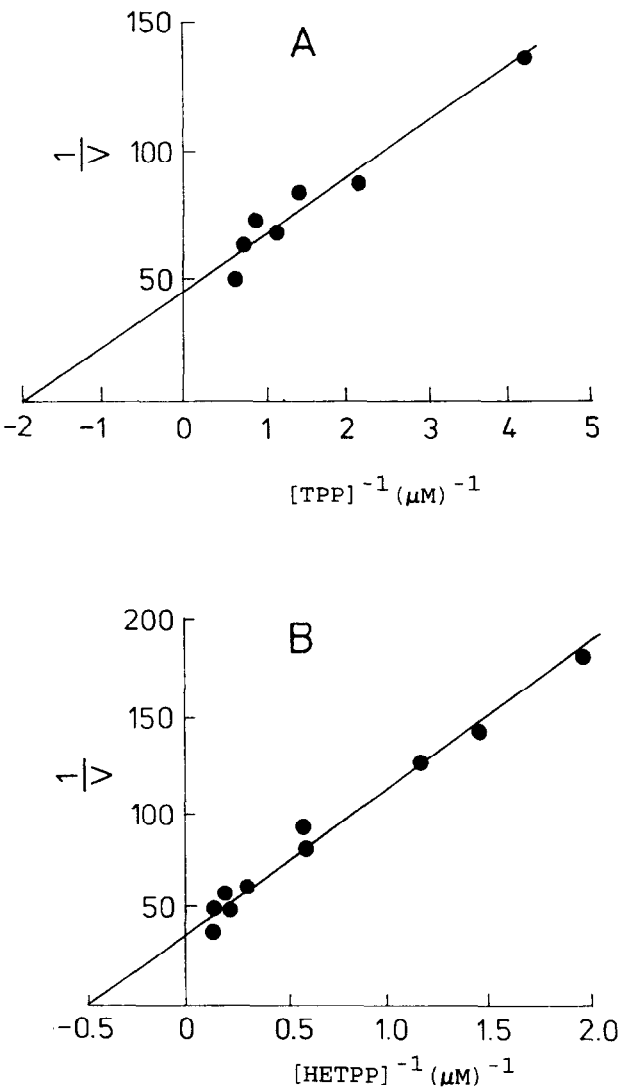

Fig. 2. Dependence of the transketolase reaction rate on TPP (A) and HETPP (B) concentration.

group at the second C-atom of the aldehyde residue dictates what path the reaction will take: either the aldehyde residue will be split out of TPP or it will be transferred onto an acceptor substrate.

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