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Structure and functioning mechanism of transketolase

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ABSTRACT

Studies of thiamine diphosphate-dependent enzymes appear to have commenced in 1937, with the isolation of the coenzyme of yeast pyruvate decarboxylase, which was demonstrated to be a diphosphoric ester of thiamine. For quite a long time, these studies were largely focused on enzymes decarboxylating α-keto acids, such as pyruvate decarboxylase and pyruvate dehydrogenase complexes. Transketolase, discovered independently by Racker and Horecker in 1953 (and named by Racker) [1], did not receive much attention until 1992, when crystal X-ray structure analysis of the enzyme from *Saccharomyces cerevisiae* was performed [2]. These data, together with the results of site-directed mutagenesis, made it possible to understand in detail the mechanism of thiamine diphosphate-dependent catalysis. Some progress was also made in studies of the functional properties of transketolase. The last review on transketolase, which was fairly complete, appeared in 1998 [3]. Therefore, the publication of this paper should not seem premature.

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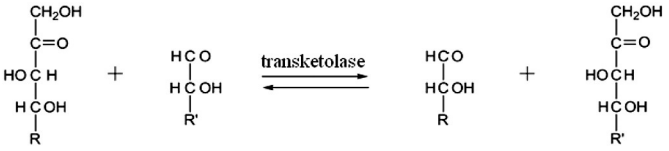
1. Introduction

Transketolase (TK, EC 2.2.1.1) is the key rate-limiting enzyme of the non-oxidative branch of the pentose phosphate pathway of carbohydrate transformation. As is the case of other enzymes of the pentose phosphate pathway, it is located in the cytoplasm. TK belongs to the group of thiamine diphosphate (ThDP)-dependent enzymes. It catalyzes two processes: cleavage of the C–C bond in a ketose (the donor substrate) and reversible transfer of the double-carbon fragment thus formed ('active glycolaldehyde') onto an aldose (the acceptor substrate) [3,4]:

Transketolase reaction: a schematic representation.

ThDP, the biologically active form of vitamin B_1 (Fig. 1), is the coenzyme of TK. Being in itself multifunctional, ThDP determines neither the direction nor the particular substrate (or substrate type) of a TK-catalyzed reaction; rather, these depend on the specific apoprotein of the enzyme. In general, TKs (with the exception of the enzymes of mammalian origin) are characterized by broad substrate specificity. Xylulose 5-phosphate (X5P), fructose 6-phosphate (F6P), erythrulose 4-phosphate, and sedoheptulose 7-phosphate are typical donor substrates of TK; ribose 5-phosphate (R5P), glyceraldehyde 3-phosphate (G3P), and erythrose 4-phosphate are typical acceptor substrates.



Conditions to be met by a donor substrate (prerequisite to its functional activity) include (1) the availability of an oxo group adjacent to the C–C bond cleaved, (2) the presence of a hydroxyl at the first carbon atom (C1), and (3) the D-threo configuration of hydroxyls at the asymmetrical third and fourth carbon atoms (C3 and C4); see the reaction scheme above. Hydroxypyruvate (HP) and dihydroxyacetone, which both lack asymmetrical carbon atoms, are the exception to the rule. When HP is used as a substrate, TK reaction becomes irreversible.

TK is widespread in nature. It is found in all organs and tissues of animals and plants studied thus far, as well as in microorganisms. The enzyme was isolated as a homogeneous protein and crystallized from a number of sources, including *Saccharomyces cerevisiae*, maize, *Escherichia coli*,*Leishmania mexicana*, *Bacillus anthracis*, *Francisella tularensis*, and human tissues [2,5–7]. TK plays an important role in life-sustaining activities of cells.

Its operation (in a concert with transaldolase) enables the interaction (Scheme 1) between the pentose phosphate pathway of carbohydrate transformation and glycolysis (which makes it possible for the cell to promptly adapt to its metabolic needs) and the formation of a number of coenzymes, vitamins, and precursors for nucleotide synthesis. Erythrose 4-phosphate, formed in the TK reaction when F6P serves as the donor substrate, is the starting material in the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan). In addition, TK is the key enzyme of the photosynthetic Calvin cycle, in which it

Abbreviations: TK, transketolase; ThDP, thiamine diphosphate; X5P, xylulose 5-phosphate; R5P, ribose 5-phosphate; F6P, fructose 6-phosphate; G3P, glyceraldehyde 3-phosphate; HP, hydroxypyruvate; GA, glycolaldehyde; DHEThDP, dihydroxyethylthiamine diphosphate; TKTL1 and TKTL2, transketolase-like proteins 1 and 2

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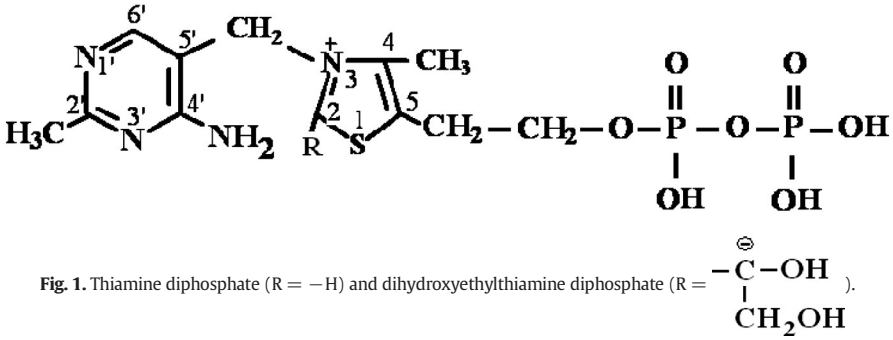

Fig. 1. Thiamine diphosphate (R = −H) and dihydroxyethylthiamine diphosphate (R = $-\overset{\ominus}{C}-OH$ with CH_2OH).

catalyzes the reactions of F6P and sedoheptulose 7-phosphate with G3P, leading to the formation, among other products, of X5P. The latter is further isomerized into ribulose 5-phosphate, which in turn undergoes phosphorylation, producing ribulose 1,5-diphosphate, the recipient of CO_2 in the photosynthetic process.

From the standpoint of biotechnology, it is interesting to use TK as a bioconversion tool, largely for the enzymatic synthesis of ketoses (particularly, in cases where HP is the substrate cleaved irreversibly) [9,10].

Being the simplest representative of ThDP-dependent enzymes, TK may serve as a good model for research into their function. As of today, the best studied TK is that isolated from *S. cerevisiae*. It is the first ThDP-dependent enzyme subjected to crystal X-ray structure analysis [2,11].

Our review will largely cover the properties of this particular enzyme, and it is worthy of note that they do not differ significantly from those of TKs of other origin.

2. General structure of transketolase

The molecule of TK from *S. cerevisiae* appears as a homodimer of structurally identical subunits, each having a molecular weight of 74.2 kDa. Each subunit comprises three α/β-type domains: the N-terminal, or PP (residues 3–322), the middle, or Pyr (residues 323–538), and the C-terminal (residues 539–680); see Fig. 2. The first two domains are involved in binding the coenzyme (ThDP), the functions of the third domain are currently unknown. It is believed to be involved in the regulation of the enzyme activity. TK from *E. coli*, however, retains its activity in the absence of the third domain [13].

The dimeric structure of TK (Fig. 3) is largely formed *via* the close interaction of PP and Pyr domains. The coenzyme is embedded into the deep cleft between the subunits in such a way that (in the quiescent state) only C2 of the thiazole ring (which ensures the donor substrate binding to ThDP) and 4′-amino group of the aminopyrimidine ring remain fully accessible to the solvent. The thiazole ring of ThDP is located in the cleft between the PP domain of one subunit and the Pyr domain of the other subunit, interacting with amino acid residues of both. The aminopyrimidine ring is lodged in a hydrophobic pocket largely formed by aromatic amino acid residues of Pyr domain (Phe-442, Phe-445, and Tyr-418); (see Fig. 4). The side chain of Phe-445 is in stacking interaction with the aminopyrimidine ring of the coenzyme.

Fig. 5 provides a schematic representation of ThDP interaction with functional groups of the active site of TK. The aminopyrimidine ring forms a series of hydrogen bonds, of which the most important is that formed between the N1′-atom of the ring and the side chain of Glu-418, playing the key role in the mechanism of ThDP-dependent catalysis (Scheme 5). The pyrophosphate group of the coenzyme interacts with the PP domain of the apoprotein both directly (by forming hydrogen bonds with His-69, His-263, and Lys-158) and indirectly (*via* the bound calcium ion); see Fig. 5. In fact, the pyrophosphate group of ThDP performs an anchoring function, ensuring tight binding of the coenzyme to the apoprotein [15]. Since the active site of TK is formed by both subunits, the dimeric form of the enzyme should be viewed as a functional catalytic unit.

3. Cofactors of transketolase

TK has two functionally identical active sites [16,17]. It is isolated as a holoenzyme, in which each active site is matched by one ThDP molecule and one calcium ion (Ca^{2+}) [18]. The coenzyme, loosely bound to the apoprotein, readily dissociates, particularly at pH above 6.5 and in the presence of ammonium sulfate in the medium [19,20]. Ca^{2+} is likewise readily released from one (the second[1]) active site. Binding of Ca^{2+} to the other (first) active site is stronger; the ion is removed under fairly stringent conditions [21]. Bivalent cations other than Ca^{2+} may also perform the function of the cofactor of TK reaction [22]. In such cases, the activity of the enzyme remains essentially the same; it is the rate of the coenzyme interaction with the apoprotein (leading to the formation of the holoenzyme) that is affected by the change. Rank order of potency of bivalent cations I increasing this rate is as follows: $Ca^{2+} > Mn^{2+} > Co^{2+} > Mg^{2+} > Ni^{2+}$ [23].

The presence of Ca^{2+} in the active site of native holoTK was somewhat unexpected. It was believed Mg^{2+} functions as the natural cofactor in all ThDP-dependent enzymes. It is for this reason that this particular cation was always used in experiments with such enzymes, including TK. However, only in the presence of Ca^{2+}, but not Mg^{2+}, was it possible to obtain crystals of TK from *S. cerevisiae*, which were appropriate for X-ray structure analysis [2]. This could not be accomplished when Mg^{2+} was substituted for Ca^{2+}.

It is interesting to note in this connection that benzoylformate decarboxylase (another ThDP-dependent enzyme) is also crystallized only in the presence of Ca^{2+}. It was suggested [24] that, in the course of the crystallization, Ca^{2+} replaces Mg^{2+} in the active site. Calcium ions are required for both the growth of the crystals and their stabilization. TK isolated from human tissues was found to contain Mg^{2+} [25]. Apparently, the ions were bound by the active sites of the enzyme. But in this case, as well, crystals appropriate for X-ray structure analysis were obtained only in the presence of Ca^{2+} [5]. No evidence has been reported thus far of the ability of the enzyme to form crystals under the conditions of Mg^{2+} substitution for Ca^{2+}.

The observed rate of TK reaction in the system TK + Ca^{2+} + ThDP + excess substrates remains constant over time only if the concentrations of both cofactors are saturating. When ThDP concentration is low, a lag preceding the steady-state phase is observed; the duration of this lag is inversely proportional to the concentration of the coenzyme [26]. The appearance of the lag is accounted for by the low rate of ThDP interaction with the apoprotein, which encompasses at least two stages (see Scheme 2).

The first stage (the interaction of the diphosphate residue of ThDP with the apoprotein), fast and readily reversible, results in the formation of the primary complex TK···ThDP, which lacks catalytic activity. The

[1] To simplify the description, let us arbitrarily assign numbers two and one, respectively, to the active sites harboring loosely and tightly bound Ca^{2+}.

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